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ACHIEVEMENTS AND PROSPECTS OF MICROBIOLOGY



II International Scientific Conference,
dedicated to 90th anniversary of the Department of Microbiology,
Faculty of Biology, Ivan Franko National University of Lviv

Lviv, October 12–14, 2026

MINISTRY OF EDUCATION AND SCIENCE OF UKRAINE
IVAN FRANKO NATIONAL UNIVERSITY OF LVIV
SERHII VYNOGRADSKY SOCIETY OF MICROBIOLOGISTS OF UKRAINE
INSTITUTE OF CELL BIOLOGY OF THE NAS OF UKRAINE
D. K. ZABOLOTNY INSTITUTE OF MICROBIOLOGY AND VIROLOGY
OF THE NAS OF UKRAINE
MINISTRY OF SCIENCE AND HIGHER EDUCATION OF THE REPUBLIC OF POLAND
FACULTY OF BIOTECHNOLOGY MEDICAL COLLEGE
UNIVERSITY OF RZESZÓW

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**II INTERNATIONAL SCIENTIFIC CONFERENCE,
DEDICATED TO 90TH ANNIVERSARY OF THE DEPARTMENT
OF MICROBIOLOGY, FACULTY OF BIOLOGY,
IVAN FRANKO NATIONAL UNIVERSITY OF LVIV
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MICROBIOLOGICAL RESEARCH AT LVIV UNIVERSITY: HISTORICAL EMERGENCE, DEVELOPMENT, AND MODERN DIRECTIONS

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Abstract. This paper traces the evolution of the Department of Microbiology at Ivan Franko National University of Lviv, highlighting key milestones in its organisational structure, research focus, and academic programs. It traces early microbiological investigations at Lviv University, the founding of the Department of Microbiology in 1936, its administrative reorganisations, and its post–World War II re-establishment within the Faculty of Biology. The contributions of the Department's leading scientists to the development of microbiology, focusing on research into microbial physiology and biochemistry, nitrogen fixation, biosynthesis of biologically active substances, plant–microbe interactions, and microbial communities in natural ecosystems, are analysed. The study outlines the Department's pivotal contribution to shaping scientific disciplines, educating qualified professionals, and advancing microbiology in Ukraine. It illustrates how the Department's research agenda has adapted to contemporary priorities in biology and microbiology. Ultimately, it underscores the Department's essential role in sustaining and enriching Lviv University's academic traditions.
Keywords: Lviv University, Department of Microbiology, history of microbiology, microbiological research, microorganisms, Faculty of Biology.

Origin and Development of Microbiological Research at Lviv University. The history of microbiological research in Lviv is intertwined with advances in botany, plant physiology, medicine, hygiene, sanitary science, and soil science. In the late 19th and early-to-mid 20th centuries, scientists at Lviv University and other scientific institutions in the city studied interactions between plants and microorganisms, soil microbiota, and the causative agents of infectious diseases, and developed practical methods for combating dangerous diseases. Their achievements significantly contributed to the establishment of microbiology as an independent science. These studies took place against a backdrop of constant social change – from the emergence of modern European science to the First and Second World Wars. Scientists not only conducted fundamental research but also applied their knowledge to solve practical problems, ranging from increasing soil fertility and preserving natural areas to preventing rabies, typhus, and other infections that plagued humanity. By bridging fundamental studies of the microworld with practical societal demands, they established a microbiological research tradition at Lviv University that extended well beyond its walls.

Franciszek Dionizy Kamieński: From Botany to the Study of Mycorrhiza. Among the first scientists in Lviv to initiate research closely aligned with modern microbiology was the botanist, Doctor of Philosophy, and Professor Franciszek Dionizy Kamieński (1851–1912). From 1877 to 1883, he worked as a private docent (Privatdozent) at Lviv University, Lviv Polytechnic, and the Lviv Veterinary Academy, later continuing his scientific career in Odesa as a professor at Imperial Novorossiysk University and director of the Botanical Garden. F. D. Kamieński's scientific interests spanned plant anatomy, morphology, and taxonomy, as well as plant parasites and plant diseases. Research on the plant Monotropa hypopitys is a prominent part of his academic legacy. In 1880, while studying its root system, the scientist noted the coexistence of fungi and higher plants – a phenomenon later termed mycorrhiza. Mycorrhiza is considered an example of a symbiotic interaction in nature. Fungal hyphae

interact with plant roots and can significantly expand the absorption zone for water and mineral nutrients, while the fungus receives organic compounds from the plant. Consequently, F. D. Kamieński's findings represented a significant milestone in comprehending the intricate interactions between plants and microorganisms. However, his work extended far beyond laboratory research. He joined extensive botanical expeditions, investigating the flora of Crimea (1887–1888), England (1889), India, Ceylon, Java, and Egypt (1892). Later, moving to Odesa while affiliated with Imperial Novorossiysk University, he described Metschnikowia artemiae – a fungus isolated from local estuaries that parasitises small crustaceans. To support this work, he developed a specialized microscope with a movable stage for stereoscopic photography (1908) (Kalynovych et al., 2011). Thus, his scientific work encompassed fundamental botany and microbiology of the time and to advance microscopic methodologies.

Microbiology in the Service of Human Health: The Fight Against Rabies and Other Infections. Alongside fundamental research, applied microbiology evolved with a focus on preventing infectious diseases. A key event in the advancement of this field in Ukraine was the establishment of the Pasteur Station in Odesa in 1886, the world's second such facility. At this station, the anti-rabies vaccination technique developed by L. Pasteur and his co-workers was put into practical application. The work of the physician and microbiologist, Professor of Hygiene Paweł Ludwig Kuczera (1872–1928), was especially significant for Lviv. In 1911, he launched rabies vaccine production in Lviv, and in 1913, he founded the first Pasteur station in Galicia to fight this fatal infection. Establishing such a station had not only medical but also social significance. Rabies, which is transmitted primarily through the bites of infected animals, is almost invariably fatal once clinical symptoms appear. That is why timely post-exposure vaccination became a fundamentally important preventive measure. P. Kuczera also worked on developing methods to combat cholera, typhus, and typhoid fever. From 1906 to 1919, he headed the Department of Hygiene, and in 1912–1913, he served as acting dean of the Medical Faculty at the University of Lviv. His interests also extended beyond the laboratory. From 1902, P. Kuczera served on the board of the General University Lectures at the University of Lviv, and in 1913, he became a member of the Supreme Health Council of Austria. In 1915–1916, P. Kuczera served as President of the Lviv Doctoral Society and was later granted the title of Honorary Citizen of Lviv. In 1919, he moved to Brno (today the Czech Republic), where he helped establish the Medical Faculty at Masaryk University (Hnatush & Nadraha, 2011).

Seweryn Józef and Helena Józefa Krzemieniewski: From Plant Physiology to New Species of Microorganisms. In the early 20th century, an important discipline emerged at the intersection of plant physiology, soil science, and microbiology. One of its leading representatives was the plant physiologist, Doctor of Philosophy, and Professor Seweryn Józef Krzemieniewski (1871–1945). S. J. Krzemieniewski studied at Warsaw and Jagiellonian Universities and completed research internships in Delft under the renowned microbiologist M. Beijerinck and in Leipzig under the plant physiologist W. Pfeffer – such training combined classical botany with what were then cutting-edge experimental methods in microbiology and plant physiology. From 1919 to 1941, S. J. Krzemieniewski served as a professor and head of the Department of Plant Anatomy and Physiology at the University of Lviv. From 1931 to 1932, he served as Rector of the Lviv University. One of his key research areas was the biology of soil microorganisms. The scientist studied nitrogen-fixing bacteria. In his work, he showed that molybdenum, in the presence of iron, can enhance the nitrogen-binding capacity of nitrogen-fixing bacteria. These studies had implications extending beyond theoretical value. Nitrogen fixation is a key natural process by which atmospheric nitrogen enters the biological cycle. Soil fertility and the productivity of plant communities

largely depend on its efficiency. S. J. Krzemieniewski also proposed an original method for isolating myxobacteria from soil and studied their morphology. Myxobacteria are a distinctive group of bacteria, many of which can exhibit complex collective behaviour: under adverse conditions, they can aggregate to form multicellular fruiting structures. Studying such organisms broadened understanding of the diversity and organisation of the microbial world. The professor's scientific interests were not confined to microbiology. He investigated humus formation processes, mineral and organic fertilisers, grassland management, plant physiology and taxonomy, and the history of science. S. J. Krzemieniewski initiated the creation of a steppe flora reserve in Kryvchytsi near Lviv, combining scientific research with the preservation of natural heritage (Terek, 2011).

His wife, Helena Józefa Krzemieniewska (1878–1966), was a talented plant physiologist and microbiologist. Her scientific work is an important part of the history of microbiology, even though women's contributions to science remained less visible in general historiography for a long time. H. J. Krzemieniewska was educated in Kraków, studying at the Faculties of Mathematics and Natural Sciences at the Jagiellonian University. From 1900 to 1914, she worked as an assistant in the Department of Plant Physiology at the Jagiellonian University and at the Academy of Agriculture in Dubliany. After the First World War, H. J. Krzemieniewska taught at Lviv Polytechnic, and from 1924, she served as a research assistant to the professor in the Department of Plant Physiology at the University of Lviv. Soil microbiology became the primary focus of her scientific work. H. J. Krzemieniewska investigated nitrogen-fixing bacteria, but her most significant contribution lay in the study of myxobacteria. She developed a technique for isolating them from soil, studied their morphology and taxonomy, and isolated and described 13 new species of myxobacteria. In recognition of her contributions, scientists named a bacterial species *Cytophaga krzemieniewskae*. This vividly illustrates how a researcher's individual contributions are enshrined in scientific nomenclature. H. J. Krzemieniewska lived through an era of major social and political turbulence. During the First World War, she worked as a nurse in military hospital, and in 1920, she participated in a vaccination campaign against smallpox and typhus. The period of World War II was particularly significant. From 1941 to 1944, H. J. Krzemieniewska worked alongside her husband at Rudolf Weigl's laboratory. Her work centered on maintaining louse cultures needed for research and producing the typhus vaccine. Thus, fundamental microbiology and practical medicine became directly interconnected under wartime conditions. After the war, H. J. Krzemieniewska continued her scientific work in Kraków and Wrocław. In 1946, she became a professor at the University of Wrocław, where she headed the Department of Plant Physiology, and later worked at the Higher School of Agriculture and the Institute of Botany of the Polish Academy of Sciences (Bilinska, 2011d).

Zdzisław Karol Steusing and Sanitary Microbiology. Another representative of the Lviv school of microbiology was the physician Professor Zdzisław Karol Steusing (1883–1952). Between 1922 and 1939, he was an extraordinary professor (profesor nadzwyczajny) and chaired the Department of Hygiene from 1923 to 1939, while also serving as dean (1930–1931) and vice-dean (1931–1932) of the Medical Faculty. Prof. Z. K. Steusing studied the microbiota of potable water. These studies were of direct sanitary-hygienic significance, as water quality was and remains a key factor in public health. During the 1941–1944 German occupation of Lviv, he lectured at the Ukrainian Underground University (Kolisnyk & Bilinska, 2011). Thus, at the beginning of the twentieth century, microbiological studies in Lviv moved beyond the traditional scope of botany and plant physiology to establish closer ties with medicine, epidemiology, and public health. During this period, research into the causative agents of infectious diseases, laboratory diagnostic approaches, and the development of specific prophylactic methods advanced significantly. The fundamental studies by

Rudolf Weigl, which laid the groundwork for modern rickettsiology, gained widespread international recognition.

Rudolf Stefan Weigl, founder of the world-renowned school of rickettsiology. One of the central figures in the history of microbiology at Lviv University was Rudolf Stefan Weigl (1883–1957) – a zoologist, microbiologist, PhD, and professor, regarded as one of the foremost rickettsia researchers of the first half of the 20th century. R. Weigl was born in 1883 in Moravia and received his higher education at Lviv University. His early research focused on zoology, cytology, and histology. From 1907 to 1913, he worked as an assistant in the Department of Zoology and Comparative Anatomy, and from 1913 onward as an Associate Professor of Zoology at Lviv University. His experience in studying cellular structures and working with microscopic objects later became an important prerequisite for his success in researching intracellular parasites.

World War I marked a turning point in R. Weigl’s scientific biography. Conscripted into the Austrian Army, he was involved in combating epidemic typhus outbreaks in prisoner-of-war and refugee camps across Bohemia, Moravia, and Poland. Working in a military bacteriological laboratory, Weigl directly encountered epidemic typhus – an infection that caused large-scale epidemics during wartime, famine, mass population displacement, and poor sanitary conditions. This practical experience in epidemic control defined the subsequent trajectory of his research. After his demobilisation, R. Weigl returned to Lviv and, in 1919, was appointed head of the laboratory in Przemyśl, where he began systematic research on epidemic typhus. In 1920, he was appointed Professor and Chair of the Department of General Biology within the Medical Faculty at Lviv University. A dedicated center of epidemic typhus research gradually coalesced within the department, and in 1921, its members were integrated into the newly created typhus research laboratory at the University. The laboratory earned broad international recognition as the ‘Weigl Institute’. At the same time, it is important to clarify the terminology: within Lviv University’s contemporary structure, no separate unit was officially designated as the ‘Weigl Institute’ as we understand it today. In 1940, the Lviv Sanitary-Bacteriological Institute was established on its basis. Rudolf Weigl’s foremost scientific breakthrough was the development of a method to artificially infect body lice with *Rickettsia prowazekii* – the causative agent of epidemic typhus. Rickettsiae are a specialised group of microorganisms that reproduce predominantly inside host cells. This trait long hindered their laboratory cultivation. R. Weigl proposed using the human louse as a biological model to cultivate the pathogen. Controlled infection of lice made it possible to obtain sufficient yields of rickettsiae for both scientific research and vaccine preparation. Lice, which feed on human blood, were sustained on the bodies of human donors, who were referred to as ‘feeders’. This approach formed the basis of Weigl’s method and fundamentally transformed the capacity to study epidemic typhus experimentally. Utilising this developed system, R. Weigl not only obtained material for vaccine production but also investigated the biological properties of other members of the Rickettsia group. He worked with the causative agent of endemic (murine) typhus, known in the scientific literature as Mooser’s rickettsia, examined Rocha-Lima rickettsia associated with other rickettsial diseases, and studied the pathogens responsible for Rocky Mountain spotted fever and trench fever (also called Volhynian fever). The key practical result of these studies was the development of an epidemic typhus vaccine. Rudolf Weigl succeeded not only in designing the production method but also in establishing its mass production. Deployed in epidemic hotspots throughout Europe, Africa, and Asia, the vaccine effectively elevated the university laboratory to an international center for research on and prevention of rickettsial diseases.

Equally significant was the development of a laboratory diagnostic method for epidemic typhus – the microagglutination test. This assay relied on an antigen from infected lice and allowed detection of a specific immune response. The Nobel laureate Charles Nicolle referred to this diagnostic test as the Weigl reaction, demonstrating its international scientific recognition. C. Nicolle also visited Weigl's laboratory at the University of Lviv. R. Weigl's achievements naturally resulted from combining thorough foundational training with extraordinary experimental ability. His earlier works in cytology, histology, and experimental biology shaped his technical proficiency in handling cellular structures and microscopic preparations, which proved indispensable for studying intracellular parasites.

During the interwar period, Weigl's laboratory emerged as one of Europe's leading centers for research on epidemic typhus. It hosted not only permanent staff but also numerous young scientists, physicians, and researchers from various countries. Lviv thus became a key center for international exchange of scientific knowledge in rickettsiology.

The international recognition of Weigl's research was likewise reflected in his educational and scientific outreach activities. In 1937, invited by the League of Nations, Professor Weigl presented a series of lectures in Geneva dedicated to strategies for controlling epidemic typhus. In 1939, he delivered lectures in Sweden and undertook a mission to Ethiopia to advise on setting up vaccine manufacturing. Weigl was nominated for the Nobel Prize in Physiology or Medicine on multiple occasions, notably in 1930, 1932, and 1936–1938. In 1940, the Soviet authorities established the Sanitary and Bacteriological Institute on the basis of R. Weigl's laboratory. Sergei Terekhov was appointed director, while R. Weigl became deputy director for scientific affairs and effectively continued to provide scientific leadership for research on epidemic typhus. This building houses the State Institution 'Lviv Research Institute of Epidemiology and Hygiene', whose work is linked to the scientific legacy of R. Weigl's laboratory (now the Research Institute of Epidemiology and Hygiene at the Danylo Halytsky Lviv National Medical University). The institute succeeded the Sanitary and Bacteriological Institute. This institute houses a museum exhibition dedicated to the eminent physician, epidemiologist and microbiologist Heinrich Mosing, which contains documents, photographs, scientific materials and the scholar's personal items, as well as materials relating to his teacher R. Weigl and the staff of his laboratory, in particular Zbigniew Stuchla, Jan Starżyk and other scientists who worked on research into typhus and the development of a vaccine using Weigl's method. A separate and particularly difficult phase in the history of Lviv's rickettsiology coincided with World War II. After the German occupation of Lviv was established, research institutions were subjected to terror and the systematic persecution of the intellectual elite. In July 1941, the German authorities carried out mass executions of Lviv university professors and their families. Weigl was thus faced with a complex moral and practical choice. He agreed to continue vaccine production, notably for the needs of the German army, while simultaneously leveraging the opportunities afforded by his position to protect scientists, students, and other persecuted individuals by employing them as lice feeders. Weigl secured the authority to select his staff independently and effectively assumed personal responsibility for them before the occupying authorities. Employment at the laboratories gave individuals official status and some protection against deportation, forced labor, and other forms of persecution. According to modern estimates, the employment system within institutions associated with R. Weigl helped thousands of individuals, including members of the scientific and academic communities, university and high school students, and members of the resistance movement. Prominent figures in Weigl's laboratory included the mathematicians Stefan Banach and Bronisław Knaster, the geographer Alfred Jahn, the botanist Stanisław Kulczyński, the microbiologists Heinrich Mosing and Henryk Meisel, the writer Zbigniew Herbert, the composer and conductor Stanisław Skrowaczewski, and other

representatives of Lviv's intellectual elite. At the same time, laboratory staff leveraged opportunities in vaccine manufacturing to help the civilian population. Heinrich Mosing – one of R. Weigl's closest students and collaborators, and a leading specialist in typhus epidemiology – helped organise the production and clandestine distribution of the vaccine during the occupation. Despite the prohibitions of the occupying authorities, the vaccine was clandestinely delivered to populations at risk of epidemic typhus, the Polish underground, as well as to ghettos and concentration camps. This dimension of the Lviv laboratory's work is an essential chapter not merely in the history of microbiology, but equally in the history of humanitarian rescue during the Holocaust and Nazi rule. In 2003, R. Weigl was posthumously honored with the title of Righteous Among the Nations for saving Jews during the Nazi occupation of Lviv.

In 1944, R. Weigl left Lviv. In the post-war period, he held positions at the Jagiellonian University in Kraków and later at the University of Poznań. In 1951, Professor Weigl set up a private laboratory in Kraków to further study epidemic typhus. The scientific tradition he established in Lviv persisted even after political borders shifted and research centers relocated. After the Second World War, R. Weigl's name was subjected to political persecution: in communist Poland, he was accused of collaborating with the German occupiers, which negatively affected his scientific reputation and official recognition. In Ukraine, the scientist's memory remained largely obscure for a long time. However, in recent years, Ivan Franko National University of Lviv, where R. Weigl studied, worked, and made his most significant discoveries, has actively restored his legacy to the scientific and public spheres (Hnatush, 2011).

Napoleon Jan Gąsiorowski and the Organisation of the Department of Microbiology. Alongside rickettsiology, classical medical bacteriology was actively developing at Lviv University. Napoleon Jan Gąsiorowski (1876–1941), a medical bacteriologist and professor of medical microbiology, played a crucial role in its institutionalization. N. J. Gąsiorowski graduated from the Medical Faculty at Lviv University. Early in his career, he worked in anatomical, histological, and bacteriological laboratories, and from 1904 to 1917 at the State Bacteriological and Epidemiological Station in Lviv. From 1920 to 1937, he headed the State Institute of Hygiene in Lviv. His career combined scientific, pedagogical, and organizational work. Between 1919 and 1929, he served as a docent in bacteriology and serology and became a professor of medical microbiology at Lviv University in 1929. Following the establishment of an independent Department of Microbiology in 1936, N. J. Gąsiorowski became its first head and remained in that post until 1939. In 1938–1939, he was Dean of the Medical Faculty. N. J. Gąsiorowski's scientific investigations encompassed numerous infectious diseases. His research focused on the agents responsible for cholera, tetanus, dysentery, and scleroma. He devoted substantial effort to the serological diagnostics of syphilis, devising a novel technique for pathogen detection. This method was applied during screening examinations of the population in the Lviv region. His contribution to the study of dysentery was equally significant. N. J. Gąsiorowski proposed an original classification of its causative agents, establishing Lviv as a leading centre for dysentery research in Galicia. Equally remarkable were his administrative capabilities: Gąsiorowski founded the Lviv school of microbiology, successfully bridging scientific inquiry and the education of future experts. The Second Congress of Microbiologists and Epidemiologists, which he convened in Lviv, served as a milestone for the advancement of the professional community (Bilinska & Danyleichenko, 2011).

Medical Microbiology and Dermatology: The Research of Jan Tadeusz Lenartowicz. Issues in infectious pathology were likewise explored at the intersection of microbiology, dermatology, and clinical medicine. One of the leading figures in this field

was Professor Jan Tadeusz Lenartowicz (1877–1959), Head of the Department of Dermatology and Venereology, who served as Dean of the Medical Faculty at Lviv University from 1931 to 1932. His scientific works covered the etiology, pathogenesis, and treatment of gonorrhea, syphilis, pemphigus, sarcoidosis, and cutaneous tuberculosis. Professor Lenartovych proposed a new method for the microscopic detection of *Treponema pallidum*, the causative agent of syphilis. He also explored the possibility of transmitting the syphilis agent to laboratory animals experimentally and developed an original treatment method for pemphigus. Another key area of his work involved investigating skin allergic reactions. He described a food-induced allergodermatosis linked to the consumption of certain plants (Borsukevych & Turkevych, 2011).

Jan Mieczysław Badian and research into the cellular organization of microorganisms. The fundamental strand of microbiology in Lviv was also represented by the research of the young scientist Jan Mieczysław Badian (1904–1942). His research focused on the structure and development of bacterial cells, particularly myxobacteria, cyanobacteria, and yeasts. A graduate of the Faculty of Mathematics and Natural Sciences, J. M. Badian worked as an assistant and later as an associate professor at the Department of Plant Anatomy and Physiology. In 1940, he was appointed head of the Department of Plant Anatomy and Cytology. His research into the cellular organisation of bacteria gained particular significance. J. M. Badian improved methods for staining microorganisms to reveal intracellular structures and was among the first to suggest the existence of a nucleoid in the bacterial cell – a structure containing genetic material and functionally corresponding to the nuclear region of a eukaryotic cell. These works reflect an important trend in the development of microbiology at that time: the gradual transition from the descriptive study of microorganisms to the investigation of their cellular organization and physiological processes. The life of J. M. Badian was tragically cut short in 1942 under the conditions of the German occupation (Bilinska, 2011a).

Thus, the development of microbiology at Lviv University in the first half of the 20th century demonstrates the gradual formation of a cohesive scientific school, in which fundamental research was directly combined with practical medicine. While the previous stage focused primarily on botanical and physiological studies of microorganisms, in the 1920s and 1930s the focus increasingly shifted toward agents that cause infectious diseases, mechanisms of transmission, laboratory diagnostics, and specific prevention.

Post-war restoration of the department and the development of agricultural microbiology. The reorganisation of the university structure in the late 1930s hindered further development of microbiological research at Lviv University. In 1939, the Department of Microbiology was transferred to the Lviv Medical Institute, created through the reorganisation of the medical faculty of Lviv University after Soviet authorities took control of Lviv. Following the end of the Second World War, in 1946, the department was re-established at the Faculty of Biology of Lviv University. Over the following decades, it developed as an important centre not only for general microbiology but also for agricultural and industrial microbiology. Until 1969, the department was called the Department of Microbiology and Soil Science, reflecting its specific research focus.

The department's first post-war stage of development was closely associated with Professor Lev Samiylovych Lytvynov (1901–1963), who chaired the Department of Microbiology and Soil Science at Lviv University from 1945 to 1961. L. Lytvynov was a microbiologist and plant physiologist, Doctor of Biological Sciences (1940), and Professor (1946). Before moving to Lviv, he possessed substantial research and teaching experience: between 1929 and 1931, he headed the Division of Plant Physiology and Agrochemistry at the Trans-Ural Zonal Agricultural Research Station; from 1931 to 1933, he was employed at the

Research Institute of Soybeans and Special Crops in Moscow; and from 1933 to 1945, he was a professor and chaired the Department of Plant Physiology and Microbiology at the Molotov Agricultural Institute. L. S. Lytvynov's scientific interests included plant physiology, mineral nutrition, plant tolerance to adverse environmental factors, and agricultural microbiology. These areas became the main focus of research at the Department of Microbiology and Soil Science at Lviv University, led by L. S. Lytvynov. The research focused on the interrelationships between soil microorganisms, soil fertility, and the physiological state of plants. Studies also explored the potential of targeted microorganism application in agriculture, particularly for developing bacterial fertilisers. This research scope was logical for the post-war period, as science faced the challenge of increasing crop yields. Soil microbiology enabled investigation of the natural processes of organic and mineral matter transformation, the role of microorganisms in supplying plants with bioavailable nutrients, and the prospects for using microbial preparations in agriculture. L. S. Lytvynov also left a notable mark on plant physiology. His works included studies on osmotic water uptake by plant root systems, soil drought, and plant tolerance to its effects. At the university, he integrated physiological studies with issues in soil science and microbiology. This interdisciplinarity was a hallmark of the department during that era and created the prerequisites for the further development of applied microbiology (Kostruba, 2011b).

In the 1950s and 1960s, the department gradually developed a research complex on the application of microorganisms in agriculture. Research focused on nitrogen-fixing bacteria, their contribution to plant nutrition, and the prospects for formulating microbial inoculants. The development of bacterial fertilisers was one of the department's first applied research directions, directly linking fundamental microbial studies with the practical needs of agricultural production. Professor Oleksandra Gustavivna Gebhardt (1905–1982) continued this line of research and headed the department from 1961 to 1969. Under her leadership, research focused on nitrogen-fixing bacteria, vitamin biosynthesis, and developing technological approaches to producing bacterial fertilisers. One outcome of this work was establishing the stimulatory effect of methylene blue on plant growth (Kostruba, 2011a).

Thus, during the 1940s–1960s, the department gradually shifted its focus from traditional soil microbiology to the study of microorganisms' physiological and biochemical functions. This transition paved the way for a new phase: studying microbial metabolism, the biosynthesis of biologically active compounds, and the regulatory mechanisms governing these processes.

Heorhii Shavlovskyi and the Foundations of Research in Microbial Biochemistry. A new chapter in the department's history began with the work of Heorhii Mykhailovych Shavlovskyi (1925–1996), a prominent microbiologist and biochemist, Doctor of Biological Sciences, Professor, and Honoured Scientist of the Ukrainian SSR. H. M. Shavlovskyi received his higher education at Lviv University and graduated in 1951. Thereafter, his academic career was directly tied to the University: from 1954 to 1970, he worked at the Department of Microbiology, rising through the ranks from a young researcher to Professor and Head of the Department. From 1968 to 1970, Professor H. M. Shavlovskyi headed the Department of Microbiology. At the same time, his work at Lviv University extended beyond administrative duties. In the 1950s, during his tenure at the department, H. M. Shavlovskyi began studying the biosynthesis of vitamin B_2 by microorganisms. This research became central to his later work and laid the foundation for one of the most prominent Lviv schools of microbial biochemistry. The studies on riboflavin marked a pivotal departure from the department's earlier scope of research. While attention had previously focused on the interaction of microorganisms with soil and plants, research now turned to microorganisms' intracellular biochemical processes. Research into vitamin biosynthesis helped move beyond

describing microbial physiological characteristics to unravelling the mechanisms that govern metabolic pathways. During this period, the department trained students whose names would later achieve international scientific recognition, such as A. A. Sibirny and V. O. Fedorenko. Under the supervision of H. M. Shavlovskyi, the department studied the flavinogenic activity of various yeast species in detail, developed methods for flavin determination, examined the effects of diverse factors on riboflavin biosynthesis, and established the role of iron in regulating this process. Considerable attention was also paid to the role of precursors in stimulating riboflavin synthesis and the relationship between this process and the biosynthesis of purine compounds. The culmination of years of research was H. M. Shavlovskyi's doctoral dissertation, which he defended in 1967 at the Institute of Microbiology of the Academy of Sciences of the USSR in Moscow; he received the academic title of Professor in 1969. A gradual shift in the fundamental nature of microbiological research carried special importance. In H. M. Shavlovskyi's studies, microorganisms were regarded not simply as subjects of taxonomy or physiology, but as complex biochemical systems wherein interrelated enzymatic and genetic mechanisms govern metabolite synthesis. For this reason, his work can be seen as a key factor driving the shift from traditional physiological and agricultural research toward molecular biology and biotechnology (Hudz, 2011). Among H. M. Shavlovskyi's students was S. P. Hudz, who would later serve as Head of the Department of Microbiology and Dean of the Faculty of Biology, and whose work will be detailed below. In the following years, this field expanded significantly beyond the Department of Microbiology.

Under the supervision of H. M. Shavlovskyi, the chemistry and enzymology of flavinogenesis; the mechanisms regulating riboflavin and flavin coenzyme biosynthesis across various microorganisms; riboflavin transport mechanisms; and the role of iron in regulating flavinogenesis were investigated. Furthermore, mutant yeast strains with impaired regulation of riboflavin synthesis and transport were obtained, and molecular genetic studies were conducted on the structural genes involved in flavinogenesis. An important organisational outcome of H. M. Shavlovskyi's activity was the establishment in 1969 of the Lviv Division of Cell Regulatory Systems of O. V. Palladin Institute of Biochemistry of the Academy of Sciences of the Ukrainian SSR. He headed the Department of Regulation of Cellular Synthesis of Low-Molecular-Weight Compounds, combining scientific research at an academic institution with pedagogical activity at the University. Members of the Division of Cell Regulatory Systems participated in the Department of Microbiology's educational process, while students had the opportunity to engage in advanced biochemical and molecular biological investigations of the time. This approach created close synergy between microbiology and biochemistry, paving the way for new disciplines in Lviv, such as molecular biology, microbial genetics, and biotechnology (Fedorovych, 2025).

Research on Microorganisms Producing Biologically Active Substances. The next stage in the Department's development is associated with the work of Associate Professor Nina Makarivna Datsiuk (1926–2021), who headed the Department from 1971 to 1981. N. M. Datsiuk graduated from the Faculty of Biology of Lviv University in 1950 and, after a period working as a microbiologist at the Lviv Brewery, returned to academic research at the university. From 1953 to 1965, she served as Assistant Professor, and from 1965 to 1971, as Associate Professor. Her scientific interests focused on issues of industrial microbiology, microbial ecology, and microbial physiology, particularly the biosynthesis of B vitamins. Research on vitamin B_{12} and tetrapyrrolic compounds held a prominent position. At that time, the Department maintained active collaboration with the vitamin B_{12} production plant located in Kurgan. Department researchers implemented strains of *Propionibacterium shermanii* that they had selected for industrial production (Podopryhora & Bilinska, 2011). During this

period, the Department's academic and research staff included R. O. Kuznetsova, S. P. Hudz, M. F. Kostruba, V. D. Ruryk, I. S. Bilinska, O. V. Poluliakh, S. A. Yelisieiev, O. I. Podopryhora, Y. S. Shakh, T. V. Vyhovska, and T. M. Pavlovych. Thus, the biochemical research direction initiated by H. Shavlovskyi was further advanced through investigations into microorganisms as producers of valuable bioactive compounds.

Associate Professor Roman Vasylovych Kucheras (1934–2013) later continued this line of research. After graduating from the Faculty of Biology of Lviv University and completing postgraduate studies, he worked in different research laboratories before joining the Department of Microbiology in 1972. From 1978 to 2000, he served as Associate Professor and, in some years, as Acting Head of the Department. From 1986 to 1995, R. V. Kucheras also served as Deputy Dean of the Faculty of Biology. His main research focus was the physiology of microorganisms that produce biologically active substances, specifically vitamins B_2 and B_{12}. R. Kucheras studied the amino acid metabolism in *P. shermanii* depending on the intensity of vitamin B_{12} production. These works had a pronounced applied character, since microbial producers were used as biological platforms to produce valuable compounds (Bilinska, 2011c). Associate Professor Roza Oleksandrivna Kuznetsova also contributed to advancing this line of research. Having graduated from the Faculty of Biology at Lviv University in 1959, she worked at the Department of Microbiology for a long period as an Assistant and later as an Associate Professor. Her research focused on regulating guanylic compound metabolism in the flavinogenic yeast *Pichia guilliermondii* and on regulating beta-galactosidase biosynthesis in yeasts.

A significant contribution to the Department's investigations in the field of industrial microbiology was made by Associate Professor Mykhailo Fedorovych Kostruba (1939–2022), who served as an Associate Professor of the Department from 1971 to 2007. His research interests encompassed industrial microbiology and microbial ecology; notably, he introduced to the Department the concept of studying bacteria involved in the sulfur cycle (Bilinska, 2011b). This line of study broadened the Department's traditional research profile from single-species microbial physiology to their broader role within ecosystem-scale biogeochemical processes.

The scientific tradition initiated at Lviv University through studies of microbial biosynthesis and metabolic regulation by H. M. Shavlovskyi was further developed by his student, Candidate of Biological Sciences, Professor, and Honoured Professor of Ivan Franko National University of Lviv, Stepan Petrovych Gudz (1940–2018). S. P. Hudz graduated from the Faculty of Biology at Lviv University in 1963, and after completing his postgraduate studies, he returned to his research and teaching work at the university. Starting in 1973, he served as Associate Professor in the Department of Microbiology, became Head of the Department in the mid-1980s, and received the academic title of Professor in 2000. From 1996 to 2002, S. P. Hudz served as Dean of the Faculty of Biology. S. P. Hudz's early scientific research directly continued the biochemical tradition of H. M. Shavlovskyi's scientific school. His Candidate of Sciences dissertation, defended in 1971, focused on regulating purine nucleotide biosynthesis in the yeasts *Candida guilliermondii* and *Candida pelliculosa*. In these works, the microorganism was no longer viewed merely as a producer of a specific substance, but as a complex metabolic system in which individual biochemical pathways are interconnected and regulated. One of S. P. Hudz's early publications was a study on *Candida guilliermondii* mutants with impairments in various steps of inosinic and adenylic acid biosynthesis.

Over the following decades, the Department's research scope expanded significantly. Under the leadership of S. P. Gudz, a comprehensive complex of research on yeast physiology and biochemistry was established, focusing in particular on patterns of β-galactosidase

biosynthesis, the effects of ethanol on carbohydrate metabolism enzymes, an the specific characteristics of lipid composition in yeast cells with different metabolic types. These studies were conducted with the participation of B. M. Borsukevych, O. V. Hirna, O. I. Tretyak, S. O. Hnatush, K. O. Tretyak, A. Ye. Zakalsky, I. B. Rusyn, Ya. I. Kolisnyk, and other researchers. Studies on lactose-utilising yeasts were a prominent part of the Department's scientific work. Studying ethanol production by these microorganisms made it possible to trace the interrelationship between carbohydrate utilisation, ethanol synthesis, and the regulation of enzymatic systems. This specific subject later laid the groundwork for S. O. Hnatush's Candidate thesis: "Ethanol production in lactose-utilising yeasts and its role in regulating the synthesis of enzymes involved in carbohydrate metabolism". Concurrently, an applied research direction emerged focused on utilising food and dairy industry waste as substrates for yeast cultivation. This approach bridged fundamental research into microbial metabolism with biotechnology applications: industrial waste was viewed not merely as an environmental waste-management issue, but as a potential source of organic substrates for producing microbial biomass and high-value biological products. Importantly, research on yeast metabolism during this period was not limited to studying carbohydrate metabolism enzymes. Researchers at the Department also explored cellular organelles and microorganisms' and their adaptive mechanisms to different carbon substrates.

From Yeast Physiology to Environmental Microbiology. At the turn of the 21st century, the Department of Microbiology's scientific trajectory took another pivotal turn. S. P. Gudz began systematically studying microbial involvement in the sulfur cycle. Bridging microbial physiology, ecology, biogeochemistry, and applied biotechnology, this interdisciplinary field became a hallmark of the modern Lviv school of microbiology. Significant contributions to the expansion of this field were made by M. F. Kostruba, S. O. Hnatush, I. M. Baran, L. Ya. Kit, T. B. Peretiatko, I. V. Kushkevych, M. B. Horishnyi, A. A. Halushka, I. R. Klym, O. M. Moroz, O. I. Podopryhora, alongside other investigators.

The research focused on sulfur- and sulfate-metabolising bacteria, which play a critical role in the biogeochemical sulfur cycle. Focusing on the water reservoirs of the Yavoriv and Rozdil sulfur deposits, scientists examined the distribution, taxonomic composition, and physiological and biochemical characteristics of these microorganisms. The authors conducted ecological monitoring of sulfur- and sulfate-metabolising bacteria, studied their utilisation of sulfur compounds under different conditions, and established the specific features of hydrogen sulfide assimilation by purple and green sulfur bacteria. During that period the department actively collaborated with the Institute of Cell Biology of the National Academy of Sciences of Ukraine. D. V. Fedorovych, Doctor of Biological Sciences, Professor and a Leading Research Fellow in the Department of Molecular Genetics and Biotechnology at that institute, serves as a Professor in the department.

Since 2014, Candidate of Biological Sciences, Professor, and Honoured Professor of Ivan Franko National University of Lviv Svitlana Oleksiivna Hnatush has headed the Department of Microbiology. Today, the Department's academic staff includes Candidates of Biological Sciences, Associate Professors Halyna Ivanivna Zvir, Andrii Andriiovych Halushka, Olha Dmytrivna Maslovska, Taras Bohdanovych Peretyatko, and Halyna Vasylivna Yavorska; Leading Research Fellow and Senior Researcher Oksana Mykhailivna Moroz; and Junior Research Fellow Solomiia Yaroslavivna Komplikevych. The Department hosts the Interdisciplinary Educational Laboratory of Virtual Methods in Biology, led by Iryna Mykhailivna Izhovska. Leading Specialist Iryna Romanivna Klym has served the Department for many years. The Department's premises also house the Laboratory of Instrumental Research Methods within the Interuniversity Center for Collective Use of Cell Biology and Bioenergy, managed by A. A. Halushka.

The Department of Microbiology is a vital educational and scientific centre for training specialists in biology, microbiology, ecology, and laboratory diagnostics in Ukraine. Throughout its development, the Department has adopted a comprehensive approach that integrates fundamental microbiological education, practical student training, and relevant scientific research to address contemporary biological and environmental challenges.

The Department's current educational activities encompass the training of higher education students at the first (Bachelor's) level in speciality E1 Biology and Biochemistry, and speciality A4.05 Secondary Education (Biology and Human Health) in both full-time and extramural study modes, as well as speciality G21 Biotechnology and Bioengineering, and speciality E2 Ecology in the full-time study mode. At the second (Master's) level, the Department offers training under the Educational and Professional Programs "Microbiology" and "Biotechnology and Bioengineering" in full-time study mode, and under the Educational and Professional Program "Laboratory Diagnostics of Biological Systems" in extramural study mode. The department participates in the preparation of PhD candidates in specialty E1 Biology and Biochemistry. Over the past 15 years I. V. Kushkevych, A. A. Halushka, O. V. Vasyliv, K. V. Sholiak, O. D. Maslovska, S. V. Diakiv, T. B. Segin, N. S. Verkholiak, O. V. Tarabas, and S. Ya. Komplikevych have successfully defended their dissertations. This educational program structure reflects the interdisciplinary nature of modern microbiology and its close connection with biology, ecology, medicine, biotechnology, and pedagogy.

The department's lecturers place considerable emphasis on helping students develop a systematic understanding of the processes that govern microbial function and their role in natural and anthropogenically altered ecosystems. The core components of the educational and research work include the study of microbial physiology and biochemistry, their diversity and ecology, bacteriological analysis methods, the biological basis of infectious processes, biotechnological approaches, and the methodology of teaching biology. Combining fundamental and applied aspects in students' training promotes the acquisition of professional competencies required for employment in research or educational institutions, diagnostic or medical laboratories, industrial companies, and other organisations. An important aspect of the department's work is involving students in research activities. The department hosts a Microbiological circle and an Immunological circle, which unite members of the student scientific society, as well as a Microbiological circle of the Junior Academy of Sciences. These circles aim to develop research skills, deepen interest in microbiology, and give young people practical experience conducting experimental research. T. B. Peretyatko, a participant in the 28th and 30th Ukrainian Antarctic expeditions, has done significant work to popularise scientific research among youth. The department's current research activities span a wide range of areas, encompassing both fundamental aspects of microbial function and the potential for practical applications to mitigate environmental challenges. Much emphasis is placed on studying the adaptations of microorganisms that transform sulfur compounds in natural environments to chemical pollutants as stress factors. The results of such studies provide a scientific framework for developing biological approaches to assessing the state of, and restoring, polluted ecosystems. A promising applied direction is developing methods to bioremediate environments contaminated with organic and inorganic pollutants using microorganisms. Microorganisms capable of biodegrading fire-fighting foam agents were isolated, the impact of pesticides on soil microbiota was investigated, and the effect of medicinal plant extracts on microorganisms was determined. The department also studies exoelectrogenesis processes in prokaryotes and the potential of using microorganisms in microbial fuel cells. This direction combines fundamental research into the mechanisms of microbial electrogenesis with the search for promising biotechnological solutions in energy generation and environmental remediation. A separate research block focuses on

microbiocenoses in coal mine spoil heaps and aquatic ecosystems in ecologically hazardous areas. These studies are important for assessing the impact of anthropogenic load on microbial communities and understanding the role of microorganisms in the functioning of transformed ecosystems. In addition, the department characterises the physiological and biochemical properties of microorganisms isolated from diverse Antarctic habitats, thereby expanding the understanding of microbial adaptive potential in extreme environments. Particular attention is paid to projects that develop approaches to restore the fertility of war-affected soils using microbial biopreparations. This research has been extended to explore the plant growth-promoting properties of the isolated bacteria, particularly under adverse environmental stress conditions such as heavy metal compounds and NaCl.

An important milestone in the development of the department's international and innovative activities was its participation in 2013–2017 in the Tempus project "Establishment of Multidisciplinary Innovative Centres for the Development of Virtual Laboratories (MICVL) in Biology and Medicine", carried out by eight European and two Ukrainian universities. The project fostered the development of modern educational technologies and the incorporation of virtual methodologies into academic practice. Furthermore, the department's academic staff have engaged in various other international projects.

Members of the department have authored a range of textbooks and study guides, including Microbiology, Industrial Microbiology, Practical Course in Microbiology, Virology, Sanitary Microbiology, among other publications.

Thus, the extensive history and activity of the Department of Microbiology at Ivan Franko National University of Lviv demonstrate its significant contribution to the development of microbiological education and science in Ukraine. The combination of established scientific traditions, modern research approaches, interdisciplinary cooperation, and the training of young specialists ensures continuity across generations. It lays a solid foundation for the department's further development. An important outcome of its activities is not only the acquisition of new fundamental knowledge, but also the application of scientific developments to practical solutions aimed at environmental preservation, the restoration of disturbed ecosystems, and the advancement of modern biotechnologies. The preservation of the collective's scientific potential, the expansion of international cooperation, and the engagement of young researchers provide strong grounds to regard the department as a vital centre for developing modern microbiology and shaping its future in Ukraine.

References

1. Bilinska, I. (2011a). Badiyan Yan Miechyslav [Jan Mieczysław Badian]. In *ENCYCLOPEDIA. Lvivskyi natsionalnyi universytet imeni Ivana Franka* [ENCYCLOPEDIA. Ivan Franko National University of Lviv] (Vol. 1, p. 168). Lvivskyi natsionalnyi universytet imeni Ivana Franka. (In Ukrainian)
2. Bilinska, I. (2011b). Kostruba Mykhailo Fedorovych [Kostruba Mykhailo Fedorovych]. In *ENCYCLOPEDIA. Lvivskyi natsionalnyi universytet imeni Ivana Franka* [ENCYCLOPEDIA. Ivan Franko National University of Lviv] (Vol. 1, pp. 657–658). Lvivskyi natsionalnyi universytet imeni Ivana Franka. (In Ukrainian)
3. Bilinska, I. (2011c). Kucheras Roman Vasylovych [Kucheras Roman Vasylovych]. In *ENCYCLOPEDIA. Lvivskyi natsionalnyi universytet imeni Ivana Franka* [ENCYCLOPEDIA. Ivan Franko National University of Lviv] (Vol. 1, p. 701). Lvivskyi natsionalnyi universytet imeni Ivana Franka. (In Ukrainian)
4. Bilinska, I. S. (2011d). Kshemenevska Helena Yuzefa [Helena Józefa Krzemieniewska]. In *ENCYCLOPEDIA. Lvivskyi natsionalnyi universytet imeni Ivana Franka* [ENCYCLOPEDIA. Ivan Franko National University of Lviv] (Vol. 1, p. 704). Lvivskyi natsionalnyi universytet imeni Ivana Franka. (In Ukrainian)

5. Bilinska, I., & Danyleichenko, V. (2011). Honsyorovskyi Napoleon Yan [Napoleon Jan Gąsiorowski]. In *ENCYCLOPEDIA. Lvivskyi natsionalnyi universytet imeni Ivana Franka* [ENCYCLOPEDIA. Ivan Franko National University of Lviv] (Vol. 1, p. 411). Lvivskyi natsionalnyi universytet imeni Ivana Franka. (In Ukrainian)
6. Borsukevych, B., & Turkevych, O. (2011). Lenartovych Yan Tadeush [Jan Tadeusz Lenartowicz]. In *ENCYCLOPEDIA. Lvivskyi natsionalnyi universytet imeni Ivana Franka* [ENCYCLOPEDIA. Ivan Franko National University of Lviv] (Vol. 2, p. 32). Lvivskyi natsionalnyi universytet imeni Ivana Franka. (In Ukrainian)
7. Fedorovych, D. V. (2025). Shavlovskyi Heorhii Mykhailovych [Shavlovskyi Heorhii Mykhailovych]. In I. M. Dziuba, A. I. Zhukovskyi, & M. H. Zhelezniak (Eds.), *Entsyklopediia Suchasnoi Ukrainy* [Encyclopedia of Modern Ukraine]. Instytut entsyklopedychnykh doslidzhen NAN Ukrainy. https://esu.com.ua/article-891198. https://doi.org/10.5281/zenodo.19718309 (In Ukrainian)
8. Hnatush, S. (2011). Vaihl Rudolf Stefan [Rudolf Stefan Weigl]. In *ENCYCLOPEDIA. Lvivskyi natsionalnyi universytet imeni Ivana Franka* [ENCYCLOPEDIA. Ivan Franko National University of Lviv] (Vol. 1, p. 260). Lvivskyi natsionalnyi universytet imeni Ivana Franka. (In Ukrainian)
9. Hnatush, S., & Nadraha, M. (2011). Kuchera Pavel Ludvih [Pawel Ludwig Kucera]. In *ENCYCLOPEDIA. Lvivskyi natsionalnyi universytet imeni Ivana Franka* [ENCYCLOPEDIA. Ivan Franko National University of Lviv] (Vol. 1, p. 701). Lvivskyi natsionalnyi universytet imeni Ivana Franka. (In Ukrainian)
10. Hudz, S. (2011). Shavlovskyi Heorhii Mykhailovych [Shavlovskyi Heorhii Mykhailovych]. In *ENCYCLOPEDIA. Lvivskyi natsionalnyi universytet imeni Ivana Franka* [ENCYCLOPEDIA. Ivan Franko National University of Lviv] (Vol. 2, p. 663). Lvivskyi natsionalnyi universytet imeni Ivana Franka. (In Ukrainian)
11. Kalynovych, N., Kit, L., & Bilinska, I. (2011). Kamenskyi Frantsishek Dionizii [Franciszek Dionizy Kamieński]. In *ENCYCLOPEDIA. Lvivskyi natsionalnyi universytet imeni Ivana Franka* [ENCYCLOPEDIA. Ivan Franko National University of Lviv] (Vol. 1, p. 580). Lvivskyi natsionalnyi universytet imeni Ivana Franka. (In Ukrainian)
12. Kolisnyk, Ya., & Bilinska, I. (2011). Shtoizynh Zdzislav Karol [Zdzisław Karol Steusing]. In *ENCYCLOPEDIA. Lvivskyi natsionalnyi universytet imeni Ivana Franka* [ENCYCLOPEDIA. Ivan Franko National University of Lviv] (Vol. 2, pp. 692–693). Lvivskyi natsionalnyi universytet imeni Ivana Franka. (In Ukrainian)
13. Kostruba, M. (2011a). Hebhardt Oleksandra Hustavivna [Hebhardt Oleksandra Hustavivna]. In *ENCYCLOPEDIA. Lvivskyi natsionalnyi universytet imeni Ivana Franka* [ENCYCLOPEDIA. Ivan Franko National University of Lviv] (Vol. 1, pp. 329–330). Lvivskyi natsionalnyi universytet imeni Ivana Franka. (In Ukrainian)
14. Kostruba, M. (2011b). Lytvynov Lev Samailovych [Lytvynov Lev Samailovych]. In *ENCYCLOPEDIA. Lvivskyi natsionalnyi universytet imeni Ivana Franka* [ENCYCLOPEDIA. Ivan Franko National University of Lviv] (Vol. 2, p. 43). Lvivskyi natsionalnyi universytet imeni Ivana Franka. (In Ukrainian)
15. Podopryhora, O., & Bilinska, I. (2011). Datsiuk Nina Makarivna [Datsiuk Nina Makarivna]. In *ENCYCLOPEDIA. Lvivskyi natsionalnyi universytet imeni Ivana Franka* [ENCYCLOPEDIA. Ivan Franko National University of Lviv] (Vol. 1, p. 425). Lvivskyi natsionalnyi universytet imeni Ivana Franka. (In Ukrainian)
16. Terek, O. (2011). Kshemenevskyi Severyn Yuzef [Seweryn Józef Krzemieniewski]. In *ENCYCLOPEDIA. Lvivskyi natsionalnyi universytet imeni Ivana Franka* [ENCYCLOPEDIA. Ivan Franko National University of Lviv] (Vol. 1, p. 704). Lvivskyi natsionalnyi universytet imeni Ivana Franka. (In Ukrainian)

BIOTECHNOLOGICAL APPLICATION OF MICROORGANISMS

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EFFECT OF *AZOTOBACTER VINELANDII* AND *BACILLUS SUBTILIS*, THE COMPONENTS OF THE AZOGRAN BIOPREPARATION, ON THE FORMATION OF WHEAT ROOT EXUDATES

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Abstract. The effect of different concentrations of *Azotobacter vinelandii* and *Bacillus subtilis*, the bacterial components of the Azogran biopreparation, on the formation of wheat root exudates under sterile hydroponic conditions was investigated. Increasing the concentration of each bacterial strain in the culture medium to 10^7 CFU/mL resulted in the highest accumulation of root exudate dry matter accompanied by a decrease in total carbohydrate content, suggesting intensive utilization of exuded carbohydrates by the bacteria. Gas chromatography-mass spectrometry (GC-MS) revealed alterations in the monosaccharide composition of root exudates, including increased proportions of galactose and mannose as well as the appearance of several unidentified compounds absent from the control. These findings indicate that bacterial concentration in the growth medium is an important factor regulating the metabolism of wheat root exudates and plant-microorganism interactions. The most pronounced effects were observed at bacterial concentrations of 10^6-10^7 CFU/mL.
Keywords: wheat, root exudates, *Azotobacter vinelandii*, *Bacillus subtilis*, carbohydrates, monosaccharides.

Objective. The aim of this study was to investigate the effect of different concentrations of *Azotobacter vinelandii* and *Bacillus subtilis*, the bacterial components of the Azogran biopreparation, on dry matter accumulation, total carbohydrate content, and the monosaccharide composition of wheat root exudates.

Materials and Methods

Experiments were conducted using wheat (*Triticum aestivum* L.) cv. Shestypalivka. Seeds were surface-sterilized and germinated on potato agar. Sterile seedlings were transferred onto stainless-steel mesh supports placed in 1.5-L glass vessels containing 80 mL of Fåhraeus medium. Plants were cultivated hydroponically in the presence of a mixed culture of *A. vinelandii* and *B. subtilis* at concentrations ranging from 10^5 to 10^7 CFU/mL for each strain. Plants grown without bacterial inoculation served as the control.

At the end of cultivation, the culture medium containing root exudates was separated by centrifugation, freeze-dried, and the dry matter yield was determined. Total carbohydrates were quantified by the phenol–sulfuric acid method (Dubois et al., 1956). Monosaccharide composition was determined by gas chromatography–mass spectrometry after acid hydrolysis according to Albersheim et al. (1967).

Results. The concentration of the bacterial inoculum significantly affected both the accumulation of root exudate dry matter and their carbohydrate composition. In the control

treatment, the dry matter yield was 19.0 mg (0.059 mg/mL of exudate). Inoculation with the *A. vinelandii-B. subtilis* consortium progressively increased dry matter accumulation depending on bacterial concentration. At inoculum concentrations of 10^5-10^6 CFU/mL, dry matter increased to 66.0–70.0 mg, whereas at 10^7 CFU/mL it reached a maximum of 256.0 mg (1.1 mg/mL of exudate), indicating enhanced physiological and biochemical activity of plants during intensive interactions with the introduced bacteria.

Simultaneously, total carbohydrate concentration in the culture medium decreased markedly from 0.67 μg/mL in the control to 0.019–0.015 μg/mL in inoculated treatments. This reduction is most likely associated with active bacterial utilization of root-derived carbohydrates as the primary carbon source. Similar patterns have been reported for rhizosphere associations where plant growth-promoting bacteria accelerate carbon exchange between plants and microorganisms (Badri & Vivanco, 2009; Canarini et al., 2019; Zhalnina et al., 2018).

GC-MS analysis identified rhamnose, fucose, mannose, galactose, glucose, and several unidentified compounds (X_1-X_5) in wheat root exudates. Galactose predominated in the control treatment (31.91 %), whereas the remaining monosaccharides were detected in relatively low amounts. In the presence of bacteria, the monosaccharide profile was substantially modified. At an inoculum concentration of 10^6 CFU/mL, the proportion of galactose increased to 62.89 %, while at 10^5 CFU/mL mannose content increased to 18.17 %. Moreover, unidentified compounds X_1-X_5 were detected only in inoculated treatments, suggesting the formation of new plant- or microbially derived metabolites during active plant-bacteria interactions.

These results are consistent with current concepts of the dynamic nature of root exudation. According to Zhalnina et al. (2018), rhizosphere bacteria not only utilize specific exudate components but also modify their composition by inducing the synthesis of novel metabolites involved in signaling and regulation of rhizosphere microbial community assembly. The elevated galactose content observed at 10^6 CFU/mL may reflect enhanced synthesis of cell wall polysaccharides, whereas increased mannose levels may indicate intensified glycosylation processes associated with plant-bacterial interactions.

Overall, inoculation of wheat with *A. vinelandii* and *B. subtilis*, the bacterial components of the Azogran biopreparation, stimulated the accumulation of root exudate biomass and substantially modified both carbohydrate and monosaccharide composition. The strongest response was observed at inoculum concentrations of 10^6–10^7 CFU/mL, corresponding to maximal metabolic activity of the plant–microorganism system and the greatest diversity of root exudate components.

Conclusions. Application of *A. vinelandii* and *B. subtilis* effectively modifies the composition of wheat root exudates. The highest dry matter yield (256.0 mg) and the lowest total carbohydrate concentration (0.015 μg/mL) were obtained at an inoculum concentration of 10^7 CFU/mL, indicating high bacterial metabolic activity. Bacterial concentrations of 10^6–10^7 CFU/mL produced the greatest changes in the monosaccharide profile of root exudates and may therefore be considered optimal for promoting beneficial interactions between wheat plants and rhizosphere microorganisms.

References

1. Albersheim P., Nevins D. J., English P. D., Karr A. A method for analysis of sugars in plant cell wall polysaccharides by gas-liquid chromatography. *Carbohydrate Research*. 1967; 5(3):340-345.
2. Badri D.V., Vivanco J.M. Regulation and function of root exudates. *Plant, Cell & Environment*. 2009; 32(6):666-681.

3. Canarini A., Kaiser C., Merchant A., Richter A., Wanek W. Root exudation of primary metabolites: Mechanisms and their roles in plant responses to environmental stimuli. *Frontiers in Plant Science*. 2019; 10:157.
4. Dubois M., Gilles K. A., Hamilton J. K., Rebers P. A., Smith F. Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*. 1956;28(3):350-356.
5. Zhalnina K., Louie K.B., Hao Z., et al. Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nature Microbiology*. 2018; 3:470-480.

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BIOTECHNOLOGICAL APPLICATIONS OF LACCASE AND LACCASE-LIKE NANOZYMES FOR BIOSENSOR DEVELOPMENT

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Abstract. This study explores the bioanalytical application of natural laccase, surface-displayed recombinant cells, and laccase-like nanozymes for biosensor development. Trametes zonata 1525 laccase was characterized and effectively utilized for the spectrophotometric and electrochemical determination of 4-hydroxyindole and 5-hydroxyindole-3-acetic acid. A cell-based biosensor featuring genetically modified yeast with surface-displayed laccase enabled sensitive 5-HIAA quantification in urine samples. Furthermore, laccase-mimicking nanomaterials (nAuCePt, nAuCu, nPtCu, nCu-HCF, nPtMn, Ag/MnO_2@PDA–B, FeMn-MOF and nMnO_2) demonstrated high catalytic efficiency for dopamine, adrenaline, 5-HIAA, 4-HI and phenolic metabolite detection. The nAuCePt nanozyme achieved a 380-fold increase in sensitivity compared to natural enzyme-based sensors. Overall, combining natural laccases with artificial mimetics offers a robust platform for modern diagnostic biosensors.
Keywords: laccase, laccase mimetics, fungi, hydroxyindole derivatives, analysis, electrochemical biosensors.

Laccases (EC 1.10.3.2) are copper-containing oxidases with a broad substrate specificity, capable of catalyzing the oxidation of a wide range of phenolic and aromatic compounds using molecular oxygen as the terminal electron acceptor. These properties have stimulated considerable interest in laccases as biocatalysts for enzymatic analytical methods, including electrochemical biosensors. In particular, laccases are promising for the determination of biologically important catechol and indole compounds, while laccase-like artificial catalysts can serve as alternatives to natural enzymes.

In our studies, laccase from *Trametes zonata* 1525, selected from 38 basidiomycete strains as the most productive strain for extracellular laccase production, was characterized. Optimization of cultivation conditions and the use of an inducer resulted in a higher enzyme productivity. The purified laccase was shown to oxidize indole compounds, including 4-hydroxyindole (4-HI) and 5-hydroxyindole-3-acetic acid (5-HIAA), with catalytic efficiencies of $1.1 \cdot 10^4$ and $5.4 \cdot 10^3$ $M^{-1} \cdot s^{-1}$, respectively. A laccase-based spectrophotometric method for

5-HIAA determination was developed, and its applicability was confirmed using real samples. To develop cell-based biosensors for the detection of analytically important compounds, a recombinant yeast strain with surface-displayed laccase was constructed. A biosensor based on these genetically modified cells was developed for 5-HIAA determination, with a limit of detection of 0.25 μM at a potential of −0.075 V. Analysis of real urine samples demonstrated the applicability of the developed system for 5-HIAA determination in biological samples.

Laccase-like nanozymes were synthesized and investigated as alternative artificial catalysts for the determination of biologically important compounds. nAuCePt was selected for adrenaline determination, whereas nAuCu and nPtCu were used for dopamine determination. The use of nAuCePt resulted in a 380-fold increase in sensitivity compared with a sensor based on natural laccase. Among the investigated hexacyanoferrate nanomaterials, nCu-HCF demonstrated the highest activity and was used for the spectrophotometric determination of adrenaline based on adrenochrome formation. Comparison of natural *T. zonata* laccase with the laccase-like nanomaterials nPtMn and $nMnO_2$ confirmed the ability of these catalysts to oxidize 5-HIAA with the generation of an electrochemical signal. Thus, the combination of natural laccases, genetically engineered yeast cells, and laccase-like nanozymes provides a promising basis for the development of enzymatic and biosensor methods for the determination of 4-HI, 5-HIAA, dopamine, adrenaline, catechol, and other biologically important compounds.

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A YEAST-BASED PLATFORM FOR THE DEVELOPMENT OF COVID-19 VACCINE PROTOTYPES

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Abstract. Yeast-based vaccine platforms represent a promising approach for the development of accesssible vaccines against emerging pathogens. In this study, vaccine prototypes against SARS-CoV-2 were developed using the yeast *Komagataella phaffii*. Recombinant fragments of the SARS-CoV-2 spike protein were expressed and purified from engineered yeast strains and evaluated in a mouse immunization model. Among the tested constructs, FP-HR1-HR2 induced the highest virus-neutralizing activity, reaching 93 %. An oral vaccine prototype was also developed by displaying the RBD on the surface of *K. phaffii* cells. Oral immunization with inactivated recombinant yeast cells resulted in neutralizing antibody levels of up to 93 %. These results demonstrate the potential of yeast-based platforms for vaccine development against SARS-CoV-2 and other emerging pathogens.
Keywords: *Komagataella phaffii*, SARS-CoV-2, recombinant vaccine, spike protein, oral vaccine.

The COVID-19 pandemic was caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which emerged in late 2019. In response to the pandemic, extensive research efforts were initiated to develop safe and effective vaccines. Hundreds of vaccine candidates have since been proposed, with more than one hundred evaluated in clinical trials.

Despite the end of the pandemic, the development of accessible and effective vaccine platforms remains relevant for addressing potential future infectious disease challenges.

At the Institute of Cell Biology of the National Academy of Sciences of Ukraine, protein-based vaccine prototypes against SARS-CoV-2 were developed. A collection of transformants was generated using a humanized strain of the yeast *Komagataella phaffii*. The transformants carried genomic expression cassettes encoding fragments of the S1 and S2 subunits of the SARS-CoV-2 spike glycoprotein under the control of the methanol-inducible *AOX1* promoter of *K. phaffii*. The spike protein fragments included RBD, NTD-RBD, FP-HR1-HR2, and NTD-RBD-FP-HR1-HR2. Each construct contained a C-terminal trimerization domain derived from the ferritin protein, an immunological adjuvant hIgG1-Fc2, a TEV protease cleavage site, and a 6-His tag for metal affinity purification of the target proteins. Each construct was additionally generated in two versions, with or without the N-terminal secretion signal derived from the yeast α-factor.

The purified proteins were used for intraperitoneal immunization of mice. The highest virus-neutralizing activity was detected in sera from animals immunized with FP-HR1-HR2, with a neutralization efficiency of 93 %. In addition, an oral vaccine prototype based on *K. phaffii* was developed. The RBD of the SARS-CoV-2 spike protein was displayed on the yeast cell surface using the anchor domain of the Sag1 protein from *Saccharomyces cerevisiae*. The inactivated yeast cells were used for oral immunization of mice, resulting in neutralizing antibody levels of up to 93 %.

The developed prototypes demonstrate the potential of yeast-based platforms for the development of vaccines against SARS-CoV-2 and other emerging pathogenic threats.

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CONSTRUCTION OF *CANDIDA FAMATA* STRAINS WITH ENHANCED TOLERANCE TO LIGNOCELLULOSIC HYDROLYSATE INHIBITORS

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Abstract. Lignocellulosic biomass is a promising source of sugars, however, during hydrolysis the toxic compounds are released, that can inhibit the producing strain. For today there is no data on the genetic regulation in *C. famata* of tolerance to inhibitors (furfural, hydroxymethylfurfural (HMF), and acetic acid), as well as whether increased resistance would correlate with increased riboflavin production. Overexpression of *ADH6* (coding for alcohol dehydrogenase), *HAA1* (coding for transcription factor), and *GRE2* (coding for aldehyde reductase) genes were performed for *C. famata* BRPI strain. Resulted strains were characterized by increased in riboflavin production.
Keywords: riboflavin, *Candida famata,* yeast, riboflavin overproducers, lignocellulose hydrolysate, inhibitors.

Riboflavin is a precursor of flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD), which are coenzymes of flavoproteins involved in numerous redox

reactions (You et al., 2021). Vitamin B_2 is currently biotechnologically produced by the engineered strains of the bacterium *Bacillus subtilis* and filamentous fungus *Ashbya (Eremothecium) gossypii.* Flavinogenic yeast *Candida famata* have significant prerogatives in biotechnological processes compared to fungi and bacteria and also have the status of GRAS, however, still need improvement to compete with current industrial producers (Abbas &Sibirny, 2011).

Inhibitors presented in lignocellulosic hydrolysates belong to three major groups: weak acids, originate from the breakdown of hemicellulose, phenolic compounds are breakdown products of lignin and furan derivatives: furfural and 5-hydroxymethylfurfural (HMF) are derived from elimination reactions of pentose and hexose sugars, respectively. At a cellular level inhibitors trigger various stress responses. Cells strive to regain and maintain pH, homeostasis, and activates signaling pathways that promote metabolic changes and apoptosis. Detoxification of toxic compounds leads to intracellular redox imbalance, and diverting energy to adaptive responses, resulting in reduced performance (Paes et al., 2021).

Methods. The standard methods of molecular genetics were used.

Findings. In this work, the overexpression of *ADH6*, *HAA1* and *GRE2* genes in the recombinant strain *C. famata* BRPI was carried out. The phenotype of the obtained transformants was studied for tolerance to the main inhibitors contained in lignocellulose. The level of riboflavin production on YNB medium with the addition of inhibitors was determined. In medium with addition of 10 mM and 3 mM of HMF the recombinant strains demonstrated higher riboflavin production. Furfural had a strong inhibitory effect, and none of the recombinant strains showed a clearly pronounced effect in the detoxification of this inhibitor. On YNB medium with 25 % of bagasse hydrolysate the recombinant strains were characterized by greater biomass accumulation, riboflavin production, as well as higher productivity.

Conclusion. Data on the regulation of tolerance to lignocellulosic hydrolysate inhibitors in *C. famata* were obtained, and the effect of overexpression of the *ADH6*, *HAA1*, and *GRE2* genes on riboflavin production was established, which improves the understandding of cellular defense mechanisms.

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References:

1. Abbas, C. A., & Sibirny, A. A. (2011). Genetic control of biosynthesis and transport of riboflavin and flavin nucleotides and construction of robust biotechnological producers. Microbiology and Molecular Biology Reviews, 75(2), 321–360. https://doi.org/10.1128/mmbr.00030-10
2. Paes, B. G., Steindorff, A. S., Formighieri, E. F., Pereira, I. S., & Almeida, J. R. M. (2021). Physiological characterization and transcriptome analysis of Pichia pastoris reveals its response to lignocellulose-derived inhibitors. AMB Express, 11(1), Article 2. https://doi.org/10.1186/s13568-020-01170-9
3. You, J., Pan, X., Yang, C., Du, Y., Osire, T., Yang, T., Zhang, X., Xu, M., Xu, G., & Rao, Z. (2021). Microbial production of riboflavin: Biotechnological advances and perspectives. Metabolic Engineering, 68, 46–58. https://doi.org/10.1016/j.ymben.2021.08.009

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ENGINEERING STRATEGIES FOR ENHANCED RIBOFLAVIN PRODUCTION BY *CANDIDA FAMATA* YEAST

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Abstract. Riboflavin is an essential compound for humans and animals. Industrial riboflavin production is achieved through the use of recombinant strains of the bacterium *Bacillus subtilis* and filamentous fungus *Ashbya gossypii.* Some mutants of *Candida famata* yeast also belong to the most famous riboflavin overproducers but there great flavinogenic potential remains unrealized. Metabolic engineering approaches have been successfully applied for construction of riboflavin overproducer in *C. famata*. A new factor of negative regulation of riboflavin biosynthesis in yeast *C. famata* was identified, which is encoded by the gene *SEF2*. We are also focused on extension of the substrate range to produce the vitamin B_2 such as whey and lignocellulose. Further studies should be accelerated for the effective utilization of substrates and supplements, facilitating better methods for bioconversion with an economic and industrial perspective.
Keywords: vitamin B_2, riboflavin, yeast, *Candida famata*, metabolic engineering; riboflavin overproducers.

Riboflavin (vitamin B_2) is a water-soluble vitamin, which is produced by all plants and most microorganisms and is essential for growth and reproduction of humans and animals Riboflavin performs its biochemical function as a precursor for the coenzymes, flavin adenine dinucleotide and flavin mononucleotide, which are mostly involved in redox reactions of all organisms. Riboflavin is involved in involved in important processes: cellular respiration, Krebs cycle, beta oxidation of fatty acids, amino acid metabolism, oxidative stress response, the mobilization of iron etc. It is mostly used as component of feed premixes for animals (80 %), in food industry as food colorant, in medicine and component of multivitamins mixtures and as drug for treatment of some diseases. Riboflavin deficiency raises concerns in both developing and developed countries. The global riboflavin market is estimated to reach USD 508.6 million in 2025. To meet the increasing demand for riboflavin, researchers are focusing on construction of more productive strains, optimizing microbial fermentation processes, and exploring alternative feedstocks.

Fungi *Eremothecium ashbyii* and *Ashbya gossypii*, and bacteria, including *Bacillus subtilis*, and lactic acid bacteria, are ideal cell factories for riboflavin overproduction. Fermentation processes have replaced the chemical synthesis of riboflavin because they are cost-effective. and environmental considerations of the latter. The yeast *C. famata* is a natural riboflavin-producing species, which are able to oversynthesis riboflavin under conditions of iron starvation. Some *C. famata* mutants are among the most efficient riboflavin producers, but their full flavinogenic potential has yet to be fully exploited. The process of fermentation with the use of yeast producers has a range of advantages (simple media and cheap renewable carbohydrates as a carbon sources, resistance against phage infection and other). To enhance riboflavin production, strains were improved through genetic and metabolic engineering.

Using classic mutagenesis and several chemicals in six stages, genetically stable riboflavin-overproducing strain, *C. famata* AF-4 was isolated, which accumulated nearly 680 mg riboflavin/L. Key transcriptional regulator *SEF1* has been identified, which enhances riboflavin production by upregulating *RIB* gene expression, Deletion of the *SEF1* gene led to inability to overproduce riboflavin in *C. famata*. The same strain, AF-4, was further improved by inserting an additional copy of *SEF1*, *IMH3* gene encoding for inosine monophosphate dehydrogenase, and two genes from the first and last steps of riboflavin synthesis: *RIB1* (GTP cyclohydrolase II) and *RIB7* (riboflavin synthetase), respectively. This strain, named BRP (Best Riboflavin Producer), is capable of producing 1 g riboflavin/L in flask culture and up to 16.4 g/L in optimized medium during fed-batch cultivation. Overexpression of *RIB1* and *RIB6*, encoding GTP cyclohydrolase II and 3,4-dihydroxy-2-butanone 4-phosphate synthase, respectively, increased riboflavin production 1.3-fold, from 653 mg/L in the parental strain to 849 mg/L.

A new factor of negative regulation of riboflavin biosynthesis in yeast *C. famata* has been identified, which is encoded by the gene *SEF2*. Introduction of an additional copy of this gene into riboflavin overproducing strain led to a decrease in riboflavin production, and its deletion on the wild-type background led to a 2-3-fold increase in riboflavin accumulation in the culture medium. In *C. famata*, deletion of the *SFU1* gene (coding for GATA-type transcription factor Sfu1) in a wild-type strain similarly triggered riboflavin overproduction, suggesting a potential role in regulation of riboflavin synthesis. Furthermore, disruption of the *VMA1* gene, which encodes the vacuolar ATPase subunit A, also led to enhanced riboflavin synthesis in *C. famata*.

Riboflavin synthesis was further improved due to overexpression of genes *PRS3* and *ADE4* coding enzymes of *de novo* purine biosynthesis, or homolog of mammal gene *BCRP* coding for riboflavin excretase. *BCRP* gene was expressed under control of promoter of *TEF1* gene in the the best flavinogenic strain of the yeast *C. famata*. Resulted transformants produced 1.3–1.5 times more riboflavin relative to the parental strain.

All these studies were carried out using glucose as a carbon source. The price of riboflavin significantly depends on the cost of the used carbon substrate. *C. famata* can efficiently utilize not only glucose but also other sugars. It was shown that the overexpression *SEF1* gene under control of promoter of the *LAC4* gene encoding β-galactosidase in *C. famata* led to increase in riboflavin production on media with lactose and cheese whey. Our data indicate that the waste whey from milk industry can be a promising substrate for riboflavin production by *C. famata.*

Engineered strains of the yeast *C. famata* have successfully overproduced riboflavin on lignocellulosic hydrolysate. Riboflavin accumulation increased in the recombinant strains *C. famata* overexpressing the *XYL1* and *XYL2* genes, which code for xylose reductase and xylitol dehydrogenase, respectively. The possibility of using other industrial waste and semi-products of food industry for production vitamin B_2, is also discussed.

Reducing costs, increasing riboflavin yield and productivity, and efficiently utilizing resources will remain key research priorities in the future. Further studies should be accelerated for the effective utilization of substrates and supplements, facilitating better methods for bioconversion with an economic and industrial perspective.

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NOVEL POSITIVE SELECTION APPROACH FOR THE YEAST *OGATAEA POLYMORPHA*

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Abstract. A novel positive selection approach for *Ogataea polymorpha* based on *CAT8* overexpression and metformin-mediated growth inhibition was investigated. Overexpression of *CAT8* conferred a growth advantage under selective conditions with glycerol as the carbon source, demonstrating the potential of this system for selection of recombinant *O. polymorpha* strains.
Keywords: *Ogataea polymorpha*, *CAT8*, metformin, positive selection, metabolic engineering.

The thermotolerant methylotrophic yeast *Ogataea polymorpha* is widely used as a model organism for studying methanol metabolism, peroxisome biology and thermotolerance and represents a promising platform for heterologous protein production and high-temperature fermentation. Further development of genetic engineering approaches for this yeast requires additional selection systems that could complement conventional antibiotic-resistance markers.

In our previous studies, metformin was identified as a potential selective agent for the development of a metabolic selection system in *O. polymorpha*. Growth of the parental NCYC495 strain on glycerol-containing medium was strongly inhibited by metformin, with complete growth inhibition observed at 50 mg/mL. Since utilization of glycerol requires an active gluconeogenic pathway, overexpression of the transcription factor gene *CAT8*, involved in the regulation of gluconeogenesis and utilization of non-fermentable carbon sources, was investigated as a basis for positive selection.

Transformation of *O. polymorpha* with a construct carrying a *CAT8* overexpression cassette restored growth under selective conditions on YNB medium containing glycerol and metformin. The presence of the target DNA sequence in the obtained transformants was confirmed by PCR. These results demonstrate that *CAT8* overexpression provides a selective growth advantage under conditions in which growth of the parental strain is inhibited by metformin.

The developed approach provides a basis for a positive metabolic selection system in *O. polymorpha* and may complement conventional selection based on antibiotic-resistance markers. Further optimization of the selective conditions and validation of the system with different genetic constructs could facilitate its application in metabolic engineering of this thermotolerant yeast.

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IDENTIFICATION OF NOVEL SUBSTRATE FOR THE YEAST ALCOHOL OXIDASE AND APPLICATION OF THIS ABILITY FOR ASSAY OF NITRATE ESTER EXPLOSIVE – PETN

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Abstract. For the first time, it was shown that pentaerythritol is a substrate for the yeast alcohol oxidase (AOx). This phenomenon was exploited to develop the novel optical methods for detection of an explosive material, pentaerythritol tetranitrate (PETN), using pentaerythritol tetranitrate reductase–alcohol oxidase (PETNR-AOx) cascade. The sensing concept relies on PETNR-catalyzed reductive denitration of PETN to generate alcohol-containing intermediates, which are subsequently oxidized by AOx with concomitant formation of hydrogen peroxide, monitored in peroxidase reaction.
Keywords: yeast *Komagataella phaffii* (*Pichia pastoris*), alcohol oxidase, pentaerythritol, pentaerythritol tetranitrate (PETN), enzymo-fluorometric assay.

Alcohol oxidase (AOx, EC 1.1.3.13) is a flavin-dependent enzyme that catalyzes the oxidation of primary alcohols to the corresponding aldehydes, accompanied by the reduction of molecular oxygen to hydrogen peroxide. Owing to its high catalytic activity and direct generation of hydrogen peroxide, AOx has been widely employed in biosensing, biocatalysis, and analytical applications. Although methanol is considered the natural substrate of AOx, the enzyme exhibits a relatively broad substrate specificity and is capable of oxidizing a variety of primary alcohols and polyhydroxylated compounds. The hydrogen peroxide generated during AOx-catalyzed oxidation has been extensively exploited in coupled enzymatic systems employing horseradish peroxidase and chromogenic or fluorogenic substrates for sensitive optical detection of target analytes, first of all, methanol and ethanol.

Despite the broad substrate scope reported for AOx, no information is currently available regarding its ability to oxidize pentaerythritol (2,2-bis(hydroxymethyl)propane-1,3-diol), a tetrahydroxylalcohol that represents the complete denitration product of pentaerythritol tetranitrate (PETN). Establishing whether pentaerythritol and PETN-derived hydroxyl-containing intermediates can serve as AOx substrates is important not only from a biocatalytic perspective but also for the development of novel enzymatic sensing strategies based on peroxide generation.

PETN is one of the most powerful nitrate ester explosives widely used in military formulations, detonating cords, blasting caps, and plastic explosive mixtures because of its high detonation velocity, chemical stability, and explosive performance.

For the first time, we demonstrate that pentaerythritol serves as a substrate for alcohol oxidase (AOx) and exploit this newly identified enzymatic activity to develop the novel optical methods for PETN determination based on a coupled pentaerythritol tetranitrate reductase–alcohol oxidase (PETNR-AOx) cascade. The sensing concept relies on PETNR-catalyzed reductive denitration of PETN to generate alcohol-containing intermediates, which are subsequently oxidized by AOx with concomitant formation of hydrogen peroxide. The generated H_2O_2 was quantified using either a horseradish peroxidase (HRP)/chromogen system or an HRP/fluorogen one, providing spectrophotometric and fluorometric detection formats, respectively.

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BIOLOGICAL ACTIVITY OF PEDIOCOCCI ISOLATED FROM TRADITIONAL UKRAINIAN FERMENTED FOODS

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Abstract. Some technological properties of *Pediococcus* spp. strains were investigated in this study to evaluate their potential for practical use in the food industry. The antagonistic activity towards opportunistic pathogens, anti-listeria activity, NaCl resistance and fermentative activity suggest that *Pediococcus* spp. strains have great potential as starters for the production of fermented foods.
Keywords: *Pediococcus*, antagonism, *Listeria monocytogenes*, fermented foods.

Bacteria of the genus *Pediococcus* are Gram-positive, catalase-negative cocci belonging to the family *Lactobacillaceae*. Compared with other members of the lactic acid bacteria (LAB) group, pediococci are not as widely used, but are no less promising for the food and pharmaceutical industries. Bacteria of the genus *Pediococcus* are frequently found in fermented milk products, meat, sourdoughs, and fermented vegetables and fruits (Lu et al., 2024). Due to their biological activity, bacteria of the genus *Pediococcus* have a wide range of applications (Plessas et al., 2020; Nakashima et al., 2024).

As well as providing typical organoleptic characteristics to fermented foods, *Pediococcus* spp. are known as producers of effective biopreservatives, as demonstrated in numerous studies (Porto et al., 2017). This property is mediated by pediocins – peptide bactericidal agents involved in the mechanisms of interspecific competition. In addition to its antagonistic activity against common opportunistic and enteric pathogens, *Pediococcus* spp. inhibits the growth of *Listeria monocytogenes* – the pathogen that causes listeriosis, a serious form of foodborne infection (de Azevedo et al., 2020). The fermentation of foods by *Pediococcus* spp. not only improves their organoleptic qualities but also prevents the proliferation of undesirable microbiota in raw materials, thereby preventing spoilage and food poisoning.

Sixteen *Pediococcus* spp. strains from the LAB culture collection of the Department of Physiology of Industrial Microorganisms, isolated from spontaneously fermented milk, cottage cheese, tomato juice, cabbage and apples, were used in this work. Some of these strains were previously selected as potential producers of gamma-aminobutyric acid (GABA) based on results of a rapid colourimetric glutamatedecarboxylase assay, which evaluates the ability for intracellular GABA synthesis. The aim of the study was to determine the technological characteristics of *Pediococcus* spp. strains to evaluate their potential for use as starter cultures in the food industry.

The results of the antagonistic activity assay using the well diffusion method showed inhibition of *L. monocytogenes* and varying degrees of growth inhibition of Gram-positive and Gram-negative opportunistic bacteria, such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enterica*, *Klebsiella pneumoniae*, *Bacillus cereus* and *Staphylococcus aureus*. All *Pediococcus* spp. strains used were resistant to 6 % NaCl and fermented fructose and maltose. Sucrose was fermented by 75 % of the *Pediococcus* strains, whilst lactose was fermented by only 25 % of the strains. The low β-galactosidase activity was confirmed by a slight increase in total titrable acidity (TTA) of milk during growth in milk; with the exception of some *Pediococcus* strains, the TTA of the milk inoculated with them reached

33–40 °T. Due to the ability to ferment maltose, the *Pediococcus* strains used are promising candidates for use in sourdough starters; during the fermentation of a flour/water mixture, the acidity levels reached the required values (12–16 °T, depending on the type of flour).

Thus, pediococci isolated from traditional fermented foods in Ukraine exhibit important technological properties and are promising candidates for the development of bacterial preparations for the production of fermented foods. Further research is required to assess their safety for human health and their probiotic properties.

References

1. Plessas, S., Mantzourani, I., & Bekatorou, A. (2020). Evaluation of Pediococcus pentosaceus SP2 as starter culture on sourdough bread making. Foods, 9(1), Article 77. https://doi.org/ 10.3390/ foods9010077
2. Nakashima, Y., Onuki, K., Hibi, T., Ohno, R. I., Sugawa, H., Tominaga, Y., Yasuda S., & Kinoshita, H. (2024). Soymilk yogurt fermented using Pediococcus pentosaceus TOKAI 759m improves mice gut microbiota and reduces pro-inflammatory cytokine production. Bioscience, Biotechnology, and Biochemistry, 88(11), 1349–1361. https://doi.org/10.1093/bbb/zbae112
3. Lu, K., Wang, X., Zhou, Y., & Zhu, Q. (2024). Genomic characterization and probiotic potential assessment of an exopolysaccharide-producing strain Pediococcus pentosaceus LL-07 isolated from fermented meat. BMC Microbiology, 24(1), Article 142. https://doi.org/10.1186/s12866-024-03304-6
4. Porto, M. C., Kuniyoshi, T. M., Azevedo, P. O., Vitolo, M., & Oliveira, R. P. (2017). Pediococcus spp.: An important genus of lactic acid bacteria and pediocin producers. Biotechnology Advances, 35(3), 361–374. https://doi.org/10.1016/j.biotechadv.2017.03.004
5. de Azevedo, P. O. S., Mendonça, C. M. N., Seibert, L., Domínguez, J. M., Converti, A., Gierus, M., & Oliveira, R. P. S. (2020). Bacteriocin-like inhibitory substance of Pediococcus pentosaceus as a biopreservative for Listeria sp. control in ready-to-eat pork ham. Brazilian Journal of Microbiology, 51(3), 949–956. https://doi.org/10.1007/s42770-020-00245-w

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BACTERIA FROM HOSTILE HABITATS AS PRODUCERS OF AUXIN-LIKE COMPOUNDS

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Abstract. The ability of four bacterial strains isolated from habitats with adverse conditions (leachate from the Lviv municipal solid waste landfill, Antarctic substrates, and soils disturbed by military operations) to synthesize auxin-like compounds was investigated. We used LB and NB media for culturing the bacteria. It was found that *Pseudarthrobacter* sp. 3B-K-54 accumulated up to 100 μg/mL of auxins in both media, whereas *Kocuria* sp. S309 was more efficient on LB. Oxygen conditions affected biosynthesis: under stationary cultivation, auxin levels decreased 3–7 times compared to aerated samples. Optimizing the L-tryptophan content (1–5 %) stimulated biosynthesis and increased product yield by 5.5–9.7 times, confirming the biotechnological potential of the studied strains as a basis for PGPR preparations.
Keywords: auxin-like compounds, *Kocuria*, *Micrococcus*, *Ochrobactrum*, *Pseudarthrobacter*.

Background. Auxins, in particular indole-3-acetic acid, are key phytohormones that regulate plant growth and development (Etesami & Glick, 2024). The use of rhizosphere and endophytic bacteria capable of synthesizing phytohormones is considered an environmentally safe alternative to chemical fertilizers and growth stimulants (Zhang et al., 2022;

Khianngam et al., 2023). Microorganisms isolated from ecological niches with adverse or extreme living conditions are of particular interest. They are characterized by physiological and biochemical adaptations and a stable metabolism (Krempaská et al., 2026), making them promising agents for biotechnologies aimed at restoring and promoting plant growth.

The aim of this study was to estimate the ability of *Ochrobactrum* sp. K3, *Pseudarthrobacter* sp. 3B-K-54, *Micrococcus* sp. Pn45, and *Kocuria* sp. S309, which were isolated from ecotopes with unfavorable conditions, to synthesize auxin-like compounds and to optimize their cultivation conditions (medium composition, aeration level, L-tryptophan concentration) to increase product yield.

Materials and Methods. We used bacterial strains isolated at the Department of Microbiology of Ivan Franko National University of Lviv from extreme habitats: *Ochrobactrum* sp. K3 (leachate from the Lviv municipal solid waste landfill), *Pseudarthrobacter* sp. 3B-K-54, *Micrococcus* sp. Pn45 (Antarctic substrates), and *Kocuria* sp. S309 (soil damaged by military operations).

The bacteria were cultured for 7 days in test tubes containing 5 ml of nutrient broth (NB) or Luria-Bertani (LB) liquid medium, to which 0.15 % L-tryptophan was added. For the bacteria that produced the highest levels of auxins, we determined how changes in culture conditions would affect the formation of these compounds. Specifically, the bacteria were grown in Erlenmeyer flasks in media volumes of 50, 150, and 300 mL. To assess the effect of aeration on the production of auxin-like compounds by these bacteria, one set of cultures was grown on an orbital shaker, while the other was grown under stationary conditions. In addition, the concentration of L-tryptophan in the medium was varied for these strains (0.15, 1, 2, 3, 4, and 5 %). The presence and total concentration of auxin-like compounds in the culture medium were determined photometrically using Salkowski's reagent.

Results. It was found that the accumulation of auxin-like compounds by strains grown in a 5-mL volume depends on the composition of the culture medium and the biological characteristics of the producers. The *Pseudarthrobacter* sp. 3B-K-54 strain synthesized up to 100 μg/mL of auxins regardless of the medium used. For *Ochrobactrum* sp. K3, no significant difference was observed between the media either – the auxin content in the medium reached 20 μg/mL. At the same time, *Micrococcus* sp. Pn45 produced slightly more auxins on day 4 of growth in NB medium, but during subsequent cultivation, the concentration of these compounds was the same in both media. *Kocuria* sp. S309 bacteria produced more auxins in LB medium.

Further studies confirmed the effect of aeration: under stationary culture conditions, the auxin content in the culture medium of all strains studied was 3–7 times lower than under shaker condition.

An increase in the concentration of L-tryptophan in the medium had a mild stimulatory effect on the synthesis of auxin-like compounds. The addition of 1–5 % of this compound led to a 5.5–9.7-fold increase in the content of auxin-like compounds, depending on the strain and the initial concentration of L-tryptophan.

Conclusions. The bacterial strains studied, which were isolated from habitats with adverse conditions, have a high potential for synthesizing auxin-like compounds. Oxygen influenced auxin biosynthesis, and optimizing the L-tryptophan content in the medium increases the yield of auxin-like compounds by 5.5–9.7 times, confirming the biotechnological potential of the studied strains as a basis for PGPR preparations..

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References

1. Etesami, H., & Glick, B. R. (2024). Bacterial indole-3-acetic acid: A key regulator for plant growth, plant-microbe interactions, and agricultural adaptive resilience. Microbiological Research, 281, 127602. https://doi.org/10.1016/j.micres.2024.127602
2. Khianngam, S., Meetum, P., Na Chiangmai, P., & Tanasupawat, S. (2023). Identification and Optimization of Indole-3-Acetic Acid Production of Endophytic Bacteria and Their Effects on Plant Growth. Tropical Life Sciences Research, 34(1). https://doi.org/10.21315/tlsr2023.34.1.12
3. Zhang, M., Gao, C., Xu, L., Niu, H., Liu, Q., Huang, Y., Lv, G., Yang, H., & Li, M. (2022). Melatonin and Indole-3-Acetic Acid Synergistically Regulate Plant Growth and Stress Resistance. Cells, 11(20), 3250. https://doi.org/10.3390/cells11203250
4. Krempaská, V., Murgašová, M., & Vesteg, M. (2026). Metabolic adaptations of extremophiles and their applications in environmental biotechnology. World Journal of Microbiology and Biotechnology, 42(8). https://doi.org/10.1007/s11274-026-05124-4

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THE RELATIONSHIP BETWEEN ELECTROGENESIS AND BIOFILM FORMATION IN ELECTROACTIVE BACTERIA

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Abstract. This study correlates biofilm formation with redox activity to assess the electrogenic potential of *Pseudomonas* strains (*P. aeruginosa*, *P. chlororaphis*, *P. fluorescens*) for microbial fuel cells. Cultivation showed *P. aeruginosa* developed dense, uniform biofilms, while others formed moderate, uneven ones. *P. aeruginosa* also exhibited a DREAM coefficient 4.6–5.8 times higher than the others within 4 h, indicating superior electron production. Ultimately, *P. aeruginosa* ATCC 27853 showed 3–8 times greater electron emission intensity, confirming a direct relationship between solid-surface biomass accumulation and redox capacity, validating these parameters for evaluating microbial current generators.
Keywords: microbial fuel cells, electrogenesis, biofilm, electroactive microorganisms, redox activity.

The search for efficient microorganisms for use as the basis of microbial fuel cells (MFCs) is based on evaluating their ability for electrogenesis and the formation of microbial associations. Key criteria include the intensity of microbial biomass accumulation on solid surfaces in the form of a biofilm and the ability of strains to produce compounds involved in redox reactions (Askitosari et al., 2019). This study focuses on analyzing the electrogenic potential of representatives of the genus *Pseudomonas* (specifically *P. aeruginosa*, *P. chlororaphis*, and *P. fluorescens* strains) through the quantitative determination of biofilm formation intensity and their level of redox activity. Establishing a correlation between these morphological and quantitative parameters allows for the identification of the most promising strains as current generators for MFCs.

The ability of microorganisms to form a biofilm was determined using crystal violet at 582 nm (O'Toole & Kolter, 1998). The redox potential was determined by the DREAM method, where methylene blue served as an indicator, and its reduction was recorded spectrophotometrically at 660 nm (Vishwanathan et al., 2015). The reliability of the results was evaluated using Student's t-test ($p \leq 0.05$) (Mavrodi et al., 2006).

The cultivation of microorganisms in polystyrene plates demonstrated an intensive accumulation of their biomass. The *P. aeruginosa* strains (ATCC 27853, ATCC 15692, ATCC 10145) formed a high-density biofilm with uniform cell layering. In contrast,

P. chlororaphis ONU 111 and *P. fluorescens* ONU 110 were characterized by a moderate intensity of biofilm formation, featuring an uneven cell distribution and the presence of gaps.

The ability of the studied microorganisms to function as the basis for microbial fuel cells (MFCs) was confirmed by their capacity to produce electrons and reduce methylene blue to a colorless leuco form (Rahimnejad et al., 2011).

The *P. aeruginosa* strains demonstrated a DREAM coefficient 4.6–5.8 times higher compared to *P. chlororaphis* and *P. fluorescens* as early as the 4th hour of the experiment. Over time, the redox activity increased, with the maximum intensity of electron emission recorded for the *P. aeruginosa* ATCC 27853 strain, showing values that exceeded the results of the other studied cultures by 3–8 times.

The analysis of the results established a direct correlation between the intensity of microbial biomass accumulation on a solid surface and the ability of the strains to engage in redox reactions. The *Pseudomonas* strains with a high level of biofilm formation and a mature, uniform biofilm (*P. aeruginosa*) demonstrated the highest capacity for electrogenesis. Accordingly, microorganisms with a lower rate of biofilm formation exhibited a significantly lower level of redox activity. The morphological and quantitative parameters of microbial association formation can be effectively utilized as criteria for evaluating microorganisms as current generators for MFCs.

References

1. Askitosari, T. D., Boto, S. T., Blank, L. M., & Rosenbaum, M. A. (2019). Boosting heterologous phenazine production in *Pseudomonas putida* KT2440 through the exploration of the natural sequence space. *Frontiers in Microbiology*, *10*. https://doi.org/10.3389/fmicb.2019.01990
2. Mavrodi, D. V., Blankenfeldt, W., & Thomashow, L. S. (2006). Phenazine Compounds in Fluorescent *Pseudomonas* spp. Biosynthesis and Regulation. *Annual Review of Phytopathology*, *44*(1), 417–445. https://doi.org/10.1146/annurev.phyto.44.013106.145710
3. O'Toole, G. A., & Kolter, R. (1998). Initiation of biofilm formation in *Pseudomonas fluorescens* WCS365 proceeds via multiple, convergent signalling pathways: A genetic analysis. *Molecular Microbiology*, *28*(3), 449–461. https://doi.org/10.1046/j.1365-2958.1998.00797.x
4. Rahimnejad, M., Najafpour, G. D., Ghoreyshi, A. A., Shakeri, M., & Zare, H. (2011). Methylene blue as electron promoters in microbial fuel cell. *International Journal of Hydrogen Energy*, *36*(20), 13335–13341. https://doi.org/10.1016/j.ijhydene.2011.07.059
5. Vishwanathan, A. S., Devkota, R., Siva Sankara Sai, S., & Rao, G. (2015). DREAM assay for studying microbial electron transfer. *Applied Biochemistry and Biotechnology*, *177*(8), 1767–1775. https://doi.org/10.1007/s12010-015-1852-3

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BIOTECHNOLOGICAL SCREENING OF PLANT-ASSOCIATED BACTERIA FOR L-TRYPTOPHAN-DEPENDENT INDOLE-3-ACETIC ACID PRODUCTION

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Abstract. Indole-3-acetic acid (IAA) production by plant-associated bacteria represents a promising trait for agricultural biotechnology. This study evaluated strain-specific responses of six bacterial

isolates to exogenous L-tryptophan (0–0.5 g/L) as an IAA biosynthesis precursor. Auxin production showed pronounced concentration- and strain-dependent variation, with *Bacillus* sp. 5 demonstrating the highest IAA production (45.89 mg/g dry biomass) at 0.5 g/L L-tryptophan. The findings support the hypothesis that IAA biosynthesis is governed by strain-specific metabolic responses to precursor availability and provide a basis for optimising bacterial auxin production for plant-growth-promoting biotechnology.
Keywords: indole-3-acetic acid, L-tryptophan, plant-associated bacteria, *Bacillus*, auxin biosynthesis.

Indole-3-acetic acid (IAA) is one of the major auxins involved in the regulation of plant growth, root system architecture, nutrient acquisition, and plant–microbe interactions. The ability to synthesise IAA is widespread among plant-associated bacteria and is regarded as an important functional trait when selecting microorganisms for plant-growth-promoting biotechnology (Spaepen et al., 2007; Duca & Glick, 2020). Bacterial IAA biosynthesis is metabolically complex and may proceed through several tryptophan-dependent routes, including pathways involving indole-3-pyruvate, indole-3-acetamide, and indole-3-acetonitrile. The activity of these routes can be affected by precursor availability, environmental conditions, and strain-specific regulatory mechanisms (Patten et al., 2013).

The present study researched the effect of exogenous L-tryptophan on IAA biosynthesis by six plant-associated bacterial isolates: *Paenibacillus* sp. 1, *Ochrobactrum* sp. 2, *Paenibacillus* sp. 3, *Bacillus* sp. 4, *Bacillus* sp. 5, and *Pseudomonas* sp. 6. The microorganisms were cultivated under control conditions and in media supplemented with L-tryptophan at concentrations of 0.1, 0.2, and 0.5 g/L. IAA production was normalised to absolutely dry bacterial biomass and expressed as mg/g. The central working hypothesis was that the effect of L-tryptophan on IAA production is determined not only by precursor concentration but also by strain-specific metabolic properties.

The experimental data demonstrated pronounced heterogeneity in IAA production among the strains. The strongest overall response was observed at 0.5 g/L L-tryptophan, whereas concentrations of 0.1 and 0.2 g/L did not produce a statistically significant overall effect compared with the control in the original analysis. The microorganisms were cultivated under control conditions and in media supplemented with L-tryptophan at concentrations of 0.1, 0.2, and 0.5 g/L. IAA production was normalised to absolutely dry bacterial biomass and expressed as mg/g. The central working hypothesis was that the effect of L-tryptophan on IAA production is determined not only by precursor concentration but also by strain-specific metabolic properties.

At 0.5 g/L, *Bacillus* sp. 5 exhibited the highest reported IAA production, reaching 45.89 mg/g of absolutely dry biomass. Under the same conditions, *Ochrobactrum* sp. 2, *Paenibacillus* sp. 1, *Bacillus* sp. 4, and *Paenibacillus* sp. 3 produced 16.14, 14.11, 11.03, and 7.83 mg/g, respectively. These differences indicate that increasing extracellular precursor availability does not elicit an equivalent biosynthetic response across taxonomically distinct bacteria.

To describe the concentration–response relationship quantitatively, the experimental means were approximated using a second-order polynomial model. The most pronounced nonlinear pattern was observed for *Bacillus* sp. 5 and could be represented as $Y = 2.77 - 13.42X + 198.99X^2$, with $R^2 = 0.998$. The large positive quadratic coefficient reflects the sharp increase in IAA production between 0.2 and 0.5 g/L.

The response of *Bacillus* sp. 5 supports a hypothesis of threshold-like precursor dependence. In this scenario, lower L-tryptophan concentrations may be insufficient to produce a major increase in metabolic flux toward IAA, whereas higher precursor availability may per-

mit a substantial redistribution of tryptophan metabolism toward auxin formation. In contrast, the weak or irregular response of *Pseudomonas* sp. 6 suggests that precursor availability alone is insufficient to determine IAA productivity. Such differences could theoretically reflect strain-specific variation in L-tryptophan uptake, abundance or activity of IAA-biosynthetic enzymes, competition between alternative tryptophan-utilising pathways, feedback regulation, or IAA degradation.

The observed strain-specific response is particularly relevant from a biotechnological perspective. Selection of microorganisms for auxin-based plant-growth-promoting applications should not rely exclusively on basal IAA production, because the capacity to respond to precursor supplementation may provide an additional indicator of metabolic flexibility and production potential. Bacterial IAA can influence root development and other plant physiological processes, which explains the growing interest in IAA-producing microorganisms as components of sustainable agricultural technologies (Duca et al., 2014). In the present dataset, *Bacillus* sp. 5 therefore represents the most promising candidate for further optimization of precursor-driven IAA production.

At the same time, the current experiment does not permit identification of the exact molecular route responsible for increased IAA formation. Although several tryptophandependent bacterial pathways are well established in the literature, including the indole-3-pyruvate, indole-3-acetamide, and indole-3-acetonitrile routes, assigning the observed phenotype to a specific pathway would require complementary genomic, transcriptomic, enzymatic, or targeted metabolomic evidence (Patten et al., 2013; Duca & Glick, 2020).

Overall, the findings demonstrate that L-tryptophan-dependent IAA biosynthesis is characterized by substantial strain-specific heterogeneity. The strongest response was observed in *Bacillus* sp. 5 at the highest tested L-tryptophan concentration, while other isolates displayed quantitatively distinct response profiles. The results support the hypothesis that precursor-dependent auxin biosynthesis should be treated as a strain-specific quantitative trait and optimized individually for each microbial producer.

References

1. Duca, D. R., & Glick, B. R. (2020). Indole-3-acetic acid biosynthesis and its regulation in plant-associated bacteria. *Applied Microbiology and Biotechnology, 104*(20), 8607–8619. https://doi.org/10.1007/s00253-020-10869-5
2. Duca, D., Lorv, J., Patten, C. L., Rose, D., & Glick, B. R. (2014). Indole-3-acetic acid in plant–microbe interactions. *Antonie van Leeuwenhoek, 106*(1), 85–125. https://doi.org/10.1007/s10482-013-0095-y
3. Patten, C. L., Blakney, A. J. C., & Coulson, T. J. D. (2013). Activity, distribution and function of indole-3-acetic acid biosynthetic pathways in bacteria. *Critical Reviews in Microbiology, 39*(4), 395–415. https://doi.org/10.3109/1040841X.2012.716819
4. Spaepen, S., Vanderleyden, J., & Remans, R. (2007). Indole-3-acetic acid in microbial and microorganism–plant signaling. *FEMS Microbiology Reviews, 31*(4), 425–448. https://doi.org/10.1111/j.1574-6976.2007.00072.x

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SIDEROPHORE PRODUCTION IN PHOSPHATE-SOLUBILIZING *PENICILLIUM* SPP.

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Abstract. Siderophores are essential compounds that contribute to iron availability and play a broader role in nutrient utilization, including phosphate solubilization. In this study, siderophore production by phosphate-solubilizing *Penicillium* spp. was investigated, and the relationship between siderophore production and phosphate-solubilizing activity was evaluated. The siderophore activity varied from 3.2 ± 1.0 (*P. vulpinum* 2878) to 57.4 ± 3.8 (*P. funiculosum* 16783). The *Penicillium funiculosum* strains were characterized as high siderophore producers and demonstrated potential for further investigation.
Keywords: siderophores, *Penicillium*, phosphate-solubilization, plant-growth promoting microorganisms, sustainable agriculture.

Introduction. Siderophores are low-molecular-weight compounds produced in various organisms that facilitate iron acquisition through Fe^{3+} chelation and increase its availability for uptake (Swayambhu, 2021). However, the role of siderophores extends beyond iron acquisition for the nutritional needs of the producer. Some siderophores exhibit antimicrobial activity and may contribute to microbial competition as they limit iron availability to phytopathogenic microorganisms (Gu, 2020). Another, less investigated role of siderophores is their involvement in phosphate solubilization, potentially enhancing the availability of phosphorus for plants (Ahash, 2025). Therefore, this study aimed to investigate siderophore production among phosphate-solubilizing strains of the genus *Penicillium*.

Materials and methods. The study included 106 strains representing 37 species of the genus *Penicillium* Siderophore production was quantitatively evaluated in 7-day culture filtrates obtained from cultures grown in NBRIP medium. Phosphate-solubilizing activity of the experimental strains had previously been investigated using the same culture samples. Siderophore production was assessed using the Chrome Azurol S (CAS) assay according to the following formula:

$$Siderophore\ activity, \% = \frac{Ar - As}{Ar} \times 100,$$

where Ar is the absorbance of the reference sample (CAS reagent with uninoculated medium) and As is the absorbance of the tested sample. Absorbance was measured at 620 nm (Schwyn, 1987). All experiments were performed in triplicate. Statistical analyses were conducted at a significance level of $p \leq 0.05$ using STATISTICA 7. Data visualization was performed using OriginLab 2026.

Results. All experimental strains demonstrated the ability to produce hydroxamate-type siderophores, as indicated by a color change of the CAS reagent from blue to violet or pink, depending on the amount of siderophore produced. Siderophore activity varied among the tested strains, with 2.8 %, 16.0 %, 28.3 %, 26.4 %, 25.5 % and 0.9 % of strains exhibiting activity levels of x – 0–10 %, 10–20 %, 20–30 %, 30–40 %, 40–50 %, and 50–60 %, respectively. Seven strains of *P. funiculosum*, along with one strain each of *P. hirsutum*, *P. lanosum*, and *P. rubrum*, exhibited the highest siderophore activity. The significant positive correlation (r = 0.3) between intensity of phosphate-solubilization and siderophore production was identified.

Conclusions. The study demonstrated the ability of phosphate-solubilizing *Penicillium* strains to produce hydroxamate-type siderophores and revealed significant correlations between siderophore production and phosphate-solubilizing activity. A pronounced strain-specific pattern of siderophore production was also observed. The experimental strains were indicated as promising for further studies related to phosphate solubilization and siderophore production and for potential application in agriculture.

References:

1. Ahash, S., Manikandan, K., Sivasankari Devi, T., Elamathi, S., Maragatham, S., & Subrahmaniyan, K. (2025). Phosphate-solubilizing microorganisms for sustainable phosphorus management in rice. Rhizosphere, 34, 101096. https://doi.org/10.1016/j.rhisph.2025.101096
2. Gu, S., Wei, Z., Shao, Z., Friman, V. P., Cao, K., Yang, T., Kramer, J., Wang, X., Li, M., Mei, X., Xu, Y., Shen, Q., Kümmerli, R., & Jousset, A. (2020). Competition for iron drives phytopathogen control by natural rhizosphere microbiomes. Nature microbiology, 5(8), 1002–1010. https://doi.org/10.1038/s41564-020-0719-8
3. Schwyn, B., & Neilands, J. B. (1987). Universal chemical assay for the detection and determination of siderophores. Analytical biochemistry, 160(1), 47–56. https://doi.org/ 10.1016/0003-2697(87)90612-9
4. Swayambhu, G., Bruno, M., Gulick, A. M., & Pfeifer, B. A. (2021). Siderophore natural products as pharmaceutical agents. Current opinion in biotechnology, 69, 242–251. https://doi.org/10.1016/j.copbio.2021.01.021

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PRODUCTION OF AUXIN-LIKE COMPOUNDS AND SIDEROPHORES BY *OCHROBACTRUM RHIZOSPHAERAE* IMV B-7956 UNDER T THE INFLUENCE OF NaCl

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Abstract. The ability of *Ochrobactrum rhizosphaerae* IMV B-7956 bacteria to produce auxin-like compounds and siderophores at various NaCl concentrations was investigated. The bacteria, isolated from filtrates of the Lviv municipal solid waste landfill, were cultured in LB liquid medium containing NaCl concentrations of 0.5, 2.5, 4.5, and 6.5 % for 5 days. On days 3, 4, and 5 biomass accumulation and the content of auxin-like compounds and siderophores in the culture medium were determined. Auxin-like compounds and siderophores were determined using a spectrophotometric method, while biomass was determined by weighing. It was found that the NaCl concentration affected both bacterial growth and the production of the studied metabolites; the highest biomass accumulation was observed at 4.5 % NaCl, whereas the addition of 6.5 % NaCl significantly inhibited culture growth. The highest production of siderophores was recorded at 4.5 % NaCl on days 4 and 5 of cultivation, whereas the highest levels of auxin-like compounds were predominantly observed at lower NaCl concentrations. The results obtained confirm the promise of further study of the biotechnological potential of *O. rhizosphaerae* IMV B-7956 bacteria.
Keywords: plant growth-promoting bacteria, phytohormones, siderophores, halotolerance.

Introduction. Auxins, particularly indole-3-acetic acid (IAA), play a key role in plant growth and development by the regulation of cell division, elongation, and root system

formation. Auxins are synthesized not only by plants but also by bacteria and are used in interactions with the plant or in the regulation of their own physiological processes (Etesami, 2024; Duca et al., 2020). The bacterial production of auxin-like compounds promotes plant growth, in particular by modifying the architecture of the root system and increasing nutrient absorption (Etesami, 2024; Poria et al., 2022). Siderophores are low-molecular-weight compounds with a high affinity for Fe^{3+}, synthesized primarily under conditions of available iron deficiency (Kramer, 2020). They facilitate the mobilization and absorption of iron by bacteria and may also enhance its availability to plants and inhibit the growth of phytopathogens (Cai et al., 2026). The synthesis of auxins and siderophores is an important component of bacteria's ability to stimulate plant growth. Bacteria of the genus *Ochrobactrum* are capable of synthesizing IAA and siderophores and stimulating plant growth (Yu et al., 2017). Study of the production of these metabolites by strains isolated from extreme environments contributes to the study of their adaptation mechanisms and the search for microorganisms with promising biotechnological properties.

The aim of this study was to evaluate the ability of *O. rhizosphaerae* IMV B-7956 bacteria, isolated from the leachate of the Lviv municipal solid waste landfill, to synthesize auxin-like compounds and siderophores under the influence of different concentrations of NaCl.

Methods. In this study *O. rhizosphaerae* IMV B-7956 bacteria were used. Bacteria were isolated at the Department of Microbiology of Ivan Franko National University of Lviv. The bacteria were cultured in Luria-Bertani (LB) liquid medium with a baseline concentration of NaCl 0.5 %, which was used as a control. The ability of bacteria to synthesize auxin-like compounds and siderophores was studied under the influence of concentrations of 2.5 %, 4.5 %, and 6.5 % NaCl. Bacteria were cultivated for 5 days under constant aeration. On days 3, 4 and 5 of growth culture liquid was collected and centrifuged (5,000 rpm for 10 min). The supernatant was used to determine auxin-like compounds and siderophores, and the bacterial cell pellet was used to determine biomass by the weight method. The content of auxin-like compounds was determined spectrophotometrically after mixing the supernatant with Salkowski's reagent in a 1:1 ratio and measuring the optical density at wavelength 536 nm. Siderophore production was determined spectrophotometrically after mixing the supernatant with Chrome Azurol S (CAS) reagent in a 1:1 ratio and measuring the optical density at 630 nm. The obtained values were used to calculate the concentrations of the studied metabolites and biomass. The results were processed and calculated using Microsoft Excel.

Results. NaCl concentration significantly affected the biomass accumulation of *O. rhizosphaerae* IMV B-7956 and the production of auxin-like compounds and siderophores. The highest biomass values were observed at 4.5 % NaCl (2.514, 2.814, and 2.443 mg/mL on days 3, 4, and 5, respectively), whereas at 6.5 % NaCl, biomass was the lowest – 0.714, 0.743, and 0.857 mg/mL on days 3, 4, and 5, respectively. In the control (0.5 % NaCl) biomass was 2.043 mg/mL on 3 day and decreased slightly to 1.814 mg/mL on days 4 and 5, whereas at 2. 5 % NaCl, a gradual decrease in biomass was observed from 2.514 to 2.086 mg/mL.

Thus, a moderate increase in salinity to 2.5–4.5 % did not inhibit the growth of the strain, and the greatest accumulation of biomass was observed at 4.5 % NaCl, whereas 6.5 % NaCl had a pronounced inhibitory effect.

The production of auxin-like compounds by *O. rhizosphaerae* IMV B-7956 also depended on the duration of cultivation and the NaCl concentration: maximum values on day 3 were observed at 0.5 % NaCl (5.85 μg/mL), whereas at 6.5 % salt their level was minimal (0.04 μg/mL). The highest production of auxin-like compounds was recorded at 0.5 % NaCl: 2.78 μg/mL on day 4 and 3.30 μg/mL on day 5 of growth. The siderophore content showed a different trend: on day 3, the highest value was observed under the influence of 2.5 % NaCl

(13.38 a.u.), on day 4 – at 4.5 % (17.57 a.u.), and on day 5– again at the 4.5 % NaCl (21.16 a.u.). Under the influence of 6.5 % NaCl, siderophore production remained the lowest throughout the entire study period.

Conclusions. The obtained results indicate that the bacterium *O. rhizosphaerae* IMV B-7956 is capable of producing auxin-like compounds and siderophores under the influence of NaCl.

References

1. Cai, J., Fang, Y., Liu, X., Adjei, M. O., & Fan, B. (2026). Revealing Microbial Siderophores: From Genes to Applications. Microorganisms, 14(2), 393. https://doi.org/10.3390/ microorganisms14020393
2. Duca, D. R., & Glick, B. R. (2020). Indole-3-acetic acid biosynthesis and its regulation in plant-associated bacteria. Applied microbiology and biotechnology, 104(20), 8607–8619. https://doi.org/10.1007/s00253-020-10869-5
3. Etesami, H., & Glick, B. R. (2024). Bacterial indole-3-acetic acid: A key regulator for plant growth, plant-microbe interactions, and agricultural adaptive resilience. Microbiological research, 281, 127602. https://doi.org/10.1016/j.micres.2024.127602
4. Kramer, J., Özkaya, Ö., & Kümmerli, R. (2020). Bacterial siderophores in community and host interactions. Nature reviews. Microbiology, 18(3), 152–163. https://doi.org/10.1038/s41579-019-0284-4
5. Poria, V., Dębiec-Andrzejewska, K., Fiodor, A., Lyzohub, M., Ajijah, N., Singh, S., & Pranaw, K. (2022). Plant Growth-Promoting Bacteria (PGPB) integrated phytotechnology: A sustainable approach for remediation of marginal lands. Frontiers in plant science, 13, 999866. https://doi.org/10.3389/fpls.2022.999866
6. Yu, X., Li, Y., Cui, Y., Liu, R., Li, Y., Chen, Q., Gu, Y., Zhao, K., Xiang, Q., Xu, K., & Zhang, X. (2017). An indoleacetic acid-producing Ochrobactrum sp. MGJ11 counteracts cadmium effect on soybean by promoting plant growth. Journal of applied microbiology, 122(4), 987–996. https://doi.org/10.1111/jam.13379

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OPTIMIZATION OF THE ISOLATION METHOD FOR EXOPOLYSACCHARIDES OF PROBIOTIC *BACILLUS VELEZENSIS* STRAINS

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Abstract. This study focused on the implementation and adaptation of new approaches for extracting exopolysaccharides (EPS) from the probiotic bacterial strains *Bacillus velezensis* UCM B-7242 and *B. velezensis* UCM B-7243. The primary objective of this research was to optimize the EPS isolation method by eliminating the conventional alcohol precipitation stage to achieve the highest possible yield and purity of the exopolysaccharides. In the course of the study, five sequential extraction stages were conducted and optimized, resulting in concentrated EPS samples with a significantly reduced content of co-extracted impurities.
Keywords: exopolysaccharides, ethanol-free extraction, probiotic strains, *Bacillus velezensis.*

Introduction. Exopolysaccharides produced by probiotic bacteria possess significant potential for biomedical applications, serving as an effective natural alternative to synthetic

therapeutics. These biopolymers exhibit potent biological properties, offering promising prospects for their use in the treatment of various diseases (Mouro et al., 2024).

Probiotic bacteria of the genus *Bacillus*, characterized by high biosynthetic activity, serve as valuable producers of EPS. However, obtaining a highly purified EPS preparation remains a challenging task that requires continuous optimization of methodological approaches. Conventional protocols for isolating bacterial EPS rely on precipitation with large volumes of chilled ethanol. However, the use of alcohol significantly increases production costs and introduces severe explosion hazards during process scaling. Furthermore, ethanol extraction non-selectively co-precipitates various matrix contaminants, complicating subsequent sample purification (Riyamol et al., 2026).

In view of the above, the **aim** of this study was to optimize the extraction method for exopolysaccharides from the probiotic strains *B. velezensis* UCM B-7242 and UCM B-7243 without using classical ethanol precipitation, followed by the determination of EPS concentration and extract purity profiles.

Methods and Materials. The probiotic strains *B. velezensis* UCM B-7242 and UCM B-7243 served as the objects of the study. Bacterial cultivation was carried out for 48 h at 37 °C in liquid nutrient medium containing glucose and a mixture of mineral salts as nutrients.

Cell-free supernatant was obtained by centrifuging the culture broth at 5.000 rpm for 30 min at 4 °C. Deproteinization was performed by adding 50 % trichloroacetic acid (TCA) to the supernatant, followed by incubation at 4 °C for 1.5 h. Protein precipitation was achieved by centrifugation at 5.000 rpm for 30 min. The deproteinized solution was neutralized to pH 7. Dialysis was performed against distilled water for 24 h using dialysis tubing with a molecular weight cut-off of 12–16 kDa. Following dialysis, the sample was additionnally centrifuged at 5.000 rpm for 15 min to remove the precipitate. To remove hydrophobic molecules, the solution was passed through an activated charcoal column. The final EPS concentration was performed by evaporating the purified solution using a rotary evaporator at 45 °C until a 5-fold reduction of the initial volume was achieved.

The EPS content in the concentrated sample was determined by the phenol-sulfuric acid method through spectrophotometric measurement of the OD_{490} of the reaction mixture. Concentration calculations were conducted using a calibration curve of D-glucose standards. Sample purity profiles were determined based on the OD_{260}/OD_{280} values of the unconcentrated extracts. Statistical data analysis was performed using Microsoft Excel.

Results and Discussion. As a result of this research, two concentrated EPS extracts were obtained from the cell-free culture supernatants of both studied strains. The EPS concentration in the extract from *B. velezensis* UCM B-7242 was 0.31 mg/mL, whereas for the UCM B-7243, it was 0.55 mg/mL. Accordingly, under the proposed isolation method, the specific EPS yield was 0.021 g/L for strain UCM B-7242 and 0.084 g/L for strain UCM B-7243. The obtained EPS yields are in agreement with global literature data for wild-type *Bacillus* strains cultivated on glucose substrates, which yields an average EPS concentration ranging from 0.05 to 0.2 g/L (Yuan et al., 2025).

The OD_{280} values for both extracts were 0.35, corresponding to the content of residual peptide impurities of no more than 0.005 % in the concentrated extracts. This result demonstrates the high efficiency of TCA-mediated deproteinization. However, the co-extracted nucleic acid background was significant, leading to an artificial overestimation of the OD_{280} value. The OD_{260} value for the unconcentrated extracts was 0.7, corresponding to a DNA and RNA content of approximately 0.016 % in the concentrated extracts. Consequently, the proposed EPS isolation method requires the inclusion of an additional nucleic acid removal step.

Given the physiological and metabolic traits of the used strains, the alcohol-free isolation method was structurally optimized and adapted to the specific technological challenges encountered during the experiments. In particular, an additional post-dialysis centrifugation step was introduced due to the coagulation of destabilized structural peptides. These protein molecules lost their solubility and formed aggregates as a result of a rapid decrease in the ionic strength of the solution. The critical evaporation stage also underwent optimization. To protect the EPS from thermal degradation, this process was carried out at a temperature not exceeding 45 °C, with a reduction in solution volume of no more than 5-fold. Additionally, a filtration step using activated charcoal was performed to remove hydrophobic impurities and pigments, as both strains proved to be active producers of riboflavin.

Conclusions:

1. An alcohol-free scheme for the isolation of EPS from *B. velezensis* strains UCM B-7242 and UCM B-7243 was implemented and optimized.

2. It was established that under the proposed alcohol-free isolation method, the specific EPS yield was 0.021 g/L for *B. velezensis* UCM B-7242 and 0.084 g/L for the UCM B-7243. The content of residual peptide components in the concentrated extracts was strictly limited to 0.005 %, while that of nucleic acids was 0.016 %.

3. The most significant results of the methodological optimization included the enhancement of the deproteinization stage, the determination of the optimal evaporation conditions, and the implementation of an adsorption purification step.

References:

1. Mouro, C., Gomes, A. P., & Gouveia, I.C. (2024). Microbial exopolysaccharides: Structure, diversity, applications, and future frontiers in sustainable functional materials. *Polysaccharides*, *5*(3), 241–287. https://doi.org/10.3390/polysaccharides5030018
2. Riyamol, Mittal, A., Ahmad, F., Haque, S., Chengaiyan, J. G., & Yadav, D. K. (2026). A review of extraction methods, downstream processing, and green recovery innovations of microbial extracellular polysaccharides. *International Journal of Biological Macromolecules*, *370*, 152873. https://doi.org/10.1016/j.ijbiomac.2026.152873
3. Yuan, J., Zong, M., Chen, J., et al. (2025). Whole genome sequencing analysis of a novel exopolysaccharide-producing *Bacillus velezensis* and its impact on the quality of soy sauce. *Food Chemistry: X*, *28*, 102585. https://doi.org/10.1016/j.fochx.2025.102585

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INFLUENCE OF STRESS-TOLERANT BACTERIA ON THE MORPHOMETRIC AND BIOCHEMICAL PARAMETERS OF SPRING WHEAT GROWN IN SOIL WITH COPPER(II) CHLORIDE

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Abstract. This study examines the influence of inoculating spring wheat (*Triticum aestivum* L., cv. Tybalt) seeds with stress-tolerant bacterial strains isolated from Antarctic substrates, Lviv

municipal solid waste landfill leachates, and war-affected soils on seedling growth under exposure to copper(II) chloride (300 mg/kg). Copper toxicity was shown to cause a 4.5-fold reduction in root length, a 15 % decrease in the *chl a*/*chl b* ratio, and an accumulation of flavonoids and proline in the leaves of non-bacterized seedlings. Inoculation of wheat seeds with the strains *Ochrobactrum* sp. K3, *Microbacterium* sp. EO1, *Rhodococcus* sp. 37, *Bacillus* sp. S304, *Pseudomonas* sp. EO2, and *Paenibacillus* sp. 5A-101 stimulated root growth of 14-day-old seedlings. Inoculation of seeds with all studied bacterial strains mitigated adverse effects in wheat seedling leaves: the *chl a*/*chl b* ratio and proline levels were restored to those of the metal-free control, whereas flavonoid content decreased compared with non-bacterized plants grown under copper(II) chloride influence. These results confirm the potential of using stress-tolerant plant growth-promoting bacteria to enhance wheat tolerance to copper-induced stress.

Keywords: chlorophyll, flavonoids, plant growth-promoting bacteria, proline, *Triticum aestivum* L.

Introduction. Soil contamination with heavy metal ions is a severe environmental problem that threatens the stability of agroecosystems and food safety. Heavy metal input into the environment primarily stems from industrial effluents, transport, as well as the intensive application of pesticides and mineral fertilizers in farming (Adrees et al., 2015; Briffa et al., 2020). The problem has become especially critical as a result of full-scale warfare: ammunition detonation, wildfires, and the destruction of military hardware result in severe soil contamination (Solokha et al., 2024; Krainiuk et al., 2025). Copper, derived from brass and bronze projectile components, explosives, and anticorrosion coatings, enters the soil as Cu^{2+} ions, accumulating persistently in topsoil layers (Krainiuk et al., 2025). An excess of Cu^{2+} ions induces phytotoxic effects, leading to root growth inhibition, photosynthetic apparatus impairment, and oxidative stress (Adrees et al., 2015). A promising, eco-friendly approach to enhancing crop stress tolerance and mitigating the negative impact of metals is the application of plant growth-promoting bacteria isolated from extreme environments.

This study aimed to evaluate how seed bacterization with stress-tolerant bacterial strains affects the morphometric parameters, photosynthetic pigment levels, non-enzymatic antioxidant content, and osmoprotectant accumulation in spring wheat (*Triticum aestivum* L., cv. Tybalt) during early ontogenesis under exposure to copper(II) chloride.

Methods. Bacterial strains isolated from extreme habitats at the Department of Microbiology of Ivan Franko National University of Lviv during 2020–2024 were used in this study: *Ochrobactrum* sp. K3 isolated from the leachate of the Lviv municipal solid waste landfill; *Microbacterium* sp. EO1, *Pseudomonas* sp. 3B-In-57, *Lysinibacillus* sp. Km8, *Bacillus* sp. RT1, *Arthrobacter* sp. E35, and *Paenibacillus* sp. 5A-101 from various Antarctic substrates collected during the 23^{th}–27^{th} Ukrainian Antarctic Expeditions; and *Streptomyces* sp. S253 and *Bacillus* sp. S304 from soil samples damaged by military actions. Bacteria were grown in Luria–Bertani broth for 2 days, harvested by centrifugation (6000 rpm, 10 min), and resuspended in 0.9 % NaCl solution to a density of 5.0 McFarland standard. Wheat seeds were inoculated by soaking in the bacterial suspension for 18 h and subsequently sown in prepared soil. Copper(II) chloride was applied to the soil as a solution to achieve a final concentration of 300 mg/kg. Wheat seeds soaked in 0.9 % NaCl and grown in soil with or without $CuCl_2 \cdot 2H_2O$ served as controls. After 14 days of growth, root and shoot lengths, photosynthetic pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll, and *chl a*/*chl b* ratio), alongside levels of phenolic compounds, flavonoids, and proline in leaves, were measured.

Results. In non-bacterized 14-day-old wheat seedlings, copper accumulation in leaves reached up to 20 mg/kg of dry weight during growth in soil with copper(II) chloride.

Copper(II) chloride exposure caused substantial alterations in the morphometric and biochemical parameters of non-bacterized wheat seedlings. The root length of non-bacterized seedlings exposed to copper(II) chloride was 4.5-fold shorter compared to uninoculated control plants grown in metal-free soil. Copper(II) chloride exposure increased total chlorophyll content in the leaves of non-bacterized seedlings, accompanied by a 15 % reduction in the chl a/chl b ratio, a twofold increase in flavonoid content, and a 30 % increase in proline accumulation compared to plants grown in unamended soil.

Accumulation of Cu in leaves of 14-day-old seedlings was lower following seed inoculation with *Paenibacillus* sp. 5A-101 and *Ochrobactrum* sp. K3, while other evaluated strains did not significantly alter leaf Cu levels compared to the non-bacterized control under copper(II) chloride exposure. Seedlings inoculated with *Ochrobactrum* sp. K3, *Microbacterium* sp. EO1, *Rhodococcus* sp. 37, *Bacillus* sp. S304, *Pseudomonas* sp. EO2, or *Paenibacillus* sp. 5A-101 and grown in copper(II) chloride-amended soil exhibited longer roots than non-bacterized control plants exposed to copper(II) chloride. Seed inoculation with all tested bacterial strains led to a lower leaf chlorophyll content in 14-day-old seedlings compared to non-bacterized plants under copper(II) chloride exposure; nevertheless, the *chl a*/*chl b* ratio remained comparable to that of non-bacterized seedlings grown in metal-free soil. Under copper(II) chloride exposure, the leaf flavonoid content in bacterized seedlings was lower than that in non-bacterized seedlings. Proline content in the leaves of bacterized seedlings was lower compared to that in non-bacterized seedlings grown under copper(II) chloride exposure, and did not differ from levels in non-bacterized plants grown in metal-free soil.

Conclusions. Inoculation of wheat seeds with the stress-tolerant bacterial strains promoted plant development at early developmental stages, positively modulating both morphometric traits and physiological status under copper(II) chloride exposure.

This study was conducted under the State Target Scientific and Technical Research Program in Antarctica for 2011–2025 and within the project “Development of an approach to restore soil fertility in soils affected by hostilities using microbial biological preparations”, state registration No. 0124U000931.

References

1. Adrees, M., Ali, S., Rizwan, M., Ibrahim, M., Abbas, F., Farid, M., Zia-ur-Rehman, M., Irshad, M. K., & Bharwana, S. A. (2015). The effect of excess copper on growth and physiology of important food crops: a review. Environmental Science and Pollution Research, 22(11), 8148–8162. https://doi.org/10.1007/s11356-015-4496-5
2. Briffa, J., Sinagra, E., & Blundell, R. (2020). Heavy metal pollution in the environment and their toxicological effects on humans. Heliyon, 6(9), e04691. https://doi.org/10.1016/j.heliyon.2020.e04691
3. Krainiuk, O. V., Buts, Y. V., Ponomarenko, R. V., Asotskyi, V. V., Darmofal, Eleonora A., Kalynovskyi, A. Y., & Maniuk, V. V. (2025). Environmental consequences of military operations in Ukraine on the example of soil research in the Kharkiv region. Journal of Geology, Geography and Geoecology, 34(2), 304–317. https://doi.org/10.15421/112526
4. Solokha, M., Demyanyuk, O., Symochko, L., Mazur, S., Vynokurova, N., Sementsova, K., & Mariychuk, R. (2024). Soil Degradation and Contamination Due to Armed Conflict in Ukraine. Land, 13(10), 1614. https://doi.org/10.3390/land13101614

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PRELIMINARY SCREENING OF RHIZOSPHERE MICROORGANISMS FOR THEIR PLANT GROWTH-PROMOTING ACTIVITIES AND STRESS TOLERANCE

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Abstract. Among the 65 microbial isolates obtained from the rhizosphere of soybean and black locust, 35 were able to grow on N-free Ashby's agar, Pikovskaya's agar, or both, indicating their potential for nitrogen fixation and phosphorus mobilization. Three of these isolates formed clearing zones around their colonies, with an average width of 5 mm. Five isolates demonstrated tolerance to 0.5 M NaCl and one month of desiccation. Two of them (one from the soybean rhizosphere and the other from the black locust rhizosphere) produced exometabolites that increased soybean seedling weight and length by 46 % and 19 %, respectively, when used to treat the seeds, indicating the agronomic potential of these rhizobacteria under adverse environmental conditions.
Keywords: rhizosphere, microorganisms, legumes, plant growth-promoting activities, stress tolerance.

Plant growth-promoting rhizosphere microorganisms play an important role in maintaining soil quality and enhancing plant development. Rhizobacteria ensure optimal plant nutrition, stimulate growth through the synthesis of phytohormones, regulate various physiological processes, and contribute to the suppression of phytopathogens in the root zone by secreting compounds with antibiotic activity etc (Ajeethan et al., 2026). The introduction of microorganisms with multiple plant growth-promoting activities into the plant rhizosphere to enhance crop growth and productivity is one of the effective tools in sustainable agriculture (Prisa et al., 2023). The negative impact of global climate change on the yield of crop plants vital to humanity (Alotaibi, 2023) has intensified the need to search for new, effective strains of plant growth-promoting rhizobacteria that are resistant to environmental stressors and to further incorporate them into agricultural practice as components of microbial biofertilizers. Numerous studies show that rhizosphere microorganisms can mitigate the harmful effects of abiotic and biotic stresses, particularly soil salinity and drought, which are closely associated with climate change, thereby improving plant health (Fiodor et al., 2021).

The aim of this study was to perform a preliminary characterization of microorganisms isolated from the rhizosphere of soybean and black locust. The potential for nitrogen fixation and phosphate solubilization in the tested microbial isolates as well as their tolerance to salt stress and one-month desiccation were evaluated. In addition, we investigated the effect of exometabolites produced by the selected rhizosphere bacteria on soybean seed germination and seedling growth to assess their overall plant growth-promoting activity.

Aliquots of 0,01 mL of suspensions of pure microbial cultures isolated from the rhizosphere of soybean (*Glycine max* (L.) Merr.) and black locust (*Robinia pseudoacacia* L.) were applied onto the surface of Ashby's agar, Pikovskaya's agar, and yeast extract–mannitol agar (YEM) to assess their ability to grow on these media. To evaluate the resilience of rhizosphere isolates to salinity, they were grown in YEM broth supplemented with 0.1 M, 0.3 M, and 0.5 M NaCl. Desiccation tolerance was determined accordingly to Farrow et. al.

(2018) with modifications. The number of viable microbial cells that formed colonies on agar growth medium after one month of desiccation at 28 °C was calculated. In plant experiments, soybean seeds (cv. Almaz) were treated with the supernatants of the selected microbial cultures grown in YEM broth to the stationary phase and incubated for six days at 24 °C. The germination rate of the seeds and the weight and length of the seedlings were determined.

The study involved 65 bacterial isolates from the rhizosphere of soybean and black locust maintained in the microbial collection of the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine (NASU). Of these, 35 isolates showed good growth on N-free Ashby's agar, Pikovskaya's agar, or both, thereby demonstrating their ability to fix nitrogen and mobilize phosphorus from insoluble phosphates. All isolates were fast-growing microorganisms that grew well on YEM medium. They form either convex and mucoid or small and rough colonies with a diameter of 2–3 mm. A prominent clear zone was observed around the colonies of only three tested strains on Pikovskaya's agar. The width of the clear zone was about 5 mm. Evaluation of the salt tolerance of the microorganisms demonstrated that five isolates exhibited good growth in YEM broth supplemented with 0.1 M, 0.2 M, and 0.5 M NaCl after 48 h of cultivation. These isolates were also tolerant to desiccation. When the microbial cultures were subjected to desiccation for one month, the number of viable cells decreased by an average of 35 %, demonstrating their high tolerance to prolonged drying (Farrow et. al, 2018). In contrast, four isolates were sensitive to desiccation. No viable cells were detected after six days of desiccation. Rhizobacteria tolerant to salt stress and desiccation were selected to evaluate their ability to improve soybean seed germination and seedling development. None of the isolates significantly affected seed germination. However, two isolates (one from the soybean rhizosphere and the other from the black locust rhizosphere) produced exometabolites that increased the weight and length of soybean seedlings by 46 % and 19 % respectively.

Thus, two promising strains of rhizosphere microorganisms were selected during our research. They demonstrated tolerance to desiccation and high salt concentration as well as the ability to solubilize inorganic phosphate. However, further studies are required to examine their growth-promoting activity in detail and to assess the feasibility of using them in the development of microbial biofertilizers.

References

1. Ajeethan, N., Abbey, L., Yurgel, S. N. (2026). Role of Plant Growth-Promoting Microbes in Plant Growth and Development. *Applied Microbiology*, *6 (2)*. 34. doi:10.3390/applmicrobiol6020034.
2. Alotaibi, M. (2023). Climate change, its impact on crop production, challenges, and possible solutions. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 51(1).* 13020. Doi: 10.15835/nbha51113020.
3. Farrow, J. M., Wells, G., Pesci, E. C. (2018). Desiccation tolerance in Acinetobacter baumannii is mediated by the two-component response regulator BfmR. *PLoS ONE, 13(10).* E0205638. Doi: 10.1371/journal.pone.0205638.
4. Fiodor, A., Singh, S., Pranaw, K. (2021). The Contrivance of Plant Growth Promoting Microbes to Mitigate Climate Change impact in Agriculture. *Microorganisms*, *9*. 1841. Doi: 10.3390/microorganisms9091841.
5. Prisa, D., Fresco, R., Spagnuolo, D. (2023). Microbial Biofertilisers in Plant Production and Resistance: a Review. *Agriculture, 13*. 1666. Doi: 10.3390/agriculture13091666.

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ENGINEERING GLYCEROL UTILIZATION IN *CANDIDA FAMATA* FOR SUSTAINABLE RIBOFLAVIN PRODUCTION USING CRUDE GLYCEROL

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Abstract. *Candida famata* is a flavinogenic non-conventional yeast with a high capacity for riboflavin (vitamin B_2) production and represents a promising microbial cell factory. Glycerol, particularly crude glycerol generated as a by-product of biodiesel production, is an attractive low-cost carbon source for microbial fermentation. However, its use for riboflavin production by *C. famata* has not previously been investigated. In the present study, glycerol utilization was investigated in the riboflavin-producing *C. famata* BRPI strain by targeted overexpression of *STL1*, encoding a glycerol transporter, and *GUT2*, encoding a mitochondrial glycerol-3-phosphate dehydrogenase. Engineered strains were evaluated during cultivation on refined and crude glycerol to assess the potential of genetic modification of glycerol uptake and metabolism for improving riboflavin production and valorizing crude glycerol as a low-cost industrial by-product.
Keywords: *Candida famata*; riboflavin; glycerol utilization; crude glycerol; metabolic engineering.

Candida famata is a flavinogenic yeast capable of riboflavin (vitamin B_2) overproduction and represents a promising platform for the development of microbial cell factories. Riboflavin is an essential vitamin widely used in the food, pharmaceutical and feed industries, while microbial fermentation provides an efficient alternative to chemical synthesis. The economic feasibility of microbial production depends not only on the productivity of the production strain but also on the cost and availability of the carbon source. Therefore, the utilization of inexpensive and renewable substrates is an important direction in the development of sustainable biotechnological processes.

Glycerol is an attractive alternative carbon source due to its increasing availability as a major by-product of biodiesel production. In particular, crude glycerol represents a potentially valuable low-cost feedstock for microbial fermentation. Unlike refined glycerol, crude glycerol contains variable amounts of residual compounds originating from the biodiesel production process, including methanol, water, inorganic salts, soaps, ash, free fatty acids, residual glycerides, fatty acid methyl esters and catalyst-derived compounds. The composition of crude glycerol depends on the feedstock and processing conditions, and its impurities may affect microbial growth, metabolism and product formation. Crude glycerol has already been investigated as a substrate for the microbial production of various value-added compounds, including 1,3-propanediol, organic acids, ethanol and polyhydroxyalkanoates (Almeida et al., 2012). For example, *Ashbya gossypii* has been used for the production of orotic acid from crude glycerol (Silva et al., 2022). However, despite the use of crude glycerol in various microbial processes, its application for riboflavin production has received little attention. Although unpublished results have indicated that mutant strains of *C. famata* can overproduce riboflavin from glycerol, the production of riboflavin by *C. famata* using crude glycerol as a carbon source has not previously been investigated.

Glycerol utilization in yeasts involves several consecutive steps, including glycerol uptake, phosphorylation and subsequent oxidation of glycerol-derived metabolites. In *Saccharomyces cerevisiae*, the major glycerol utilization pathway involves *STL1*, encoding a

glycerol/H^+ symporter, *GUT1*, encoding glycerol kinase, and *GUT2*, encoding a mitochondrial glycerol-3-phosphate dehydrogenase. Glycerol is transported into the cell by Stl1p, phosphorylated by Gut1p to glycerol-3-phosphate and subsequently converted by Gut2p to dihydroxyacetone phosphate (DHAP), which can enter central carbon metabolism. The importance of these genes for growth on glycerol has been demonstrated in other yeast species. In *S. cerevisiae*, deletion of *STL1*, *GUT1* or *GUT2* resulted in the loss of growth on glycerol as the sole carbon source, confirming the essential role of this pathway (Swinnen et al., 2013). Similarly, *STL1* from *Candida albicans* has been shown to encode a functional glycerol transporter, and expression of *C. albicans STL1* restored the ability of an *S. cerevisiae stl1Δ* mutant to grow on minimal glycerol medium (Kayingo et al., 2009). These findings provided a rationale for investigating glycerol transport and metabolism as potential targets for improving glycerol utilization in *C. famata*.

In the present study, glycerol utilization was investigated in the riboflavin-producing *C. famata* BRPI strain (Dmytruk et al., 2020) through targeted genetic engineering of components involved in glycerol uptake and metabolism. The *STL1* gene, encoding a glycerol transporter, and the *GUT2* gene, encoding a mitochondrial glycerol-3-phosphate dehydrogenase, were introduced into the production background to investigate their contribution to glycerol utilization and riboflavin biosynthesis. The engineered strains were evaluated in fermentation experiments using 5 % (v/v) refined glycerol and 5 % (v/v) crude glycerol, allowing their performance to be compared on substrates differing in purity and composition.

Overexpression of *STL1* increased riboflavin production 1.48-fold on 5 % (v/v) refined glycerol and 1.36-fold on 5 % (v/v) crude glycerol compared with the parental BRPI strain. Similarly, *GUT2* overexpression increased riboflavin production 1.65-fold and 1.42-fold on refined and crude glycerol, respectively. These results indicate that targeting both glycerol uptake and intracellular glycerol metabolism can positively affect riboflavin production by *C. famata*. The differences observed between refined and crude glycerol highlight the potential influence of substrate composition on the performance of engineered strains and demonstrate that crude glycerol can support enhanced riboflavin production despite its variable composition.

Overall, this study combines metabolic engineering of glycerol utilization with the valorization of crude glycerol as an industrial by-product. The demonstrated effects of *STL1* and *GUT2* overexpression support glycerol transport and intracellular metabolism as promising targets for improving riboflavin production in *C. famata*. The use of crude glycerol as a low-cost carbon source may contribute to the development of more sustainable and economically attractive microbial processes for riboflavin production. Further studies are currently underway to investigate the effect of *GUT1* overexpression, which encodes glycerol kinase catalyzing the first enzymatic step in glycerol utilization, on glycerol metabolism and riboflavin production.

References:

1. Almeida, J. R. M., Fávaro, L. C. L., & Quirino, B. F. (2012). Biodiesel biorefinery: Opportunities and challenges for microbial production of fuels and chemicals from glycerol waste. Biotechnology for Biofuels, 5, Article 48. https://doi.org/10.1186/1754-6834-5-48.
2. Dmytruk, K. V., Ruchala, J., Fedorovych, D. V., Ostapiv, R. D., & Sibirny, A. A. (2020). Modulation of the purine pathway for riboflavin production in flavinogenic recombinant strain of the yeast Candida famata. Biotechnology Journal, 15(1), Article 1900468. https://doi.org/10.1002/biot.201900468.
3. Kayingo, G., Martins, A., Andrie, R., Neves, L., Lucas, C., & Wong, B. (2009). A permease encoded by STL1 is required for active glycerol uptake by Candida albicans. Microbiology, 155(5), 1547–1557. https://doi.org/10.1099/mic.0.023457-0.
4. Silva, R., Aguiar, T. Q., & Domingues, L. (2022). Orotic acid production from crude glycerol by engineered Ashbya gossypii. Bioresource Technology Reports, 17, Article 100992. https://doi.org/10.1016/j.biteb.2022.100992.

5. Swinnen, S., Klein, M., Carrillo, M., McInnes, J., Nguyen, H. T. T., & Nevoigt, E. (2013). Reevaluation of glycerol utilization in Saccharomyces cerevisiae: Characterization of an isolate that grows on glycerol without supporting supplements. Biotechnology for Biofuels, 6, Article 157. https://doi.org/10.1186/1754-6834-6-157

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SCREENING FOR TECHNOLOGICAL PROPERTIES OF CORYNEFORM BACTERIA PROMISING IN SMEAR-RIPENED CHEESE PRODUCTION

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Abstract. The technological properties (psychrotrophicity, halotolerance, and acid tolerance) of coryneform bacteria strains belonging to the genera *Arthrobacter*, *Brevibacterium*, *Corynebacterium*, and *Brachybacterium*, isolated mainly from milk and cheese, were investigated. Based on a combination of features (ability to grow at 12–15 °C, in the presence of 3–7 % NaCl, and at pH 5.5–7.0), *Brevibacterium* and *Corynebacterium* strains promising for use in smear-ripened cheese production were selected.
Keywords: coryneform bacteria, technological properties, smear-ripened cheeses.

Coryneform bacteria play an important role in the production of smear-ripened cheeses. The characteristic taste, aroma, soft texture, and edible orange smear rind of these cheeses are formed as a result of the activity of a complex surface microbiome consisting mainly of coryneform bacteria, including *Brevibacterium*, *Corynebacterium*, *Arthrobacter*, *Glutamicibacter*, *Microbacterium* and several other genera, as well as salt-tolerant yeasts (Ritschard & Schuppler, 2024). The specific ripening conditions and the biochemical and functional potential of the aerobic smear microbiota contribute to the high nutritional and biological value of these products. The ripening process of such cheeses is controlled by regular surface treatment with a brine solution inoculated with *Brevibacterium linens*, which may be supplemented by other coryneform bacteria (particularly *Corynebacterium casei* and *Arthrobacter* spp.) as well as the yeast *Debaryomyces hansenii* (Bockelmann et al., 2003). Because smear-ripened cheeses ripen at low temperatures, high salt concentrations, and moderately acidic initial pH values, pronounced psychrotrophic capacity, halotolerance, and acid tolerance are among the principal technological characteristics required of coryneform bacteria used as adjunct starter cultures.

The production of smear-ripened cheeses is mainly concentrated at small craft creameries, which actively continue their development in Ukraine despite the military action (Peshuk et al., 2025). Given that the current ripening technology for these cheeses relies on imported commercial *B. linens* starter cultures used either as monocultures or as components of multistrain starter preparations, the selection of domestic *B. linens* strains is very promising. This will allow the creation of unique, high-quality ethnic cheeses with authentic flavour profiles that can compete on the foreign market.

The purpose of this study was to screen strains of coryneform bacteria from the Ukrainian Collection of Microorganisms (UCM) whose technological properties meet the requirements for adjunct cultures used in the production of smear-ripened cheeses.

The study included 43 collection strains of saprophytic coryneform bacteria of the genera *Brevibacterium*, *Arthrobacter*, *Corynebacterium*, and *Brachybacterium* isolated from milk and dairy products. The cultures were maintained on Corynebacterium agar medium

(https://www.dsmz.de/?id=441). Strain tolerance to temperature, NaCl concentration, and initial medium pH was examined in the same medium without agar. Strain growth was evaluated after 14 days of incubation by measuring the optical density of the culture broth at 540 nm using a KFK-2MP photoelectric colorimeter; medium pH was measured with an I-160MI laboratory ion meter. Uninoculated medium served as the control. The ability of the strains to grow at different temperatures was examined at 12–37 °C; osmotolerance was evaluated at NaCl concentrations of 3–15 %; and tolerance to active medium acidity was determined in the initial pH range of 4.5–7.0. It was established that the examined strains exhibited significant thermotolerance: all of them grew at 25–28 °C, 79.0 % were capable of growth at 12–15 °C, 88.4 % at 37 °C, and 83.7 % at 42 °C. The coryneform bacteria showed a high degree of salt tolerance: all strains grew at NaCl concentrations of 3–5 %, the vast majority (91 %) were resistant to 7 % NaCl, and a significant proportion (65 %) to 10 % NaCl. The highest NaCl tolerance was observed in 53 % of the strains belonging to *B. aurantiacum*, *B. linens*, and several *Corynebacterium sp.* which grew actively at a 15 % NaCl concentration. Assessment of growth capacity at different initial pH values showed that all strains grew efficiently at pH 6.5–7.0, whereas 97 % demonstrated growth at pH 6.0. A majority of the strains (79 %) were resistant to pH 5.5, but only 21 % retained viability at pH 5.0. None of the strains grew at pH 4.5.

The study resulted in the selection of coryneform bacteria strains belonging to the genera *Brevibacterium* and *Corynebacterium* that met the main technological requirements for their use as adjunct cultures in the production of smear-ripened cheeses.

References

1. Bockelmann, W., Willems, P., Rademaker, J., Noordman, W. & Heller, K. J. (2003). Cultures for the surface ripening of smeared soft cheeses. *Kieler Milchwirtschaftliche Forschungsberichte*, 55 (4), 277–299.
2. Ritschard, J. S., & Schuppler, M. (2024). The microbial diversity on the surface of smear-ripened cheeses and its impact on cheese quality and safety. *Foods*, 13(2), 214. https://doi.org/10.3390/foods13020214.
3. Peshuk, L. V., Kudryk, N. A., Prykhodko, D. Y. (2025). Trends in the development of craft cheese production – integration into the sustainable food system. Journal of Chemistry and Technologies, 33(3), 714–727.

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VITASERGIA SVIDASOMA IMV F-100106 – A NOVEL ENDOPHYTIC YEAST FROM *TUBER MELANOSPORUM* AND ITS USE IN SUSTAINABLE AGRICULTURE AND RESTORATION OF MILITARY-DEGRADED SOILS

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Abstract A novel endophytic yeast fungus, *Vitasergia svidasoma* IMV F-100106 (PRJNA807518), belonging to the family *Debaryomycetaceae*, was isolated from the fruiting body of *Tuber*

melanosporum. The complete genome of the species is 11.195 Mb in size and consists of eight chromosomes and one mitochondrial genome. *Vitasergia svidasoma* IMV F-100106 is an active component of the soil biological preparation Mykovital (Oliferchuk et al., 2022; Oliferchuk et al., 2023a; Oliferchuk et al., 2023b). Its application in tomato cultivation technologies contributed to improved plant growth, increased yield and fruit quality, accelerated fruit ripening in the absence of mineral fertilizers, and stimulated the development of mycorrhizal symbiosis. An important practical application of *V. svidasoma* was its use in combination with biochar to facilitate the establishment of *Amaranthus tricolor* in an area damaged by artillery shelling. This technology demonstrated the potential of the endophytic yeast fungus for restoring vegetation cover and regulating microbiological processes in soils degraded by military activities.The results obtained demonstrate the considerable potential of *Vitasergia svidasoma* IMV F-100106 as a biological agent for sustainable agriculture, soil microbiome regulation, stimulation of mycorrhizal interactions, and biological remediation of ecosystems disturbed by anthropogenic and military impacts.
Keywords: *Vitasergia svidasoma* IMV F-100106 (PRJNA807518); mycorrhizal symbiosis; organic farming; soil mycobiome; military-degraded soils.

Relevance. The relevance of this study is driven by global climate change, increasing anthropogenic pressure on ecosystems, and large-scale soil degradation caused, in particular, by military activities in Ukraine. The restoration of soil fertility and ecosystem functions through the use of effective and environmentally safe biological technologies is becoming increasingly important. Microorganisms, particularly endophytic yeasts, are promising biological agents capable of promoting plant growth, enhancing mycorrhizal interactions, and regulating microbiological processes in soil. This study summarizes the genomic characteristics of V. svidasoma and the results of its application for improving tomato productivity, regulating the soil mycobiome, and restoring soils degraded as a result of military activities.

Materials and Methods. *V. svidasoma* was isolated from surface-sterilized fruiting bodies of *Tuber melanosporum* using Malt Extract Agar (MEA). The genome was analyzed using hybrid sequencing on the Illumina NovaSeq and Oxford Nanopore MinION platforms; genome assembly was performed using Flye, followed by assembly polishing with Medaka/Pilon and genome annotation using MOSGA/BRAKER. Field trials with tomato were conducted in the Vinnytsia region without the application of mineral fertilizers. Mykovital was applied through drip irrigation at rates of 1.0 and 1.5 L/ha; untreated plants served as the control, while MikePro PS3 was used as the reference preparation.The restoration of artillery-damaged soil was studied in the village of Torfiane, Kyiv region. *V. svidasoma* immobilized on biochar was applied as part of a soil restoration system that included organic substrates and the Rozum warm-bed technology. Six months after treatment, microscopic fungal abundance, species richness, and diversity were assessed. Statistical analysis was performed using two-way analysis of variance (two-way ANOVA).

Results. The complete T2T genome of *V. svidasoma* IMV F-100106 comprised eight chromosomes and a mitochondrial genome (52,458 bp), with a total size of 11.195 Mbp and GC content of 55.05 %. Genome annotation identified 4,452 protein-coding sequences, 83 tRNA genes, and 165 rRNA genes. Whole-genome phylogeny revealed no close genus-level relatives, supporting the assignment of the strain to the novel genus *Vitasergia*. The genome is deposited in GenBank (CP095433.1–CP095441.1; PRJNA807518). Application of Mykovital at 1.0–1.5 L/ha increased tomato plant density by 8.1–10.4 %, height by 13.2–14.3 %, mean fruit weight by 11.4–13.4 %, and yield by 11.1–12.1 % compared with the control. Fruit ripening began 12–14 days earlier, while treated plants developed stronger root systems and more pronounced mycorrhization.Mykovital application shifted the soil mycobiome toward ectomycorrhizal and beneficial fungi and reduced the contribution of pathogenic components. In military-degraded soil, six months after application of Mykovital + biochar, micromycete abundance reached 107–142·10⁴ CFU/g compared with 17–57·10⁴ CFU/g in

the control. Species richness and Shannon–Weaver diversity were also higher. *Amaranthus tricolor* reached approximately 1 m in the treated area compared with only 5 cm in the control.

Conclusions. *Vitasergia svidasoma* IMV F-100106 (PRJNA807518) is a novel endophytic yeast of the family *Debaryomycetaceae*, isolated from *Tuber melanosporum*. It possesses a complete T2T genome of 11.195 Mbp (PRJNA807518) and has no close relatives at the genus level. As the active agent of the Mykovital preparation, *V. svidasoma* promotes tomato growth and mycorrhizal development, increases yield (+11–12 %) and fruit quality, and accelerates fruit ripening. Application of *V. svidasoma* promotes the formation of a soil mycobiome dominated by mycorrhizal and beneficial fungi, with a reduced proportion of pathogenic components, creating conditions for reduced agrochemical use.In combination with biochar and organic mulch, Mykovital promotes the restoration of micromycete abundance and α-diversity in artillery-damaged soil and supports vigorous plant establishment, demonstrating the potential of this biotechnology for the rehabilitation of lands degraded by military activities.

References

1. Oliferchuk, V. P., Fedorovych, D. V., Samborskyi, M. V., & Samarska, M. I. (2023a). Influence on the soil metagenome of a new species of endophyte *Vitasergia svidasoma* VS 1223 (IMV F-100106) isolated from black truffle. *Bulletin of Sumy National Agrarian University. The Series: Agronomy and Biology*, (1), 79-89. https://doi.org/10.32782/agrobio.2023.1.10
2. Oliferchuk, V., Kendzora, N., Shukel, I., Samarska, M., & Olejniuk-Puchniak, O. (2023b). The role of V-strategist endophytes in stimulating the formation of mycorrhizal interactions and soil regeneration. In E. C. Rigobelo (Ed.), *Symbiosis in nature*. IntechOpen. https://doi.org/10.5772/intechopen.109912
3. Oliferchuk, V., Fedorovych, D., Samarska, M., Bunetskyi, V., Samborskyy, M., Kachor, A., Kurylenko, O., Olejniuk-Puchniak, O., Kendzora, N., & Hotsii, N. (2022). Changes in the structure of myco- and microbiocenosis of soil with use of fungi and bacteria strains immobilized on biochar as an example of ecosystem maintenance services. *Ecological Engineering & Environmental Technology, 23*(6), 42-52. https://doi.org/10.12912/27197050/152522

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EFFECT OF CO-CULTIVATION OF LACTIC ACID BACTERIA AND YEAST ON ACIDITY LEVEL OF FERMENTED PUMPKIN JUICE

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Abstract. The study investigates the ability of *Lactiplantibacillus plantarum* strains and yeast to ferment pumpkin juice as monocultures and their combinations. The acidity level is an indicator of active microbial growth and is one of the key organoleptic characteristics. The lactic acid bacteria (LAB) and yeast strains differed in their acid production levels during juice fermentation. Combinations of LAB and yeast strains were selected based on the higher pH values and titrable activity levels compared to corresponding monocultures.
Keywords*:* lactic acid bacteria, yeast, coculture fermentation, pumpkin juice, acidity.

Fermentation is a long-standing practice in the food industry, which improves the sensory qualities and enriches the nutritional composition of foods (Shah et al., 2023). It is well-known that production of organic acids by LAB during fermentation leads to a decrease in pH values and inhibition of unfavourable microbiota growth. Furthermore, LAB strains can synthesise bacteriocins that also play the role of biopreservatives of food products (Anumudu et al., 2024). The interaction between lactic acid bacteria and yeast has long been a focus of researchers' attention. It was shown that a synergistic interaction between yeast and LAB results in unique flavour profiles and increased acid production, which shortens the time to reach the desired acidity level and obtain the end product (Fang et al., 2024). In addition, the positive health effect of consuming foods containing lactic acid bacteria and yeast strains with probiotic potential has been reported (Tullio, 2024). Pumpkin is one of the most important agricultural crops in Ukraine, which contains a variety of biologically active compounds, minerals and dietary fibre (Batool et al., 2022; Gavril et al., 2024), So, pumpkin serves as an excellent matrix for the development of food fermentation biotechnology.

The aim of the work was to investigate the effect of cocultivation of LAB and yeast strains on the level of acid production during fermentation of pumpkin juice and to select the most effective strain combinations.

LAB and yeast strains of the Ukrainian Collection of Microorganisms (UCM) and the Working Collection of the Department of Physiology of Industrial Microorganisms (D. K. Zabolotny Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine) were used. Among them, 6 strains of *Lactiplantibacillus plantarum* species and *Leuconostoc sp* UCM B-2947 strain, 35 strains of *Saccharomyces cerevisiae*, 11 strains of *Kluyveromyces marxianus*, 2 strains of *Wickerhamomyces anomalus*, *S. uvarum* FF20, *Kluveromyces lactis* 327, *Yarrowia lipolytica* FF21 and *Y. lipolytica* FF24 strains. LAB and yeast were grown for 24 h in MRS medium at 37 °C and in YPD medium at 30 °C, respectively. Monocultures and strain compositions (in equal proportions) were used for inoculation of sterilised pumpkin juice. All samples were incubated for 24 h at 30 °C. Uninoculated pumpkin juice was used as a control. Monitoring of the fermentation process and selection of promising strains were carried out based on the results of pH value measurement and the total titrable acidity (expressed in Terner Degrees, °T) determination. The results are presented as M ± SD, where M is the mean and SD is the standard deviation.

Initial pH values of pumpkin juice ranged from 5.19 to 5.99, and titrated acidity ranged from 3 °T to 5 °T, depending on the raw material used. Among LAB monocultures, the highest acidification was found in juices fermented with *L. plantarum* УКМ B-2627 (pH 4.31 ± 0.30, 9.66 ± 1.15 °T), *L. plantarum* IMV B-7565 (pH 4.29 ± 0.20, 10.50 ± 86 °T), *L. plantarum* IMV B-7598 (pH 4.42 ± 0.33, 10.16 ± 0.35°T) and *L. plantarum* KM-4 (pH 4.22 ± 0.15, 10.25 ± 0.35 °T) strains, which showed a significant difference from the results of the control unfermented sample of pumpkin juice (pH 5.65 ± 0.23 and 4.10 ± 1.02 °T). Among the yeast, *K. marxianus* F21 (pH 4.57 ± 0.56, 13.00 ± 1.41 °T), *Y. lipolytica* FF21 (pH 5.12 ± 1.36, 2.75 ± 0.35 °T), and *S. cerevisiae* UCM Y-2519 (pH 4.52 ± 0.37, 12.00 ± 2.82 °T) strains characterized by a high level of acid production.

Pairwise combinations were formed between the most effective *L. plantarum* and yeast strains. No direct antagonism was observed in any combination, confirming their compatibility during fermentation. There was no statistically significant difference between the acidity values of 8 out of 12 tested combinations and juice samples obtained with a monoculture of the corresponding *L. plantarum* strains. Other combinations of strains showed higher acid production compared to juice samples fermented with *L. plantarum* and yeast monocultures. Pumpkin juice samples fermented by *L. plantarum* IMV B-7565 and *S. cerevisiae* UCM Y-2519 strains had pH 4.19 ± 0.18 and total titrable acidity 10.25 ± 1.06 °T. Furthermore, significant improvement was observed in all combinations with *L. plantarum*

KM-4 strain: *L. plantarum* KM-4 + *K. marxianus* F21 (pH 4.22 ± 0.19, 21.25 ± 2.47 °T), *L. plantarum* KM-4 + *Y. lipolytica* FF21 (pH 3.92 ± 0.01, 26.5 ± 0.7 °T), *L. plantarum* KM-4 + *S. cerevisiae* UCM Y-2519 (pH 4.12 ± 0.21, 19.5 ± 0.7 °T).

Thus, the results obtained demonstrate that selected strains of lactic acid bacteria and yeast are promising candidates for pumpkin juice fermentation to obtain a functional drink. Further research is needed to study the spectrum of biologically active compounds synthesised by microorganisms during fermentation and their interaction, and the effect of fermentation on the antioxidant potential of the pumpkin juice.

References

1. Anumudu, C. K., Miri, T., & Onyeaka, H. (2024). Multifunctional Applications of Lactic Acid Bacteria: Enhancing Safety, Quality, and Nutritional Value in Foods and Fermented Beverages. Foods (Basel, Switzerland), 13(23), 3714. https://doi.org/10.3390/foods13233714
2. Batool, M., Ranjha, M. M. A. N., Roobab, U., Manzoor, M. F., Farooq, U., Nadeem, H. R., Nadeem, M., Kanwal, R., AbdElgawad, H., Al Jaouni, S. K., Selim, S., & Ibrahim, S. A. (2022). Nutritional Value, Phytochemical Potential, and Therapeutic Benefits of Pumpkin (Cucurbita sp.). Plants, 11(11), 1394. https://doi.org/10.3390/plants11111394.
3. Fang L., Wang W., Dou Z., Chen J., Meng Y., Cai L., Li Y. (2023) Effects of mixed fermentation of different lactic acid bacteria and yeast on phytic acid degradation and flavor compounds in sourdough, LWT, Volume 174, 114438, ISSN 0023-6438, https://doi.org/10.1016/j.lwt.2023.114438.
4. Gavril, R. N., Stoica, F., Lipșa, F. D., Constantin, O. E., Stănciuc, N., Aprodu, I., & Râpeanu, G. (2024). Pumpkin and Pumpkin By-Products: A Comprehensive Overview of Phytochemicals, Extraction, Health Benefits, and Food Applications. Foods, 13(17), 2694. https://doi.org/10.3390/foods13172694.
5. Shah, A. M., Tarfeen, N., Mohamed, H., & Song, Y. (2023). Fermented Foods: Their Health-Promoting Components and Potential Effects on Gut Microbiota. Fermentation, 9(2), 118. https://doi.org/10.3390/fermentation9020118.
6. Tullio, V. (2024). Probiotic Yeasts: A Developing Reality? Journal of Fungi, 10(7), 489. https://doi.org/10.3390/jof10070489.

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EFFECT OF CULTIVATION PARAMETERS ON ANTIBIOTIC PRODUCTION IN ACTINOMYCETES OF THE GENUS *MICROMONOSPORA*

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Abstract. The effect of the composition of the ISP-9 synthetic medium on the growth and antimicrobial activity of strains of the genus *Micromonospora* was investigated. It was found that the use of arginine as a nitrogen source resulted in the highest level of activity at a concentration of 2.5 g/L. As the phosphate concentration increased, antimicrobial activity decreased, with the highest activity observed at a concentration of 1 mM. A dependence of pigment formation on the phosphate content in the medium was also observed.
Keywords: *Micromonospora,* secondary metabolism, culture medium, antimicrobial activity.

The spread of antibiotic resistance highlights the critical necessity of searching for new classes of antimicrobial compounds (Murray et al., 2022). In this context, members of the Micromonosporaceae family are regarded as extremely promising producers; however, a significant proportion of their biosynthetic gene clusters (BGCs) are cryptic, and their expression is strictly dependent on cultivation conditions (in accordance with the OSMAC concept) (Yan et al., 2022). Since traditional complex culture media often cause catabolite repression of secondary metabolism due to an oversupply of nutrients, they are unable to provide an optimal balance between biomass growth and the induction of antimicrobial activity (Ruiz-Villafán et al., 2022). Overcoming this limitation requires the use of chemically defined (synthetic) media, which allow for the control of trophic parameters, the targeted removal of repression of biosynthetic pathways, and a reliable assessment of the effect of each component on antibiotic production (Li et al., 2019). The aim of the study was to develop an optimised composition of a culture medium to enhance the biosynthetic potential of strains belonging to the *Micromonosporaceae* family, in particular those isolated from mine tailings.

ISP-9 medium was selected as the base medium for modifying the culture conditions. A combination of glucose (10 g/L) and glycerol (5 g/L) was used as carbon sources. This choice is justified by the two-phase growth model: readily available glucose provides rapid biomass accumulation in the early stages of cultivation, whilst the slower assimilation of glycerol maintains the cells' energy balance during the stationary phase, which is critical for the initiation of secondary metabolite biosynthesis. To maintain osmotic homeostasis and provide the enzyme systems with the necessary cofactors, the medium was supplemented with inorganic salts of $MgSO_4$, NaCl and $CaCl_2$ at a concentration of 0.5 g/L. Magnesium and calcium ions play a key structural and regulatory role, stabilising cell membranes and ensuring the functioning of ATP-dependent enzymes in biosynthetic cascades. A key step in the optimisation process was the targeted replacement of the nitrogen source. In the standard ISP-9 formulation, this role is fulfilled by ammonium sulphate ($(NH_4)_2SO_4$). However, for our work, it was replaced with the amino acid arginine. The theoretical basis for this decision was the need to avoid nitrogen catabolite repression (NCR). According to the literature, an excess of readily available ammonium ions activates the global transcription regulator GlnR, which, in turn, represses the expression of secondary metabolism genes and inhibits the synthesis of antimicrobial compounds (He et al., 2016). The use of arginine as a slowly assimilated source of nitrogen allows one to bypass GlnR-mediated repression and create conditions of moderate trophic stress, which acts as a trigger for the activation of 'silent' biosynthetic clusters. In order to find the optimal balance between biomass accumulation and maximum antimicrobial activity, the arginine concentration was tested within the range of 0.25–3.0 g/L. Based on the screening results, the working concentration of this component was set at 2.5 g/L, which ensured the highest level of bioactive substance production.

The next stage of the research involved optimising the phosphate content (in the form of KH_2PO_4 and K_2HPO_4 salts). The ISP-9 basal medium is characterised by high levels of these compounds, which often act as a limiting factor for the synthesis of bioactive substances. An excess of inorganic phosphorus acts as a trophic signal that stimulates primary metabolism and biomass accumulation, but at the same time represses the expression of genes responsible for the synthesis of antibiotics (a process known as phosphate repression). To establish an optimal balance between culture growth and the production of secondary metabolites, phosphates were added to the medium in the form of a potassium phosphate buffer with a molar concentration ranging from 1 to 50 mM. The screening results revealed a clear trend towards a decrease in antimicrobial activity as the phosphate concentration in the medium increased. The largest zones of growth inhibition in the test cultures were observed at the lowest concentration tested (1 mM). This concentration proved to be the

optimal trigger for the induction of biosynthetic processes; therefore, it was selected as the working concentration for further optimisation. Furthermore, during cultivation, a change in colony pigmentation was observed depending on the phosphate level. Strains that actively produced pigment under phosphate-limiting conditions completely lost this ability following an increase in phosphate concentration in the medium. Since pigment formation, like antibiotic synthesis, is a classic manifestation of secondary metabolism, the results obtained indicate a close interrelationship between these processes. It is likely that the concentration of inorganic phosphorus exerts a pleiotropic (multifactorial) effect, simultaneously regulating both biosynthetic pathways via shared molecular mechanisms (Hobbs et al., 1990).

Therefore, optimising the ISP-9 synthetic medium by incorporating arginine and limiting phosphate (to 1 mM) enabled us to effectively overcome catabolite repression. This ensured an optimal balance between culture growth and maximum activation of secondary metabolite synthesis, in particular antimicrobial compounds and pigments.

References

1. Murray, C. J., Ikuta, K. S., Sharara, F., Swetschinski, L., Aguilar, G. R., Gray, A., ... & Tasak, N. (2022). Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The lancet*, *399*(10325), 629–655.
2. Yan, S., Zeng, M., Wang, H., & Zhang, H. (2022). Micromonospora: a prolific source of bioactive secondary metabolites with therapeutic potential. *Journal of Medicinal Chemistry*, *65*(13), 8735–8771.
3. Ruiz-Villafán, B., Cruz-Bautista, R., Manzo-Ruiz, M., Passari, A. K., Villarreal-Gómez, K., Rodríguez-Sanoja, R., & Sanchez, S. (2022). Carbon catabolite regulation of secondary metabolite formation, an old but not well-established regulatory system. *Microbial Biotechnology*, *15*(4), 1058–1072.
4. Li, L., Liu, X., Jiang, W., & Lu, Y. (2019). Recent advances in synthetic biology approaches to optimize production of bioactive natural products in actinobacteria. *Frontiers in Microbiology*, *10*, 2467.
5. He, J.-M., Zhu, H., Zheng, G.-S., Liu, P.-P., Wang, J., Zhao, G.-P., Zhu, G.-Q., Jiang, W.-H., & Lu, Y.-H. (2016). Direct involvement of the master nitrogen metabolism regulator GlnR in antibiotic biosynthesis in *Streptomyces*. *Journal of Biological Chemistry, 291*(51), 26443–26454. https://doi.org/10.1074/jbc.M116.762476
6. Hobbs, G., Frazer, C. M., Gardner, D. C. J., Flett, F., & Oliver, S. G. (1990). Pigmented antibiotic production by Streptomyces coelicolor A3(2): Kinetics and the influence of nutrients. *Journal of General Microbiology*, 136(11), 2291-2296. https://doi.org/10.1099/00221287-136-11-2291

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SEF2 PROTEIN NEGATIVELY REGULATES RIBOFLAVIN PRODUCTION IN *CANDIDA FAMATA*

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Abstract. Riboflavin biosynthesis in *Candida famata* is regulated by iron availability, although its regulatory mechanisms remain poorly understood. We demonstrate that Sef2 negatively regulates riboflavin biosynthesis and is functionally linked to the Sef1-dependent iron response. These findings expand our understanding of the regulatory network controlling flavinogenesis in yeast.
Keywords: yeast, riboflavin, biosynthesis, regulation, gene expression.

Riboflavin (vitamin B_2) is an essential vitamin and the precursor of the flavin cofactors flavin mononucleotide and flavin adenine dinucleotide, which participate in redox reactions critical for energy metabolism. Due to the high demand for riboflavin in the pharmaceutical, food, and feed industries, enhancing its microbial production has become increasingly relevant. The yeast *Candida famata* is a promising candidate for this purpose because of its natural flavinogenic potential (Abbas et al, 2011).

Although the metabolic pathway of riboflavin biosynthesis is well studied in various yeast species, relatively little is known about its regulation. *SEF1*, a gene considered to be a key regulator of flavinogenesis in the yeast *C. famata,* encodes the transcription factor Sef1, which activates riboflavin overproduction under conditions of iron deficiency in the medium (Romanov et al, 2025).

In the present study we investigated the role of the gene *SEF2.* Sef2 was shown to repress riboflavin biosynthesis fivefold when the encoding gene was overexpressed in the wild-type strain VKM Y-9. A similar effect was observed in the overproducing strain AF4 harboring a defective version of the *SEF2* gene lacking 130 amino acid residues. Deletion of this gene led to a sixfold and fourfold increase in riboflavin production in iron-replete (Fe+) and iron-depleted (Fe−) media, respectively.

To study potential interactions between Sef1 and Sef2 proteins, we performed relative expression analysis in the wild-type strain L20105 and the *sef2*Δ mutant grown in Fe+ and Fe− media. The expression of the *SEF1* gene was found to be 1.25 times higher in the mutant strain compared to the wild-type under iron starvation and 1.5 times higher under normal iron supply. The expression of the structural gene *RIB6*, encoding 3,4-dihydroxy-2-butanone-4-phosphate synthase, was 8 times higher in the *sef2*Δ mutant under iron-replete conditions. The expression of *SEF2* gene did not differ significantly in iron-deplete and iron-replete media.

These results suggest that Sef2 negatively affects riboflavin biosynthesis and may interact with the previously studied Sef1. Further studies are required to fully elucidate the iron-dependent signaling pathway in flavinogenic yeasts.

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References

1. Abbas, C. A., & Sibirny, A. A. (2011). Genetic Control of Biosynthesis and Transport of Riboflavin and Flavin Nucleotides and Construction of Robust Biotechnological Producers. *Microbiology and Molecular Biology Reviews*, *75*(2), 321–360. https://doi.org/10.1128/mmbr.00030-10
2. Romanov, S., Lyzak, O., Sibirny, A., & Dmytruk, K. (2025). The Sef1 transcription factor interacts with promoters of riboflavin structural genes in *Candida famata*. *FEMS Yeast Research*, *25*. https://doi.org/10.1093/femsyr/foaf055

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IDENTIFICATION OF WILD *BRADYRHIZOBIUM* STRAINS AND THEIR PLANT GROWTH-PROMOTING EFFECT ON SOYBEAN IN FIELD CONDITIONS

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Abstract. Efficient nodulation by nitrogen-fixing rhizobia underlies sustainable soybean production and reduces the need for mineral fertilizers. Wild *Bradyrhizobium* strains were isolated from surface-sterilized root nodules of soybean cv. ES Mentor and identified by whole-genome and 16S rRNA gene sequencing, and annotated with Average Nucleotide Identity (ANI) analysis in DFAST. Two isolates, 31 and 82, were identified as *B. japonicum* and *B. diazoefficiens*, respectively, both showing 100 % identity to their closest reference type strains. A consortium of the two strains, used for seed pre-sowing inoculation, significantly increased root length (by 57.9 %) and root diameter (by 16.4 %) in a field experiment, while stimulating nitrogen-fixing and phosphate-mobilizing rhizosphere microbiota and lowering the nitrate nitrogen content in soil. The results confirm the plant growth-promoting potential of the isolated *Bradyrhizobium* strains for soybean cultivation.
Keywords: *Bradyrhizobium*, soybean, symbiotic nitrogen fixation, plant growth-promoting bacteria, rhizosphere microbiota.

Soybean (*Glycine max* (L.) Merr.) is one of the world's most important protein- and oil-yielding crops, and increasing its productivity while reducing dependence on chemical fertilizers is central to sustainable agriculture. This goal is closely linked to the efficiency of symbiotic nitrogen fixation carried out by root-nodule bacteria (rhizobia), which engage in a finely regulated molecular dialogue with the host legume during nodule formation and functioning (Yeremko et al., 2025). Screening, isolation, and identification of highly effective indigenous rhizobial strains from local agrocenoses is therefore a key step toward developing novel microbial consortia for seed inoculation and for enhancing legume-rhizobium symbiosis. The aim of this study was to isolate and identify wild strains of *Bradyrhizobium* spp. from nodules by whole-genome sequencing and 16S rRNA gene analysis, and to evaluate their phytostimulatory effect on soybean cv. ES Mentor plants under field conditions.

Microorganisms were isolated from surface-sterilized soybean root nodules by plating on YEME (Yeast Extract Malt Extract) agar. Total DNA was extracted from the resulting pure cultures. Taxonomic identification was based on PCR amplification and whole-genome sequencing (Illumina NovaSeq, 2×150 bp), together with 16S rRNA gene sequencing by the Sanger method. Average Nucleotide Identity (ANI) was calculated using the FastANI algorithm implemented in DFAST 1.3.9 (DDBJ Fast Annotation and Submission Tool) (Tanizawa et al., 2018). For the nitrogen-fixing consortium, strains were cultivated in liquid YEM medium for 4 days at 28 °C on a shaker-incubator to a titre of $1 \cdot 10^6$ CFU/mL, combined, and used for pre-sowing treatment of soybean (*G. max* L.) seeds. Morphometric parameters of the plants were measured at the pod-formation stage, together with microbiological and agrochemical analyses of the rhizosphere soil. Root length, root diameter, stem length, and leaf number were analyzed by one-way ANOVA and Welch's t-test with Bonferroni correction.

Two strains, *Bradyrhizobium* sp. 31 and 82, were isolated from soybean nodules. Strain 31 was phylogenetically related to *B. japonicum* DSM 3014 and *B. japonicum* ACCC 15027 (100 % identity for both), whereas strain 82 was related to *B. diazoefficiens* KG2-6 and *B. diazoefficiens* FK2-4 (100 % identity for both). Whole-genome sequencing identified strain 31 as *B. japonicum* and strain 82 as *B. diazoefficiens* (Delamuta et al., 2013), both recognised as effective soybean microsymbionts. Sequencing yielded a genome of 9.2 Mb in size; its annotation revealed 8,443 protein-coding regions. A check of the genome's completeness indicates that the sequence obtained is 99.24 % complete and contains 0.15 % contamination. The successful identification of slow-growing bradyrhizobia confirmed the targeted isolation of active nitrogen fixers with considerable biotechnological potential.

In the field experiment, seed inoculation with the two-strain consortium significantly increased root length by 57.9 % (179.2 mm in the control vs. 282.9 mm with the inoculant; $p = 0.0015$ after Bonferroni correction; Cohen's $d = 3.08$) and root diameter by 16.4 %, while stem length increased by 2.9 % and leaf number by 3.5 %, the latter two changes being statistically non-significant. This agrees with reports that *B. inoculation* improves nodulation and vegetative growth parameters of soybean under field conditions (Jarecki et al., 2024). Agrochemical analysis of the rhizosphere soil showed a decrease in nitrate nitrogen content from 3.8 to 2.0 mg/kg (–47.4 %), attributable to more intensive uptake by the better-developed root system, while organic matter content increased by 6.1 %. Potassium, sulfur, zinc, and iron contents were slightly lower in the inoculated variant (by 4.6–16.7 %), whereas calcium, magnesium, copper, manganese, boron, cation exchange capacity, and base saturation differed only minimally (within ±3 %). Pre-sowing inoculation increased the abundance of nitrogen-fixing, oligoazotrophic, and phosphate-mobilising microorganisms in the soil and decreased the abundance of oligotrophic microorganisms.

Thus, the identified strains *Bradyrhizobium japonicum* 31 and *B. diazoefficiens* 82 proved promising for forming an effective plant-microorganism symbiosis and showed a phytostimulatory effect on soybean in a field experiment, while positively influencing the microbial and agrochemical dynamics of the rhizosphere soil.

References

1. Delamuta, J. R. M., Ribeiro, R. A., Ormeño-Orrillo, E., Melo, I. S., Martínez-Romero, E., & Hungria, M. (2013). Polyphasic evidence supporting the reclassification of *Bradyrhizobium japonicum* group Ia strains as *Bradyrhizobium diazoefficiens* sp. nov. International Journal of Systematic and Evolutionary Microbiology, 63(9), 3342–3351. https://doi.org/10.1099/ijs.0.049130-0
2. Jarecki, W., Borza, I. M., Rosan, C. A., Vicas, S. I., & Domuța, C. G. (2024). Soybean response to seed inoculation with *Bradyrhizobium japonicum* and/or nitrogen fertilization. Agriculture, 14(7), 1025. https://doi.org/10.3390/agriculture14071025
3. Tanizawa, Y., Fujisawa, T., & Nakamura, Y. (2018). DFAST: A flexible prokaryotic genome annotation pipeline for faster genome publication. Bioinformatics, 34(6), 1037–1039. https://doi.org/10.1093/bioinformatics/btx713
4. Yeremko, L., Czopek, K., Staniak, M., Marenych, M., & Hanhur, V. (2025). Role of environmental factors in legume-rhizobium symbiosis: A review. Biomolecules, 15(1), 118. https://doi.org/10.3390/biom15010118

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SELECTION OF MICROORGANISMS CAPABLE OF MINERALIZATION OF ORGANIC MATTER AND DEVELOPMENT IN COMPOSTED SUBSTRATES

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Abstract. Bacteria, which are active cellulose destroyers *Bacillus amyloliquefaciens* ssp. were selected and identified. *B. plantarum* 201, *B. amyloliquefaciens ssp. plantarum* 293, *B. megaterium* 362, *B. amyloliquefaciens* 348, *B. amyloliquefaciens* 425, which, in addition to high cellulolytic ability, are producers of auxin substances, non-phytotoxic and compatible with the active cellulolytic micromycete *Trichoderma harzianum* PD3. The obtained results are important for the development of technologies for biocomposting of organic substrates based on bird droppings with a phased introduction to composted mixtures of active strains of *Trichoderma* and bacteria of the genus *Bacillus.*
Keywords: cellulolytic microorganisms, *Trichoderma*, *Bacillus*, chicken droppings, composting.

Composting of organic substrates occurs due to the functioning of microorganisms in them, therefore, optimization of microbiological processes during their production is crucial for obtaining high-quality composts. It is considered promising to create technologies for composting organic matter on the basis of microorganisms useful from an agronomic point of view introduced into compost mixtures. In fact, such composts can be a kind of complex microbial preparations. For example, various types of microscopic fungi and bacteria are used to compost organic substrate based on bird droppings and cattle manure (Shevchenko et al., 2023). Micromycetes of the genus *Trichoderma* and bacteria of the genus Bacillus are encouraging in this aspect, since individual representatives of these taxonomic groups have a number of valuable agronomic properties, in particular, they are powerful biodestroyers of organic matter, antagonists of a number of pathogens of cultivated plants and producers of phytohormones (Avdeeva et al., 2016; Baldrian et al., 2008).

The purpose of our research was to search for agronomically valuable microorganisms with their subsequent introduction into composted mixtures in order to speed up the process of biofermentation of the organic substrate and improve the quality indicators of the obtained composts.

At the first stage of our work, we selected the active cellulose destructor micromycete *Trichoderma harzianum* PD3, showed its ability to actively develop in a composted substrate based on bird droppings, and investigated a number of other agronomically useful properties of the strain. At the second stage of research, the selection of cellulolytic strains of spore-forming bacteria of the genus Bacillus was carried out due to the fact that the representatives of this genus are mostly non-pathogenic, stable during storage and technological in production, which, in combination with high hydrolytic activity, makes them promising for use in composting technologies of organic mixtures containing straw.

The selection of spore-forming bacteria – cellulose destroyers was carried out in laboratory conditions using a mineral medium in which the only source of carbon is cellulose (Volkogon et al., 2010). The identification of isolated bacteria to the genus was established according to Bergey's determinant. The species identification of the selected strains was carried out by the taxonomic identification method of MALDI-TOF.

From various substrates (chicken droppings, hay, straw, rhizosphere soil, plant roots, etc.), 240 isolates of spore-forming bacteria were isolated, which were selected based on their ability to destroy cellulose (a mineral nutrient medium with a single source of carbon in the form of filter paper and winter wheat straw was used). The cellulolytic ability of the isolates selected in this way was also tested during cultivation on a minimal glucose-salt medium with carboxymethyl cellulose. Among the isolated bacteria, *Bacillus* sp. 201, *Bacillus* sp. 243, *Bacillus* sp. 293*, Bacill*us sp. 348, *Bacillus* sp. 362, *Bacillus* sp. 392 and *Bacillus* sp. 425, which were selected for further research (Bondar et al., 2024).

According to the results of the compatibility study of the micromycete *T. harzianum* PD3 and isolates of active cellulolytic bacteria of the genus *Bacillus*, it was found that strains of *Bacillus* sp. 243 and 392 are incompatible with *T. harzianum* PD3 due to the fact that the micromycete completely inhibited the growth of bacillus strains, which excludes the possibility of their simultaneous use in further studies. The combined use of *T. harzianum* PD3 with strains of *Bacillu*s sp. 201, 293 and 425. Strains of *Bacillus* sp. 348 and 362, the effect of which on *T. harzianum* PD3 consisted in a slight limitation of the development of the micromycete, can also be used in further studies, but with some caveats, for example, with the phased, time-separated introduction of trichoderma and the specified strains of bacilli into composted substrates.

As a result, 5 strains of bacteria – active cellulose destroyers – were selected and identified: *Bacillus amyloliquefaciens* ssp. *plantarum* 201, B. *amyloliquefaciens* ssp. *Plantarum* 293, *B. megaterium* 362, *B. amyloliquefaciens* 348 and *B. amyloliquefaciens* 425. It was established that the selected strains of bacilli do not have phytotoxic properties and produce phytohormonal substances of auxin nature. Strains of bacteria *Bacillus amyloliquefaciens* ssp. *plantarum* 201, *B. amyloliquefaciens* ssp. *plantarum* 293, *B. megaterium* 362, *B. amyloliquefaciens* 348, *B. amyloliquefaciens* 425 are compatible with the micromycete strain *T. harzianum* PD3 and can be used in further research on the development of technologies for biocomposting of organic substrates based on bird droppings with the introduction of active cellulolytic strains of *Trichoderma* and bacteria of the genus *Bacillus* into composted mixtures.

References

1. Avdeeva, L. V., Kharhota, M. A., & Kharhota, G. V. (2016). Destruction of harvested plant residues by strains of *B. subtilis* IMV B-7516 and *B. licheniformis* MV B-7515. *Journal of microbiology*, 2, 14–19.
2. Baldrian, P., & Valaskova, V. (2008). Degradation of cellulose by basidiomycetous fungi. *FEMS Microbiol. Rev.*, 32(3), 501–521.
3. Bondar, I. M., Dimova, S. B. (2024). Properties of compost based on chicken droppings obtained by the introduction of two microorganisms. *Agricultural Microbiology*, 40, 58–69. https://doi.org/10.35868/1997-3004.40.58-69
4. Shevchenko, L. A., & Ryabukha, G. I. (2023). Biocomposting of chicken manure with the participation of introduced microorganisms (review). *Agricultural microbiology*, 37, 3–22. https://doi.org/10.35868/1997-3004.37.3-22
5. Volkogon, V. V., Nadkernychna, O. V., & Tokmakova, L. M. (2010). *Experimental soil microbiology. for sciences.* Agrarian. Science.

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PROTECTIVE AND STIMULATING PROPERTIES OF THE ENDOPHYTIC STRAIN *BACILLUS VELEZENSIS* IMV B-8134

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Abstract. The plant microbiome, particularly endophytic microorganisms, is attracting attention due to its targeted probiotic effects on plants. The development of novel bioformulations based on endophytic bacteria is of critical importance for the sustainable development of crop production under extreme climate change. Under field conditions, stimulatory and phytoprotective effects on soybean plants were observed through the synergistic action of the endophytic strain *Bacillus velezensis* IMV B-8134 and the association of soybean rhizobia. These effects persisted in the next generation of plants, ensuring higher disease resistance and soybean productivity.
Keywords: endophytes, rhizobia, biotic and abiotic stresses, soybean fungal diseases, yield.

Soybean (*Glycine max* (L.) Merr.) is one of the most important protein and oil crops in world agriculture (Ramlal et al., 2026). It plays a significant role in ensuring food and energy security. In Ukraine, there has been a tendency to expand the acreage devoted to this crop in recent years, and as of 2025, almost 2,091 thousand hectares were planted with soybeans (State Statistics Service of Ukraine). A major obstacle to obtaining high yields of high-quality soybean seeds is infection by phytopathogenic microorganisms, 50 % of which are fungal pathogens (Pospielova et al., 2020). Pathogenic fungi are capable of synthesizing toxins, the accumulation of which can lead to a reduction in the food and feed properties of the crop and to poisoning in humans and animals (Bandara et al. 2020; Salotti et al., 2024). Alternaria blight is a disease that is becoming increasingly prevalent in soybean crops due to global climate change. The dominant pathogens are *A. alternata* (Fr.) Keissler, *A. solani* (Ell. et Mart.), and *A. tenuissima* (Fr.) Wiltshire (Pospielova et al. 2020; Rani et al., 2020). The damage caused by Alternaria blight is that the disease leads to a reduction in the photosynthetic surface area of the leaves, their wilting and death. Infected plants lag behind in growth, produce small, underdeveloped beans and non-viable seeds, or produce no beans at all.

Experiments on the cultivation of the mid-early soybean variety ‘Sculptor’ were conducted at the experimental field of the D. K. Zabolotny Institute of Microbiology and Virology of the NAS of Ukraine under arid conditions in 2025. The field research design included the following seed inoculation treatments: 1 – control (no inoculation, water treatment); 2 – Ryzobin^{K} (association of *Bradyrhizobium japonicum* UCM B-6018, B-6023 and B-6035); 3 – *B. velezensis* IMV B-8134; 4 – Ryzobin^{K} + *B. velezensis* IMV B-8134; 5 – Ryzobin^{K} + *B. velezensis* IMV B-8134 bioprimed seeds (seeds from the soybean harvest inoculated with the Ryzobin^{K} + *B. velezensis* IMV B-8134 bioformulation in the previous year).

During the active vegetation period, in the flowering- early bean formation stages (BBCH 65–71), symptoms of Alternaria leaf blight were observed on infected plants – dark brown or black circular spots with a yellow margin that contrasted strongly with the green background of healthy leaf tissue. The results of the phytopathological analysis confirmed the absence of *Alternaria* infection in soybean plants in the variants with mono-inoculation

of *B. velezensis* IMV B-8134 and the complex inoculation of Ryzobin^{K} + *B. velezensis* IMV B-8134. In the variant with bioprimed plants, the number of infected plants was three times lower than the control. At the same time, soybean yield in the variants with complex inoculation and with bioprimed plants was 20.9 % and 38.4 % higher than the control, respecttively. This effect was probably achieved due to the synergistic action of the studied rhizobial and non-rhizobial endophytic bacteria, which improved nitrogen nutrition, provided protection against biotic and abiotic stresses, and stimulated soybean growth and productivity.

Thus, the obtained results indicate that the use of a complex inoculant based on the association of complementary rhizobia and the non-rhizobial endophytic strain *B. velezensis* IMV B-8134 contributed to the reduction of soybean damage by Alternaria blight under natural infection background, as well as to the increase in productivity. These effects persisted in the next generation of plants.

References

1. Bandara, A. Y., Weerasooriya, D. K., Bradley, C. A., Allen, T. W., & Esker, P. D. (2020). Dissecting the economic impact of soybean diseases in the United States over two decades. PloS one, 15(4), e0231141. Doi: 10.1371/journal.pone.0231141.
2. Pospielova, G. D., Kovalenko, N. P., Nechyporenko, N. I., & Kocherha, V. Y. (2020). The impact of agroclimatic factors on the development of major soybean diseases. [Вплив агрокліматичних факторів на розвиток основних хвороб сої]. Ukrainian Black Sea Region Agrarian Science, 3, 45-52. (in Ukrainian). Doi: 10.31521/2313-092X/2020-3(107)-6.
3. Ramlal, A., Rajendran, R. A., Lal, S. K., & Chigeza, G. (2026). Soybean: The path ahead. In Soybeans (pp. 351–363). Apple Academic Press. Doi: 10.1201/9781998511730-20.
4. Rani, N., Lal, H. C., & Kumar, P. (2020). Survey and morphological characterization of *Alternaria* spp. causing alternaria blight of pigeon pea. International Journal of Current Microbiology and Applied Sciences, 9, 437-440. Doi: 10.13140/RG.2.2.30166.68169.
5. Salotti, I., Giorni, P., & Battilani, P. (2024). Biology, ecology, and epidemiology of Alternaria species affecting tomato: ground information for the development of a predictive model. Frontiers in plant science, 15, 1430965. Doi: 10.3389/fpls.2024.1430965.
6. State Statistics Service of Ukraine. [Державна служба статистики України] (2025). Planted Areas and Yields of Major Crops. [Посівні площі та врожайність основних культур]. (in Ukrainian). Available at: https://www.ukrstat.gov.ua/operativ/operativ2019/sg/ ppsgk/arh_ppsgk_u.html

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PRODUCTION OF BIOETHANOL AND HIGH-VALUE SUBSTANCES BY NON-CONVENTIONAL YEASTS

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Abstract. The non-conventional yeasts *Ogataea polymorpha* and *Candida famata* were studied as hosts for the production of ethanol, L-lactic acid, and riboflavin from xylose, glycerol, and lignocellulosic hydrolysates. Metabolic engineering and selection improved ethanol production by

O. polymorpha from pentose sugars and enabled L-lactic acid production from lignocellulosic substrates. Metabolic engineering of *C. famata* resulted in strains with increased riboflavin production from xylose, lignocellulosic hydrolysates, and crude glycerol. The obtained strains provide a basis for further development of yeast-based production processes using lignocellulosic sugars and glycerol as carbon sources.
Keywords: *Ogataea polymorpha, Candida famata*, bioethanol, L-lactic acid, riboflavin, metabolic engineering.

Production of the bioethanol and high-value chemicals from lignocellulosic hydrolysates has important economic and environmental issues. We study ethanol, lactic acid and riboflavin synthesis by the industrially important non-conventional yeasts *Ogataea polymorpha* and *Candida famata*. Using approaches of metabolic engineering and classical selection, strains of the thermotolerant yeast *O. polymorpha* have been constructed accumulating more than 20 g/L of ethanol from abundant lignocellulosic sugar xylose which 50 times exceeded ethanol production by the wild-type strain. Ethanol production from another important pentose sugar L-arabinose was also increased. Constructed strains also accumulated increased amounts of ethanol in lignocellulosic hydrolysates. The strains of *O. polymorpha* producing enhanced amounts of ethanol from pure and crude glycerol also have been isolated.

Recombinant strains of *O. polymorpha* producing lactic acid in lignocellulosic hydrolysates were constructed. Yeast *C. famata* is capable of riboflavin overproduction under iron starvation. Previously, robust genetically stable *C. famata* riboflavin overproducers in iron-rich glucose media have been isolated. Using metabolic engineering approaches, the strains of *C. famata* accumulating elevated amounts of riboflavin from xylose, lignocellulosic hydrolysates and crude glycerol (by-product of biodiesel production) have been constructed. Future prospects of the current research and possible industrial implementation will be discussed.

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WHOLE-GENOME SEQUENCING, BIOACTIVITY, ADAPTIVE POTENTIAL AND PHYTOSTIMULATORY PROPERTIES OF *AMYCOLATOPSIS ROODEPOORTENSIS* Y13 ISOLATED FROM WAR-IMPACTED SOIL

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Abstract. The actinomycete strain *Amycolatopsis roodepoortensis* Y13, isolated from war-impacted soil, was subjected to whole-genome sequencing, biosynthetic gene clusters for secondary metabolites together with resistance determinants for heavy metals were detected in the genome. The strain tolerated elevated concentrations of copper and zinc as well as increased salinity, displayed high sensitivity to aminoglycosides and vancomycin, and exhibited antagonistic activity against several

phytopathogenic fungi, including *Sclerotinia sclerotiorum*, *Bipolaris sorokiniana*, *Alternaria arborescens*, *Fusarium graminearum* and *Aspergillus niger*. In a *Triticum aestivum* L. germination bioassay, strain Y13 significantly ($p < 0.001$) stimulated root and stem growth, increasing primary root length by 18.0 % and stem length by 27.4 %. These findings indicate that *A. roodepoortensis* Y13 combines multi-stress resistance with antimicrobial and phytostimulatory potential.
Keywords*: Amycolatopsis roodepoortensis*; whole-genome sequencing; heavy metal resistance; antagonistic activity; phytostimulation.

Actinomycetes remain the most prolific bacterial source of agriculturally valuable secondary metabolites, and genome-based approaches continue to uncover previously hidden biosynthetic potential within this group (Cibichakravarthy & Jose, 2021). They synthesise a wide range of bioactive substances, including siderophores, antibiotics and phytohormones: their secondary metabolites neutralise the effects of heavy metals, their antibiotics are actively used against phytopathogenic fungi, and their phytohormones improve plant growth and yield. Therefore, the aim of this work was to perform whole-genome sequencing of strain Y13, to determine its resistance to heavy metals, elevated salinity and antibiotics, to study its antagonistic activity against phytopathogenic fungi, and to determine its phytostimulatory effect.

Strain Y13 was isolated from soil in Kryvyi Rih, Ukraine, near the "Kryvorizhstal" plant (47°28′26.53″N, 33°38′57.62″E), a territory impacted by a ballistic missile strike. The 16S rRNA gene was sequenced by the Sanger method, and the whole genome was sequenced using Illumina NovaSeq. A neighbour-joining tree was constructed in MEGA 12.1.1 with the bootstrap method (1,000 replications) (Kumar et al., 2024). Genome annotation was performed with the DFAST and RAST pipelines, and taxonomic identification was confirmed by average nucleotide identity (ANI) analysis using DFAST_QC (Elmanzalawi et al., 2025). Biosynthetic gene clusters (BGCs) responsible for secondary metabolite production were identified with antiSMASH 8.0 (Blin et al., 2025). Antagonistic activity was assessed by the agar double-layer technique. Resistance to heavy metals was assessed by growth on potato glucose agar (PGA) supplemented with heavy metals, and salt tolerance was determined on PGA with a NaCl gradient (0, 0.1, 2, 5 and 7 %). Antibiotic sensitivity was tested by the modified Kirby-Bauer disc-diffusion method on glucose yeast medium (GYM). The phytostimulatory effect of strain Y13 was tested in a germination bioassay with *Triticum aestivum* L. cv. 'MIP Valencia' (Aramis LLC).

Based on 16S rRNA gene phylogenetic analysis, strain Y13 belongs to the genus *Amycolatopsis* and forms a clade with *A. roodepoortensis* ZEL 1. The whole-genome sequence was deposited in the NCBI database (BioProject PRJNA1215024). The ANI value relative to the reference genome of *A. roodepoortensis* DSM 46661 was 96.63 %, confirming the identification of strain Y13 as *A. roodepoortensis*. The genome comprises approximately 8.87 million base pairs across 55 sequences, with a GC content of 69.1 %, and encodes auxin biosynthesis (10 subsystems) together with resistance determinants for mercury (1), arsenic (4), cobalt-zinc-cadmium (20) and copper (15), as well as thiazole-oxazole-modified microcin (TOMM) synthesis (2). BGCs with high homology corresponded to erythromycin-related clusters (Ery-3 to Ery-9), geosmin, ectoine, mirubactin, albachelin, 2-methylisoborneol, citrulassin, ristomycin and ε-poly-L-lysine.

Strain Y13 grew actively at 600 mg/L of copper and zinc but was inhibited at 500 mg/L of chromium and 300 mg/L of nickel, and it tolerated up to 5 % NaCl. It showed the highest sensitivity to aminoglycosides (gentamicin, 11.3 mm; kanamycin, 10.3 mm; amikacin, 8.3 mm; streptomycin, 7.3 mm) and to vancomycin (8.7 mm). Fluoroquinolones produced no complete inhibition zone but a less-dense lawn (5–7 mm), indicating a bacteriostatic effect, while the strain was insensitive to β-lactams, cephalosporins and carbapenems, and

unaffected by polyenes and polymyxin B — a pattern typical of *Amycolatopsis* spp. due to endogenous β-lactamase production and/or low-affinity target proteins.

Strain Y13 exhibited antagonistic activity against *Sclerotinia sclerotiorum* SSC21 (7 mm), *Bipolaris sorokiniana* TR1 (7.7 mm), *Alternaria arborescens* TR1 (5 mm), *Fusarium graminearum* DSM 21727 (11 mm) and *Aspergillus niger* ATCC 6275 (6 mm); the biomass extract inhibited *B. sorokiniana* TR1 (9 mm), while the culture-liquid supernatant was slightly more active (13.7 mm). In the *Triticum aestivum* L. germination bioassay, all measured morphometric parameters increased significantly ($p<0.001$): primary root length rose from 107.83 to 127.23 mm (+18.0 %), the first lateral root from 94.53 to 116.07 mm (+22.8 %), the second lateral root from 81.63 to 105.00 mm (+28.6 %, the largest gain), and stem length from 58.87 to 75.00 mm (+27.4 %).

The isolated strain *A. roodepoortensis* Y13 combines resistance to several adverse factors with promising antimicrobial and phytostimulatory properties, indicating its potential for biotechnological application, including the reclamation of war-impacted soils.

References

1. Blin, K., Shaw, S., Vader, L., Szenei, J., Reitz, Z. L., Augustijn, H. E., Cediel-Becerra, J. D. D., de Crécy-Lagard, V., Koetsier, R. A., Williams, S. E., Cruz-Morales, P., Wongwas, S., Segurado Luchsinger, A. E., Biermann, F., Korenskaia, A., Zdouc, M. M., Meijer, D., Terlouw, B. R., van der Hooft, J. J. J., ... Weber, T. (2025). antiSMASH 8.0: Extended gene cluster detection capabilities and analyses of chemistry, enzymology, and regulation. Nucleic Acids Research, 53(W1), W32–W38. https://doi.org/10.1093/nar/gkaf334
2. Cibichakravarthy, B., & Jose, P. A. (2021). Biosynthetic potential of *Streptomyces rationalizes* genome-based bioprospecting. Antibiotics, 10(7), 873. https://doi.org/10.3390/antibiotics 10070873
3. Elmanzalawi, M., Fujisawa, T., Mori, H., Nakamura, Y., & Tanizawa, Y. (2025). DFAST_QC: Quality assessment and taxonomic identification tool for prokaryotic genomes. BMC Bioinformatics, 26(1), 3. https://doi.org/10.1186/s12859-024-06030-y
4. Kumar, S., Stecher, G., Suleski, M., Sanderford, M., Sharma, S., & Tamura, K. (2024). MEGA12: Molecular evolutionary genetic analysis version 12 for adaptive and green computing. Molecular Biology and Evolution, 41(12), msae263. https://doi.org/10.1093/molbev/msae263

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BIFIDOBACTERIUM BIFIDUM LVA-3 FERMENTATE AS AN ANTIBACTERIAL AND PRECONDITIONING AGENT FOR MESENCHYMAL STEM CELLS

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Abstract. The therapeutic efficacy of adipose tissue-derived mesenchymal stem cells (AD-MSCs) can be compromised by local inflammation and microbial infections at wound sites. Preconditioning MSCs with probiotic postbiotics offers a dual strategy to prime stem cell function and provide local antibacterial

protection. This study evaluated the dose-dependent effects of heat-inactivated and sterile-filtered *Bifidobacterium bifidum* LVA-3 culture fermentate on its direct antagonistic activity against opportunistic pathogens and AD-MSC functional performance. At concentrations of 5–10 %, the fermentate effectively inhibited *S. aureus*, *E. coli*, and *P. aeruginosa* while maintaining AD-MSC adhesion, boosting metabolic activity, accelerating directional migration, and modulating lineage-specific differentiation. Preconditioning AD-MSCs with *B. bifidum* LVA-3 fermentate represents a novel biotechnological approach for optimizing cell-based therapies targeting infected and impaired tissue wounds.
Keywords: *Bifidobacterium bifidum fermentate,* mesenchymal stem cells, postbiotics, preconditioning, tissue regeneration.

Cell-based therapies utilizing human adipose tissue-derived mesenchymal stem cells (AD-MSCs) are promising for tissue regeneration and wound healing. However, local microenvironmental stressors and microbial infection can compromise MSC therapeutic efficacy. Preconditioning MSCs with bioactive bacterial products (Waterman et al., 2010; Saberian et al., 2020; Kazemi et al., 2022) offers a novel strategy to prime stem cell functional performance prior to transplantation.

This study aimed to evaluate the antibacterial properties of *Bifidobacterium bifidum* LVA-3 culture fermentate and determine its potential as a preconditioning agent by assessing its dose-dependent effects on AD-MSC adhesion, metabolic activity, migration, and multilineage differentiation potential.

Materials and Methods. *B. bifidum* LVA-3 was cultured in semi-liquid nutrient medium at 37 °C for 2 days until reaching stationary phase, heat-inactivated (60 °C, 30 min), and sterile-filtered (0.22 μm). Antagonistic activity against opportunistic pathogens (*Staphylococcus aureus* 209, *Escherichia coli*, and *Pseudomonas aeruginosa* 9027) was measured via agar diffusion assay. Human AD-MSCs (passages 4–6) were isolated as described previously (Zuk et al., 2002) with informed donor consent and bioethics approval. AD-MSCs were seeded in standard medium (α-MEM, 10 % FBS, antibiotics) supplemented with 0 % (control), 5 %, 10 %, 25 %, or 50 % fermentate, cultured for 24 h, after that cell adhesion and basal metabolic activity (resazurin assay) were assessed. Migration (scratch assay) and directed osteogenic/adipogenic differentiation capacity were evaluated following culture in corresponding media (Cormier et al., 2015; Campagnoli et al, 2001) with 5 % and 10 % fermentate.

The *B. bifidum* LVA-3 fermentate demonstrated distinct antagonistic activity against all tested opportunistic pathogens. High concentrations (25 % and 50 %) lowered media pH, impaired MSC adhesion, and suppressed metabolic activity. Conversely, preconditioning AD-MSCs with 5 % and 10 % fermentate maintained cell adhesion, significantly boosted metabolic activity, and accelerated directional cell migration. Furthermore, 5 % and 10 % fermentate preconditioning enhanced adipogenic differentiation. Under osteogenic induction, 5 % fermentate preserved osteogenic differentiation capacity, whereas 10 % fermentate exerted an inhibitory effect.

Conclusion: *B. bifidum* LVA-3 fermentate acts as a effective preconditioning agent for human AD-MSCs at low concentrations (5–10 %), significantly enhancing their metabolic and migratory functions while preserving line-specific differentiation flexibility. Combined with its intrinsic antimicrobial properties, *B. bifidum* LVA-3 fermentate preconditioning offers a novel approach for optimizing MSC-based therapies targeting infected wounds, critical tissue defects, and ischemic injuries.

References

1. Campagnoli, C., Roberts, I. A., Kumar, S., Bennett, P. R., Bellantuono, I., & Fisk, N. M. (2001). Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. *Blood*, *98*(8), 2396–2402. https://doi.org/10.1182/blood.v98.8.2396

2. Cormier, N., Yeo, A., Fiorentino, E., & Paxson, J. (2015). Optimization of the Wound Scratch Assay to Detect Changes in Murine Mesenchymal Stromal Cell Migration After Damage by Soluble Cigarette Smoke Extract. *Journal of visualized experiments : JoVE*, (106), e53414. https://doi.org/10.3791/53414
3. Kazemi, A., Ataellahi Eshkoor, P., Saeedi, P., & Halabian, R. (2022). Evaluation of antioxidant and antibacterial effects of lactobacilli metabolites- preconditioned bone marrow mesenchymal stem cells in skin lesions amelioration. *Bioorganic chemistry*, *124*, 105797. https://doi.org/ 10.1016/j.bioorg.2022.105797
4. Saberian, M., Shahidi Delshad, E., & Habibi, M. (2020). The Effect of *Bifidobacterium Bifidum* Supernatant and Cell Mass on the Proliferation Potential of Rat Bone Marrow-Derived Stromal Cells. *Iranian journal of medical sciences*, *45*(4), 269–276. https://doi.org/ 10.30476/ ijms.2019.45772
5. Waterman, R. S., Tomchuck, S. L., Henkle, S. L., & Betancourt, A. M. (2010). A new mesenchymal stem cell (MSC) paradigm: polarization into a pro-inflammatory MSC1 or an Immunosuppressive MSC2 phenotype. *PloS one*, *5*(4), e10088. https://doi.org/10.1371/ journal. pone.0010088
6. Zuk, P. A., Zhu, M., Ashjian, P., De Ugarte, D. A., Huang, J. I., Mizuno, H., Alfonso, Z. C., Fraser, J. K., Benhaim, P., & Hedrick, M. H. (2002). Human adipose tissue is a source of multipotent stem cells. *Molecular biology of the cell*, *13*(12), 4279–4295. https://doi.org/10. 1091/ mbc.e02-02-0105

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ENGINEERING MICROBIAL PRODUCERS OF ETHANOL AND LACTIC ACID AS ALTERNATIVE FERMENTATION PRODUCTS

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Abstract. Improved pentose alcoholic fermentation in *Ogataea polymorpha* was achieved by positive selection and identification of the genetic determinants *IRA1* and *API1*. Heterologous expression of lactate dehydrogenase genes enabled efficient L-lactic acid production, demonstrating distinct strategies for redirecting yeast metabolism toward alternative fermentation products.
Keywords: *Ogataea polymorpha*, xylose, metabolic engineering, fermentation.

The use of renewable feedstocks for ethanol and L-lactic acid production requires microorganisms capable of efficient conversion of different carbon sources. The thermostolerant yeast *Ogataea polymorpha* utilizes a broad range of substrates, including glucose and xylose, and is capable of alcoholic fermentation at elevated temperatures.

Positive selection combined with whole-genome sequencing and reverse genetics was used to improve alcoholic fermentation of pentoses. The selected *O. polymorpha* strain A107 accumulated 20.91 g/L ethanol from xylose at 45 °C, more than 50-fold higher than the wild-type strain. Mutations affecting *IRA1* and *API1* were identified as important determinants of improved xylose and L-arabinose fermentation.

An alternative direction involved engineering *O. polymorpha* for L-lactic acid production by expression of heterologous lactate dehydrogenase genes. The most efficient recombinant strain accumulated up to 72 g/L L-lactic acid from glucose, whereas production from xylose reached 9.6 g/L.

The obtained results demonstrate that *O. polymorpha* metabolism can be redirected toward either ethanol or L-lactic acid production using complementary strain-development strategies. The identified genetic targets and constructed strains provide a basis for further improvement of both processes.

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SILVERFISH-ASSOCIATED YEASTS LINK ENVIRONMENTAL PERSISTENCE WITH EPITHELIAL-INTERACTION PHENOTYPES.

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Abstract. Synanthropic insects may represent overlooked reservoirs of viable yeasts in the built environment. Fungal profiling and culture-based characterization of the silverfish *Lepisma saccharinum* revealed a yeast-dominated mycobiota and yielded isolates of *Nakaseomyces glabratus* and *Candida zeylanoides*. The isolates differed markedly in stress tolerance, biofilm formation, and interactions with human keratinocytes. Importantly, environmental robustness did not consistently predict epithelial-interaction phenotypes, indicating distinct and isolate-specific combinations of traits relevant to environmental persistence and potential host interactions.
Keywords: *Lepisma saccharinum, Nakaseomyces glabratus, Candida zeylanoides*, fungal microbiota, epithelial interaction.

Synanthropic insects represent a largely overlooked microbial niche within the built environment, yet little is known about the viable fungi they harbor and their functional properties. We characterized the fungal microbiota associated with the silverfish *Lepisma saccharinum* and examined phenotypic diversity among cultivable yeasts recovered from the insects. ITS1 amplicon profiling of a pooled silverfish sample revealed a strongly yeast-dominated fungal community, with *Candida* accounting for approximately 83 % of assigned reads. Culture-dependent analysis yielded two *Candida glabrata* (currently *Nakaseomyces glabratus*) isolates, LS1 and LS6, and one *Candida zeylanoides* isolate, LS4. The isolates displayed pronounced strain-dependent differences in carbon-source utilization, thermotolerance, halotolerance and biofilm formation. LS1 and LS6 grew at temperatures up to 42 °C, showed greater tolerance to osmotic stress than LS4 and produced more biofilm than the *Saccharomyces cerevisiae* reference strain. Imaging flow cytometry further demonstrated association of all

three isolates with HaCaT keratinocytes and internalization-associated signals, while yeast exposure was accompanied by increased IL-6- and IL-8-associated fluorescence. Importantly, environmental robustness did not directly parallel epithelial-interaction phenotypes: LS4 showed the narrowest stress-tolerance profile and the lowest internalization-associated fraction, yet elicited the strongest IL-8-associated signal. Together, these findings reveal substantial isolate-specific functional diversity among silverfish-associated yeasts and identify *L. saccharinum* as a previously underexplored potential reservoir of viable, clinically relevant yeasts in the built environment.

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ENHANCING BIOACTIVE COMPOUND AND ENZYME PRODUCTION IN *PSEUDOPESTALOTIOPSIS THEAE* THROUGH EPIGENETIC MODIFICATION

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Abstract. The chemical modifier BRD4770 treatment enhanced secondary metabolite production, cellulase and antioxidant activity. *Pseudopestalotiopsis theae* SAL1 compared to both the control and 5-azacytidine. The treated cultures exhibited stronger $ABTS^+$ radical-scavenging activity (EC_{50} = 66.6 µg/mL) and increased cellulase production, with a 22 mm hydrolysis zone and 71.33 µg/mL glucose release. GC-MS and LC-ESI-MS/MS analyses revealed the induction of several previously undetected metabolites, indicating activation of silent biosynthetic gene clusters. Montelukast-like antioxidant compounds were identified in BRD4770-treated extracts and confirmed by RP-HPLC. These findings demonstrate that BRD4770 effectively enhances the metabolic diversity and bioactive potential of *P. theae*.
Keywords: Endophytic fungus, Microbial epigenetics, Chemical epigenetic modulator, Histone modification, Cryptic Metabolites, Metabolite profiling.

Endophytic fungi are a rich but less explored source of biologically active metabolites, particularly those associated with medicinal plants. In this study, the endophytic fungus *Pseudopestalotiopsis theae* isolate SAL1 was isolated from *Saraca asoca* (Roxb.). Using the DNA methyltransferase inhibitor 5-azacytidine (25 µM) and the histone H3K9 methyltransferase inhibitor BRD4770 (100 nM), cryptic secondary metabolite synthesis was increased by epigenetic regulation. Compared to 5-azacytidine, BRD4770 significantly increased metabolite production, enzyme activity and antioxidant potential, demonstrating its greater capacity to activate cryptic metabolic pathways. $ABTS^+$ radical-scavenging activity was markedly enhanced in BRD4770-treated cultures, with an EC_{50} of 66.6 µg/mL, compared with > 133.3 µg/mL in the control. Additionally, cellulase synthesis increased, with wider hydrolysis zones (22 mm) and higher glucose release (71.33 µg/mL), peaking between 72 and 120 h. While LC-ESI-MS/MS detected six additional compounds in BRD4770 and BRD4770 + 5-azacytidine-treated cultures, including fatty acids, phytohormones, phenolics, heterocyclic and fluorinated derivatives, indicating activation of previously silent biosynthetic gene clusters, GC-MS analysis demonstrated the induction of new metabolites following epigenetic treatment. Montelukast-like antioxidant molecules were also found in extracts treated with BRD4770 by LC-ESI-MS/MS. The increased antioxidant activity shown following BRD4770 therapy was supported by reverse-phase HPLC, which revealed retention durations similar to those of a

montelukast standard. Overall, BRD4770 successfully increased *P. theae* chemical diversity by epigenetic modification, demonstrating its promise for the synthesis of industrial enzymes, antioxidant applications and the identification of new naturally occurring bioactive chemicals.

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GROWTH DYNAMICS AND EXOPOLYSACCHARIDE PRODUCTION BY COLLECTION STRAINS OF *LACTOBACILLUS CASEI*

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Abstract. *Lactobacillus casei* subsp. *casei* L187 and *L. casei* subsp. *tolerans* L290 are highly efficient exopolysaccharide (EPS) producers in MRS medium supplemented with 5 % sucrose at 24 °C, yielding 107.5 ± 2.3 and 95.6 ± 2.1 mg/mL of biopolymers, respectively. EPS accumulation is a phase-dependent process: synthesis correlates with the logarithmic growth of the culture and reaches its maximum at 24 h of cultivation. Subsequent disruption of this relationship indicates the activation of secondary metabolism or EPS degradation at later growth phases.
Keywords: lactic acid bacteria*, Lactobacillus casei*, exopolysaccharides, growth phases.

Lactic acid bacteria (LAB) play a key role in the development of sustainable biotechnologies due to their ability to synthesize a wide range of bioactive metabolites (Loo et al., 2025). They can positively influence the gut microbiota, stimulate immune responses, and enhance wound healing (Liu et al., 2025). Among LAB metabolites, exopolysaccharides (EPS) hold a special place — high-molecular-weight biopolymers secreted by cells into the extracellular milieu. EPS perform important biological functions: they form the protective matrix of biofilms, increase resistance to stress factors, act as ion-binding agents, and modulate interactions between bacteria and the macroorganism (Kaur et al., 2023). EPS are valuable texturizers, stabilizers, and carriers for drug delivery systems. Industrial production technologies enable standardized EPS production independently of climatic conditions by precisely controlling cultivation parameters.

The aim of this study was to evaluate the growth dynamics and EPS accumulation by *Lactobacillus casei* strains.

The study was conducted using two strains from the Collection of Marine and Biotechnologically Relevant Microbial Strains of Odesa I. I. Mechnikov National University (*Lactobacillus casei subsp. casei* L187 and *L. casei subsp. tolerans* L290), which demonstrated high EPS-synthesizing activity under established optimal conditions based on preliminary screening. *Lactobacilli* were cultivated in MRS broth supplemented with 5 % D-sucrose at 24 °C for 48 h, with sampling every 6 h. Growth dynamics were evaluated by measuring the optical density of the culture suspension (OD_{600}) using a Quant spectrophotometer (BioTek, USA) and by determining viable cell counts (CFU/mL) using the serial dilution and plate count method on MRS agar. An OD_{600} value of approximately 1.0 was considered equivalent to ~$(1–3)\cdot10^9$ CFU/mL (Trabelsi et al., 2013). In parallel, EPS were isolated from cell-free supernatants at each sampling point and quantified using the phenol–sulfuric acid assay (Yadav et al., 2024). Experiments were performed in three independent replicates.

Analysis of the growth curves of the studied lactobacilli strains revealed a positive relationship between the increase in suspension optical density and the number of viable cells up

to a certain cultivation time point. For both strains, the period from 0 to 24 h was characterized by a simultaneous increase in OD_{600} and CFU/mL accompanied by intensive EPS accumulation. Maximum EPS production (107.5 ± 2.3 mg/mL for *L. casei* subsp. *casei* L187 and 95.6 ± 2.1 mg/mL for *L. casei* subsp. *tolerans* L290) coincided with the highest viable cell counts (≈ 9.5 log CFU/mL) at the transition between the exponential and stationary growth phases. This indicates a direct positive relationship between bacterial growth and EPS biosynthesis at the early stages of cultivation. After 24 h of cultivation, OD_{600} remained stable or increased only slightly, whereas viable cell counts gradually declined. At the same time, EPS concentrations no longer increased and even showed a slight decrease, indicating the cessation of active EPS biosynthesis and suggesting partial polymer degradation or reutilization. The indirect relationship between OD_{600} and viable cell counts observed during the stationary phase is typical of lactobacilli and is associated with changes in the physical properties of the culture medium, accumulation of biopolymers, and cell aggregation. The disruption of the positive correlation between growth parameters and EPS accumulation during the later cultivation stages indicates that EPS biosynthesis is not solely dependent on biomass formation but is regulated by the growth phase and the physiological state of the cells (Cui et al, 2023).

In conclusion, the conducted study revealed a clear phase-dependent accumulation of EPS by lactobacilli. During the lag and exponential phases (0–24 h), biomass increase was directly associated with intensive EPS biosynthesis, with the highest accumulation coinciding with the transition point of the cultures into the stationary phase (24 h). During the stationary phase (after 24 h), this relationship was disrupted. In *L. casei* subsp. *casei* L187, a moderate decrease in EPS concentration was observed, suggesting activation of degradative enzymes (glycosidases) or polymer reutilization under carbon-limited conditions. In contrast, *L. casei* subsp. *tolerans* L290 ceased EPS synthesis after 24 h, while the EPS concentration remained stable.

The obtained data indicate that EPS production by the studied lactobacilli is a regulated process controlled by the physiological state of the bacterial population and the growth phase, representing a secondary metabolic product. The determined regularities provide a fundamental basis for optimizing industrial EPS production processes and developing novel functional biotechnological products.

References

1. Cui, Y., Dong, S. & Qu, X. (2023). New progress in the identifying regulatory factors of exopolysaccharide synthesis in lactic acid bacteria. World J Microbiol Biotechnol, 39 (11), 301. doi: 10.1007/s11274-023-03756-4
2. Kaur, N. & Dey, P. (2023). Bacterial exopolysaccharides as emerging bioactive macromolecules: from fundamentals to applications. Research in Microbiology, 174 (4), 1–24. doi:10.1016/j.resmic.2022.104024
3. Liu, W., Wei, Y., Xiang, R. Dong, B. & Yang, X. (2025). Lactic acid bacteria exopolysaccharides unveiling multifaceted insights from structure to application in foods and health promotion. Foods, 14 (5), 823. doi:10.3390/foods14050823
4. Loo, J. S., Oslan, S. N. H., Mokshin, N. A. S., Othman, R., Amin, Z., Prihanto, A. A. & Tan, J. S. (2025). Comprehensive review of strategies for lactic acid bacteria poduction and metabolite enhancement in probiotic cultures: multifunctional applications in functional foods. Fermentation, 11 (5), 241. doi:10.3390/fermentation11050241
5. Trabelsi, I., Bejar, W., Ayadi, D., Chouayekh, H., Kammoun, R., Bejar, S. & Salah, R. B. (2013). Encapsulation in alginate and alginate coated-chitosan improved the survival of newly probiotic in oxgall and gastric juice. Int J Biol Macromol, 61. 36–42. doi:10.1016/j.ijbiomac. 2013.06.035
6. Yadav, M. K., Song, J. H., Vasquez, R., Lee, J. S., Kim, I. H. & Kang, D. K. (2024). Methods for detection, extraction, purification, and characterization of exopolysaccharides of lactic acid bacteria – A systematic review. Foods. 13 (22). 3687. doi: 10.3390/foods13223687

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OVEREXPRESSION OF PENTAERYTHRITOL TETRANITRATE REDUCTASE FROM *ENTEROBACTER CLOACAE PB2* IN *ESCHERICHIA COLI* CELLS

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Abstract. The recombinant strain of *Escherichia coli* overproducing $(His)_6$-tagged pentaerythritol tetranitrate reductase (PETNR) from *Enterobacter cloacae PB2* has been constructed. This strain can be used to develop a cost-efficient large-scale procedure for isolation and one step purification by metal-affinity chromatography of a highly specific PETNR, necessary for the construction of enzymatic kits and biosensors for assay of PETN.
Keywords: pentaerythritol tetranitrate; pentaerythritol tetranitrate reductase; gene cloning.

Pentaerythritol tetranitrate (PETN) is one of the most widely used nitrate ester explosives in military, mining, and civil engineering applications owing to its high detonation performance and chemical stability (Manner et al. 2022). At the same time, the extensive use of PETN has increased the risk of environmental contamination, accidental release, and its misuse in criminal and terrorist activities (Li et al. 2014). Therefore, the development of rapid, sensitive, and portable analytical methods for PETN determination has become an important challenge in environmental monitoring, forensic science, homeland security, and remediation technologies.

Among emerging analytical approaches, biosensors have attracted considerable attention because they combine the high selectivity of biological recognition elements with rapid signal transduction (Naresh et al. 2021). Enzyme-based biosensors are particularly attractive owing to their exceptional catalytic efficiency and substrate specificity (Alvarado-Ramirez et al. 2021).

In current report, we describe a construction of the *Escherichia coli* recombinant strain overproducing $(His)_6$-tagged pentaerythritol tetranitrate reductase (PETNR) from *Enterobacter cloacae PB2*. A codon optimized PETNR gene (GeneArt, Life Technologies) was synthesized and cloned into the pET21a expression vector. The resulting plasmid was introduced into *Escherichia coli* BL21(DE3). The recombinant strain of *E. coli* that overproduces soluble $(His)_6$-tagged PETNR has been constructed. The enzyme preparations are sufficiently stable and possess activity of 185 U/mg. The yield of the PETNR is approximately 120 mg from 1 L culture. The most prominent advantages of the obtained preparation are a high yield and simple isolation of the enzyme. These findings can be used to develop a cost-efficient large-scale procedure for isolation and one step purification by metal-affinity chromatography of a highly specific PETNR, necessary for the construction of enzymatic kits and biosensors for assay of PETN.

References

1. Manner et al. (2022). Chemical Evaluation and Performance Characterization of Pentaerythritol Tetranitrate (PETN) under Melt Conditions. *ACS Mater Au*., *2*(4), 464–473. Doi: 10.1021/acsmaterialsau.2c00022.
2. Li Zhuang et al. (2014). Laboratory and pilot-scale bioremediation of Pentaerythritol Tetranitrate (PETN) contaminated soil. *Hazard. Mater., 264*, 261–268. https://doi.org/10.1016/j.jhazmat.2013.11.035
3. Naresh et al. (2021). A Review on Biosensors and Recent Development of Nanostructured Materials-Enabled Biosensors. *Sensors*, *21*(4), 1109. Doi: 10.3390/s21041109.
4. Alvarado-Ramirez et al. (2021). Enzyme (Single and Multiple) and Nanozyme Biosensors: Recent Developments and Their Novel Applications in the Water-Food-Health Nexus. *Biosensors*, *11*(11), 410. Doi: 10.3390/bios1111.

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EFFECT OF AQUEOUS FILM-FORMING FIREFIGHTING FOAM "SOFIR AFFF" ON NITROGEN-CYCLING MICROORGANISMS

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Abstract. The study investigated the effect of "SOFIR AFFF" firefighting foam on nitrogen-cycling microorganisms in grey forest soil. The abundance of microorganisms involved in nitrogen cycling was assessed one day, 14 days, and two months after soil contamination. One day after contamination, the abundance of ammonifying and nitrifying microorganisms decreased, whereas nitrogen-fixing and oligonitrophilic microorganisms increased. After 14 days and two months, the abundance of nitrogen-cycling microorganisms increased compared with the control. The stimulatory effect may be associated with the use of certain foam components as sources of carbon, nitrogen, and energy, indicating adaptation of soil microbial communities to "SOFIR AFFF".
Keywords: film forming foam (AFFF), soil microbocenosis, nitrogen-cycling microorganisms, fire.

The full-scale war in Ukraine has caused widespread damage to fuel infrastructure, including oil depots, fuel and lubricant storage facilities, oil refineries, and logistics hubs. Fires at critical infrastructure facilities have specific characteristics, including high heat release intensity, a risk of re-ignition, and the danger of vapor–air mixture explosions. Aqueous film-forming foams (AFFF) are among the most effective firefighting agents for flammable liquids. They form an aqueous film on the surface of hydrocarbons, isolating the flammable liquid from oxygen and suppressing evaporation (Cao et al., 2022).

Aqueous film-forming foams are specialized firefighting agents developed in the 1960s for extinguishing class B fires caused by the ignition of non-polar (water-insoluble) flammable liquids, such as petroleum, gasoline, kerosene, fuel oil, lubricants, benzene, cyclohexane, etc., as well as polar (water-soluble) flammable liquids, including ethanol, methanol and other alcohols, organic acids, aldehydes, ketones, and polar solvents used in the paint and coatings industry (Peshoria et al., 2020).

AFFF formulations contain surfactants that reduce the surface tension of aqueous solutions and facilitate foam formation, as well as other additives that improve stability, provide freeze protection, prevent corrosion, and enhance other functional properties. The surfactants used in AFFF include per- and polyfluoroalkyl substances (PFAS), which are widely used in various industries as water-repellent agents, non-stick coatings for cookware, moisture- and stain-resistant coatings for textiles, food packaging, lubricants, and other applications due to their high chemical stability. Under natural environmental conditions, most PFAS do not undergo complete mineralization but instead persist, slowly transform, or migrate between environmental compartments. Consequently, the release of surfactants and other chemical components of AFFF poses a potential risk of environmental contamination. AFFF solutions primarily contaminate soils and can subsequently migrate into water bodies (Borovykov et al., 2025). The environmental risk posed by these substances is exacerbated by their high environmental persistence, potential for bioaccumulation, and potential toxic effects (Cousins et al., 2020). Nitrifying bacteria and archaea involved in ammonia oxidation, as well as denitrifying microorganisms responsible for the reduction of nitrate to molecular nitrogen, are among the most sensitive to PFAS exposure. Therefore, studying the effects of AFFF on soil microbial communities is essential for predicting environmental risks and developing strategies for the remediation of contaminated areas. Previous studies have demonstrated that fluorosynthetic firefighting foams can significantly alter the abundance and structure of soil microbial communities, including nitrogen-cycling microorganisms (Khanik et al., 2023).

The aim of this study was to investigate the effects of the fluorosynthetic aqueous film-forming firefighting foam "SOFIR AFFF" on nitrogen-cycling microorganisms in grey forest soil one day, 14 days, and two months after contamination with the foam. Soil suspension dilutions (10^{-3}–10^{-6}) were prepared and inoculated onto the following nutrient media: meat-peptone agar (MPA), Ashby's medium, and Winogradsky's medium. The plates were incubated at 28 °C for 2–7 days, after which the number of colonies was determined. All experiments were performed in triplicate. Uncontaminated soil was used as the control. After colony counting, the mean number of colonies was determined and recalculated per 1 g of soil using the formula: $K = a \times n \times 10 / (0.1 \times m)$, where K is the number of colony-forming units per gram of soil; a is the mean number of colonies per plate; n is the dilution from which inoculation was performed; 10 is the volume of the initial suspension, mL; 0.1 is the volume of the diluted suspension, mL; and m is the mass of soil used for analysis, g (Gudz et al., 2014). Statistical analysis was performed using Microsoft Excel 2003 and OriginPro 7.0.

One day after the application of "SOFIR AFFF" to the soil, a slight decrease in the abundance of ammonifying and nitrifying microorganisms was observed, which may indicate an initial toxic or stress response to the components of the firefighting foam. At the same time, the abundance of nitrogen-fixing and oligonitrophilic microorganisms under the influence of "SOFIR AFFF" was approximately twice as high as in the control.

After 14 days, the abundance of ammonifying, nitrifying, nitrogen-fixing, and oligonitrophilic microorganisms increased by approximately 2-fold, 1.5-fold, 2.4-fold, and 2-fold, respectively. An increase in the abundance of nitrogen-cycling microorganisms was also observed two months after soil contamination with "SOFIR AFFF". In our opinion, this stimulatory effect may be associated with the presence of low-toxicity short-chain perfluorocarboxylic acids in the foam formulation, as well as with the utilization of certain foam components as sources of carbon, nitrogen, and energy. It is also likely that components of the firefighting foam, including ammonium salts, amines, and quaternary ammonium compounds, provided an additional source of nitrogen, potentially stimulating ammonification and nitrification processes. Thus, "SOFIR AFFF" had predominantly a stimulatory effect on nitrogen-cycling microorganisms in grey forest soil, which may indicate adaptation of microbial communities to the stress factor and the gradual incorporation of foam components into microbial metabolic processes.

References

1. Borovykov, V. O., Slutska, O. M., Skorobahatko, T. M., et al. (2023). Rozroblennia ta zastosuvannia vitchyznianoi normatyvnoi bazy shchodo otsiniuvannia yakosti pinoutvoriuvachiv dlia hasinnia pozhezh na perekhidnyi period [Development and application of the domestic regulatory framework for assessing the quality of foam concentrates for firefighting during the transitional period]. Fire Safety, 42, 5–15. https://doi.org/10.32447/20786662.42.2023.01
2. Cao, Y., et al. (2022). Occurrence of PFASs and its effect on soil bacteria at a fire-training area using PFOS-restricted aqueous film-forming foams. iScience, 25(4), 104084. https://doi.org/10.1016/j.isci.2022.104084
3. Cousins, I. T., DeWitt, J. C., Glüge, J., et al. (2020). The high persistence of PFAS is sufficient for their management as a chemical class. Environmental Science: Processes & Impacts, 22(12), 2307–2312. https://doi.org/10.1039/D0EM00355G
4. Gudz, S. P., Hnatush, S. O., Yavorska, G. V., et al. (2014). Praktykum z mikrobiologii': pidruchnyk [Workshop on microbiology: textbook]. Lviv. Nac. Univ. imeni Ivana Franka. Ser. Biol. Stud., Lviv (in Ukrainian).
5. Khanik, Y. O., Zvir, G. I., Grynchyshyn, N. M. (2023). Vplyv ftorsyntetychnoi plivkoutvoriuvalnoi piny dlia pozhezhohasinnia na mikrobiotsenoz gruntu [Influence of firefighting fluorosynthetic film forming foam on soil microbocenosis]. Ecology and Noospherology, 34(2), 61–69. https://doi.org/10.15421/032310
6. Peshoria, S., Nandini, D., Tanwar, R. K., Narang, R. (2020). Short-chain and long-chain fluorosurfactants in firefighting foam: A review. Environmental Chemistry Letters, 18(4), 1277–1300. https://doi.org/10.1007/s10311-020-01015-8

BIOCHEMISTRY OF MICROORGANISMS

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MODULATION OF BACTERIAL CELLULOSE PRODUCTION BY THE KOMBUCHA SYMBIOTIC COMMUNITY THROUGH INOCULUM RATIO AND METABOLIC SUPPLEMENTATION

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Abstract. This study investigated how the fresh tea medium-to-SCOBY (symbiotic culture of bacteria and yeast) inoculum ratio and supplementation with α-ketoglutaric acid (α-KG), citric acid, magnesium sulfate, and glycerol affect bacterial cellulose (BC) biofilm formation, medium pH, and total polyphenol content over 14 days of static fermentation. α-KG and citric acid, both TCA cycle intermediates, produced qualitatively similar biphasic dose-response effects on BC yield: moderate supplementation doubled biofilm mass, whereas high doses were inhibitory. $MgSO_4$ and glycerol both reduced BC yield. Thus, α-KG and citric acid, at defined moderate concentrations, are promising low-cost supplements for enhancing BC yield in kombucha-based systems.
Keywords: bacterial cellulose, alpha-ketoglutaric acid, citric acid, kombucha.

Bacterial cellulose (BC) is a biopolymer produced by several bacterial species and has attracted considerable attention as a sustainable alternative to conventional textile materials, which often require energy-intensive processing and harsh chemical treatments. Among BC-producing microorganisms, acetic acid bacteria belonging to the genera *Komagataeibacter*, *Acetobacter*, and *Gluconobacter* are particularly well characterized *(*El-Gendi et al., 2022*)*. More recently, increasing attention has been directed toward the use of complex microbial communities, including the symbiotic cultures associated with kombucha, as potential platforms for BC production (Gagliardi et al., 2025). Kombucha is a fermented beverage produced through the metabolic interactions of a symbiotic culture of bacteria and yeasts (SCOBY), which typically includes acetic acid bacteria, lactic acid bacteria, and various yeast species. Previous studies have demonstrated that supplementation with specific metabolites and nutrients, including ethanol, glycerol, organic acids such as acetate, ascorbic acid, and nitrogen sources, can enhance BC production by kombucha-associated or kombucha-derived *Komagataeibacter* strains (Li et al., 2012; Agustina et al., 2025). While traditional optimization strategies for BC largely focus on upstream carbon sources like glycerol or ethanol, downstream metabolic regulators remain poorly understood.

In the present study, alpha-ketoglutaric acid (α-KG), citrate, and Mg^{2+} were selected as kombucha supplements because they influence distinct metabolic processes that may affect SCOBY-mediated bacterial cellulose formation.

A commercial kombucha starter (SCOBY) purchased from a local market in Ivano-Frankivsk (Ukraine) in 2024 was used in the experiments. The SCOBY starter was cultured

in the sweetened tea infusion to create stock cultures. The sweetened tea infusion was prepared by mixing boiled water, Monomax "Ceylon" black pekoe tea (1 tea bag per 700 mL), and sucrose (50 g/L). In the first experiment, the effects of the fresh tea-to-SCOBY inoculum ratio were determined. In the next experiment, the tea infusion was divided into several portions, supplemented with the tested compounds, and mixed with the parental culture (kombucha SCOBY inoculum). Independent experiments were conducted to evaluate the effects of supplementation with citric acid, α-ketoglutaric acid (α-KG), magnesium sulfate ($MgSO_4$), and glycerol on kombucha fermentation and bacterial cellulose production. The wet and dry masses of the BC biofilms formed were assessed on day 14. The acidity of the medium was measured with a pH meter at the beginning of cultivation (day 0) and then on day 14. Total polyphenols were measured using the Folin-Ciocalteu colorimetric method.

A higher medium-to-inoculum ratio (2:1) yielded significantly more wet and BC biomass than a lower ratio (1:2), and this ratio was selected as the baseline conditions for further experiments. Both α-KG and citric acid, tricarboxylic acid cycle intermediates, showed biphasic, dose-dependent effects on BC yield: moderate supplementation (0.25 g/L α-KG; 0.5 g/L citric acid) approximately doubled biofilm mass relative to control, while higher doses markedly suppressed or abolished BC growth, with α-KG showing stronger inhibition at high concentration than citric acid. α-KG supplementation also modestly reduced total polyphenol content in kombucha in a dose-dependent manner. In contrast, $MgSO_4$ (0.8 g/L) and glycerol (40 g/L) both significantly decreased BC yield relative to the control, and glycerol also reduced polyphenol content. Medium pH declined over cultivation in all groups regardless of treatment, indicating that supplementation affected cellulose biosynthesis rather than the overall acidification dynamics of fermentation.

The results support α-KG and citric acid, applied within a narrow, optimal concentration range, as practical, low-cost strategies for improving BC yield in kombucha fermentation, and also underscore the importance of dose optimization when implementing supplementation strategies. With regard to magnesium sulfate and glycerol, the unexpected reduction in yield observed in this study warrants "dose-response" studies in the tea-based SCOBY system, since neither compound was tested across a wide range of concentrations.

References

1. Agustina, W., Gamonpilas, C., & Boontawan, A. (2025). Effect of additives on bacterial cellulose yield and properties in Thai red tea kombucha fermentation. *International Journal of Biological Macromolecules*, *334*, 149143. Doi:10.1016/j.ijbiomac.2025.149143.
2. El-Gendi, H., Taha, T.H., Ray, J. B., & Saleh, A. K. (2022). Recent advances in bacterial cellulose: a low-cost effective production media, optimization strategies and applications. *Cellulose*, *29*, 7495–7533. Doi:10.1007/s10570-022-04697-1.
3. Gagliardi, T. R., De Farias Nascimento, A. & Valencia, G.A. (2025). Kombucha bacterial cellulose: a promising biopolymer for advanced food and nonfood applications. *Foods*, *14*, 738. Doi:10.3390/foods14050738.
4. Li, Y., Tian, C., Tian, H., Zhang, J., He, X., Ping, W., & Lei, H. (2012). Improvement of bacterial cellulose production by manipulating the metabolic pathways in which ethanol and sodium citrate are involved. *Appl Microbiol Biotechnol*, *96*, 1479–1487. Doi:10.1007/s00253-012-4242-6.

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FILAMENTOUS FUNGI AS PRODUCERS OF EXTRACELLULAR HYDROLYTIC ENZYMES

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Abstract. Filamentous fungi contribute in the degradation of organic material and help for attaching various surfaces through secretion of extracellular hydrolytic enzymes. However, the enzymatic capacity of numerous species of Dothideomycetes associated with plants stay insufficiently characterrized. In this study, five filamentous species of fungi, isolated in pure culture from infected plant leaves (*Atriplex patula, Cannabis sativa, Chelidonium majus* and *Sambucus nigra*). Were identified as *Pseudocercospora depazeoides* (strain 1), *Septoria cannabis* (2), *Septoria chelidonii* (3), *Cercospora chenopodii* (4) and *Alternaria chartarum* (5). The study of extracellular proteolytic and glycosidic activities were carried out in culture liquid supernatants. None of the istrains tested hydrolyzed elastin, fibrin, fibrinogen or collagen, while keratinolytic activity was detected in one mycelial culture. β-D-Glucosidase activity was observed in all tested fungi, whereas α-L-rhamnosidase activity was detected only in one strain, The results indicate that the associated with plants fungi could may be of interest for the study of other enzymatic activities
Keywords: *Septoria, Cercospora*, *Pseudocercospor,* glycosidases, keratinase.

Filamentous fungi are important components of plant-associated organism communities. By colonizing plant tissues, they participate in the transformation of plant-derived organic matter and contribute to nutrient cycling. The ability to exploit different ecological niches is closely associated with extracellular enzymes that release low-molecular-weight compounds from complex biopolymers. The fungal enzymes, in particualitly extracellular proteases and glycosidases have both ecological and physiological significance, and also are intensively investigated as potential biocatalysts for food, pharmaceutical, agricultural and biomass-processing technologies (Scherlach & Hertweck, 2021; Lübeck & Lübeck, 2022). Their production is particularly important for fungi as the plant tissues contain structurally complex polymers and diverse secondary metabolites. Proteolytic enzymes participate in protein turnover and nutrient acquisition, whereas glycosidases contribute to the utilization of plant carbohydrates and glycosylated compounds. So, β-D-glucosidases are involved in the final stages of cellulose hydrolysis and are of interest for lignocellulosic biomass conversion, while α-L-rhamnosidases can remove terminal rhamnose residues from glycosides and are relevant to the biotransformation of plant flavonoids and aroma precursors (Lübeck & Lübeck, 2022). Keratinases are the other specialized group of extracellular proteases capable to degrade the highly resistant keratin structures and are perspective in biological processing of keratin-containing wastes (Alwakeel et al., 2021). The aim of this study was to characterize the extracellular proteolytic and glycosidase potential of filamentous fungi and to identify isolates showing enzymatic activity.

Materials and Methods. Studied fungi were isolated from damaged leaves of *Atriplex patula, Cannabis sativa, Chelidonium majus* and *Sambucus nigra,* collected in the Kyiv region in 2024. Pure cultures were maintained on potato dextrose agar at 25 °C. Proteolytic

activity was assessed in culture liquid supernatants using elastin, fibrin, fibrinogen, collagen, casein and keratin as substrates. α-L-Rhamnosidase and β-D-glucosidase activities were determined using p-nitrophenyl-α-L-rhamnopyranoside and *p*-nitrophenyl-β-D-glucopyranoside, respectively. Naringin and neohesperidin was also used to determine α-L-rhamnosidase activity. The identification of fungi was based on macro- and micromorphological characteristics, including light microscopy and scanning electron microscopy. Enzyme assays were performed in 5–7 replicates, and differences were evaluated statistically at $P < 0.05$.

Results and Discussion. Fungal isolates isolated from the leaves of various plants (*Atriplex patula, Cannabis sativa, Chelidonium majus* and *Sambucus nigra,*) were identified as *Pseudocercospora depazeoides* (strain 1), *Septoria cannabis* (2), *Septoria chelidonii* (3), *Cercospora chenopodii* (4) and *Alternaria chartarum* (5). Thus, the enzymatic screening covered representatives of several genera of Dothideomycete and observed enzymatic activities expand the available information on the physiological characteristics of these plant-associated fungi.

All studied strains significantly differed in their extracellular enzymatic profiles. None of the tested cultures demonstrated detectable hydrolysis of elastin, fibrin, fibrinogen or collagen, remained whereas at trace levels exerted caseinolytic activity. Keratinolytic activity was detected only in *Alternaria chartarum* (5), reaching 4 U/ml. This result is importand because keratin is a structurally resistant protein stabilized by disulfide bonds, hydrogen bonding and hydrophobic interactions, and fungal keratinases are therefore of interest for environmentally compatible processing of keratinous residues.

In contrast to the limited proteolytic activity, glycosidase activity was detected more broadly. All tested strains produced β-D-glucosidase, with activities ranging from 0.2 to 0.4 U/ml. The highest values, 0.4 U/ml, were observed for from *Pseudocercospora depazeoides* (1) and *Septoria cannabis* (2). Only strains from *Pseudocercospora depazeoides* produced α-L-rhamnosidase, with an activity of 0.37 U/ml on the synthetic substrate. This strain 1 also hydrolyzed the natural substrates naringin and neohesperidin, with activities of 0.35 and 0.20 U/ml, respectively. The lower activity toward natural substrates compared with the synthetic substrate suggests differences in substrate accessibility or enzyme–substrate interaction. Nevertheless, hydrolysis of naringin and neohesperidin demonstrates the ability of this fungal strains to act on naturally occurring rhamnosylated flavonoids.

Conclusions. The studied filamentous fungi showed low or undetectable activity toward the tested protein substrates but displayed a more pronounced activity to glycosides. Particularly noteworthy are the keratinase activity of *A. chartarum* and the α-L-rhamnosidase activity of *P. depazeoides*. β-D-Glucosidase activity was detected in all isolates, with the highest activity in *P. depazeoides* and *S. cannabis*, that indicates a broader capacity for transformation of plant-derived glycosides by studied fungi. These findings indicate that less-studied plant-associated fungi may represent useful sources of extracellular hydrolases and justify further investigation of their enzyme systems, including optimization of cultivation conditions and biochemical characterization of the most active enzymes.

References

1. Alwakeel, S. S., Ameen, F., Al Gwaiz, H., Sonbol, H., Alghamdi, S., Moharram, A. M., & Al-Bedak, O. A. (2021). Keratinases produced by *Aspergillus stelliformis*, *Aspergillus sydowii*, and *Fusarium brachygibbosum* isolated from human hair: Yield and activity. Journal of Fungi, 7(6), 471. https://doi.org/10.3390/jof7060471
2. Lübeck, M., & Lübeck, P.S. (2022). Fungal Cell Factories for Efficient and Sustainable Production of Proteins and Peptides. Microorganisms. 10(4), 753, 2–24. https://doi.org/10.3390/microorganisms10040753
3. Scherlach, K., & Hertweck, C. (2021). Mining and unearthing hidden biosynthetic potential. Nature Communications, 12, 3864. https://doi.org/10.1038/s41467-021-24133-5

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SIMILARITY AND DIFFERENCE OF EXOCHITINASES ChiA AND ChiB OF *STREPTOMYCES COELICOLOR* A3(2)

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Abstract. Defining the similarities and differences in the molecular organization of precursors of exochitinases whose genes arose as a result of one ancestral gene duplication can be important for studying the evolution of *Streptomyces* enzymes. BLASTP- analysis was carried out of the primary structures of the chitinase precursors ChiA and ChiB and their individual elements. Our research has shown that as a result of various genetic processes, the sequences of *chi* genes have acquired significant differences. The biggest changes affected the terminal fragments of the *chi*A and *chi*B genes, for example, those encoding SP and CBM_4_9.
Keywords: amino acid sequence, precursors of exochitinase, domain, similarity indicator.

Defining the similarities and differences in the molecular organization of exochitinase ChiA and ChiB precursors can be important for studying the evolution of streptomycete enzymes (Bussink et al., 2007; Karlsson & Stenlid, 2008; Huang et al., 2012; Shen et al., 2020).

Chitinases (systematic name (1→4)-2-acetamido-2-deoxy-β-D-glucan glycanohydrolase, EC 3.2.1.14) are hydrolytic enzymes that break down glycosidic bonds in a polysaccharide chitin molecule. Microbial chitinases are classified into two glycosidase families – GH18 and GH19 (Kawase et al., 2006; Lv et al., 2023). All streptomycete chitinases function outside the cell and are synthesized as enzyme precursors (Saito et al., 2001; Saito et al., 2003; Owji et al., 2018).

The purpose: to identify the similarities and differences in the organization of precursors of chitinases chiA and chiB, whose genes arose as a result of one ancestral gene duplication.

Materials and methods. The paper analyzed information about amino acid sequences and the domain organization of enzyme molecules precursors ChiA (BAA75642.1) and ChiB (BAA75643.1) available in public online databases like NCBI, CAZy, KEGG, and InterPro. Sequence alignment was performed using the BLASTP program package (reference protein – refseq_protein) from NCBI. Sequence similarity indicators: Query coverage (Qc); Identity (I); Positive (P). In bioinformatic studies, the ChiA protein sequence (BAA75642.1/Q9Z9M9) was used as the query.

Research results and their discussion. The *S. coelicolor* A3(2) is the streptomycete strain, which has the most well-studied chitin hydrolysis processes (Saito et al., 2001; Saito et al., 2003; Kawase et al., 2006; Bao & Yang, 2019). As established earlier, *chi*A (RC_SR27180) and *chi*B (RC_SR30580) genes of the *S. coelicolor* A3(2) strain had a common ancestral gene because they were formed as a result of duplication (Saito et al., 2001; Saito et al., 2003). Exochitinase precursors ChiA and ChiB contain same essential elements (catalytic domains and signal peptides) and optional domains (chitin-binding domains or fibronectin type III) (Saito et al., 2001; Saito et al., 2003). Even though organizations of chitinases is similar, protein molecules have different molecular sizes: 571 aa (ChiA) and 610 aa (ChiB).

It was decided to identify (refseq_protein) the similarities and differences of the primary structures of chitinase precursors ChiA and ChiB and their individual elements.

Signal peptides (SP) are short N-terminal fragments of exoenzyme precursors that control the secretion and translocation of proteins in all living organisms (von Heijne, 1985; Owji et al., 2018). The molecular size of the SP of precursor ChiA is 36 aa, the size of the SP of precursor ChiB is 40 aa. The carried out BLASTP-analysis did not reveal any similarity in the structures of SP precursors of chitinases ChiA and ChiB. Despite the organization of signal peptides according to a single pattern, one of the noticeable features of signal sequences as a group is their extraordinary variability in overall length and amino acid sequence across organisms (von Heijne, 1985; Owji et al., 2018).

The database InterPro presents a classification of chitinase ChiA (Q9Z9M9) domains – chitin-binding domain (CBM_4_9 – PF0208), fibronectin type 3 domain (fn3 – PF00041), and catalytic domain (Glyco_hydro_18 – PF02018). It was found that in the chitin-binding domain sequences there are several small homologous fragments, each a few amino acids long. The similarity of catalytic domain sequences (Qc = 95 %, I = 38 %, P = 55 %) indicates their functional relatedness. However, the greatest similarity (Qc = 99 %, I = 63 %, P = 71 %) was found in fn3 sequences. The similarity (Qc = 96 %, I = 47 %, P = 62 %) of mature chitinase sequences (chains) confirmed their close relationship. It has been found that when chitin is added, the level of chitinase ChiA expression significantly exceeds the expression of chitinase ChiB (Saito et al., 2003).

Conclusion. Our research has shown that as a result of various genetic processes, the sequences of *chi* genes have acquired significant differences. The biggest changes affected the terminal fragments of the *chi*A and *chi*B genes, for example, those encoding SP and CBM_4_9.

References

1. Bao, L. & Yang, S-H. (2019). Microbial chitinases: properties, current state and biotechnological applications. *World Journal of Microbiology & Biotechnology,* 35(9), 130–144. https://doi.org/ 10.1007/ s11274-019-2721-y
2. Bussink, A. P., Speijer, D., Aerts, J. M., & Boot, R. G. (2007). Evolution of mammalian chitinase(-like) members of family 18 glycosyl hydrolases. *Genetics*, *177*(2), 959–970. https://doi.org/ 10.1534/genetics.107.075846
3. Huang, Q. S., Xie, X. L., Liang, G., Gong, F., Wang, Y., Wei, X. Q., Wang, Q., Ji, Z. L., & Chen, Q. X. (2012). The GH18 family of chitinases: their domain architectures, functions and evolutions. *Glycobiology*, *22*(1), 23–34. https://doi.org/10.1093/glycob/cwr092
4. Karlsson, M., & Stenlid, J. (2008). Evolution of Family 18 Glycoside Hydrolases: Diversity, Domain Structures and Phylogenetic Relationships. *Microbial Physiology,* 16(3–4), 208–223. Portico. https://doi.org/10.1159/000151220
5. Kawase, T., Yokokawa, S., Saito, A., Fujii, T., Nikaidou, N., Miyashita, K., & Watanabe, T. (2006). Comparison of enzymatic and antifungal properties between family 18 and 19 chitinases from *S. coelicolor A3*(2). *Bioscience, biotechnology, and biochemistry*, 70(4), 988–998. https:// doi.org/10.1271/bbb.70.98816.
6. Lv, J., Lv, X., Ma, M., Oh, D. H., Jiang, Z., Fu, X. (2023). Chitin and chitin-based biomaterials: A review of advances in processing and food applications. *Carbohydrate polymers*, *299*, 120142. https://doi.org/10.1016/j.carbpol.2022.120142
7. Owji, H., Nezafat, N., Negahdaripour, M., Hajiebrahimi, A. & Ghasemi, Y. (2018) A comprehensive review of signal peptides: Structure, roles, and applications. *European Journal of Cell Biology*, 97 (6), 422-441. https://doi.org/10.1016/j.ejcb.2018.06.003.
8. Saito, A., Miyashita, K., Biuković, G., & Schrempf, H. (2001). Characteristics of a *Streptomyces coelicolor* A3(2) Extracellular Protein Targeting Chitin and Chitosan. *Applied and Environmental Microbiology,* 67(3), 1268–1273. https://doi.org/10.1128/AEM.67.3.1268-1273.2001
9. Saito A., Fujii T. & Miyashita K. (2003). Distribution and evolution of chitinase genes in *Streptomyces* species: involvement of gene-duplication and domain-deletion. *Antonie Van Leeuwenhoek,* 84, 7–15. https://doi.org/10.1023/A:1024463113606.

10. Shen, D., Wang, J., Dong, Y., Zhang, M., Tang, Z., Xia, Q., Nyawira, K. T., Jing, M., Dou, D., & Xia, A. (2020). The glycoside hydrolase 18 family chitinases are associated with development and virulence in the mosquito pathogen *Pythium guiyangense*. *Fungal genetics and biology : FG & B*, *135*, 103290. https://doi.org/10.1016/j.fgb.2019.103290
11. von Heijne, G. Signal sequences: The limits of variation. (1985*). Journal of Molecular Biology,* 184(1), 99 105, https://doi.org/10.1016/0022-2836(85)90046-4

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NANOZYME CAN SUBSTITUTE A NATURAL *OGATAEA POLYMORPHA* CATALASE ENZYME *IN VIVO*

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Abstract. Nanozymes are promising artificial enzyme substitutes, but their ability to compensate for natural enzyme deficiency in living cells remains insufficiently explored. In this study, catalase-like platinum (nPt) and platinum–gold (nPtAu) nanoparticles were tested in the methylotrophic yeast *Ogataea polymorpha*. Both nanozymes exhibited catalase-like activity *in vitro*. The catalase-deficient *O. polymorpha* C-105 strain was able to grow on methanol-containing media only in the presence of nanozymes. Nanozyme treatment also reduced intracellular hydrogen peroxide levels. Transmission electron microscopy confirmed the presence of nanoparticles inside the yeast cells. These findings demonstrate that synthetic catalase-like nanozymes can functionally replace deficient natural enzymes *in vivo*.
Keywords: nanozyme; platinum nanoparticles; catalase-like activity; *Ogataea polymorpha*; hydrogen peroxide.

Reactive oxygen species (ROS) are continuously generated during cellular metabolism and play important roles in physiological redox signaling. However, excessive ROS accumulation results in oxidative stress and may damage cellular macromolecules, including DNA and proteins (Murphy et al., 2022). Catalase is an important component of the antioxidant defense system because it decomposes hydrogen peroxide into water and molecular oxygen. In methylotrophic yeasts, catalase is particularly important for growth on methanol as the sole carbon and energy source (Gonchar et al., 1990).

Nanozymes are nanomaterials exhibiting enzyme-like catalytic properties and have attracted considerable attention as potential alternatives to natural enzymes because of their high stability and tunable catalytic properties (Wu et al., 2019; Yang et al., 2021). Among them, catalase-like nanozymes are capable of decomposing hydrogen peroxide and therefore have potential applications in the regulation of intracellular oxidative stress (Xu et al., 2022). Platinum-group-element-based nanozymes are of particular interest because of their high catalytic activity, stability, and potential biomedical applications (He et al., 2021). Nevertheless, whether synthetic nanozymes can functionally replace deficient natural enzymes directly within living cells remains insufficiently investigated.

The aim of this study was to determine whether catalase-like platinum-containing nanozymes can compensate for the deficiency of natural catalase in catalase-deficient *O. polymorpha* cells. Platinum nanoparticles (nPt) were synthesized using a chemical reduction method. The resulting nPt nanoparticles were subsequently used as seeds for the

preparation of platinum–gold core–shell nanoparticles [nPt(core)Au(shell), nPtAu]. The synthesized nPt nanoparticles had sizes of 68.1 and 91.3 nm, whereas the corresponding nPtAu nanoparticles were 531.2 and 615.1 nm in size. Both nPt and nPtAu exhibited catalase-like activity in vitro, confirming their ability to decompose hydrogen peroxide (Prokopiv et al., 2023).

The catalase-deficient *O. polymorpha* C-105 strain was used to investigate whether the catalytic activity of the nanoparticles could compensate for the absence of natural catalase *in vivo*. This strain carries a defect in the *CATX* gene and is unable to grow normally under conditions requiring efficient hydrogen peroxide detoxification. Catalase-deficient mutants of methylotrophic yeasts have previously been shown to exhibit impaired regulation of alcohol oxidase synthesis and altered metabolism during growth on methanol (Gonchar et al., 1990). The synthesized nanozymes were introduced into *O. polymorpha* C-105 cells, and their effects on cell growth and intracellular hydrogen peroxide levels were investigated. In the presence of either nPt or nPtAu, the catalase-deficient yeast cells were able to grow on methanol as well as on a mixture of glucose and methanol. In contrast, no growth was observed in the absence of the nanozymes. Importantly, restoration of cell growth was accompanied by a decrease in intracellular hydrogen peroxide levels (Prokopiv et al., 2023).

The ability of nanoparticles to enter living cells is an important factor determining their biological effects. Cellular uptake of nanoparticles depends on their physicochemical properties as well as their interactions with the cell surface and intracellular structures (Behzadi et al., 2017). In the present study, transmission electron microscopy confirmed the presence of nPt and nPtAu nanoparticles within *O. polymorpha* cells, demonstrating that the nanozymes can reach the intracellular environment where hydrogen peroxide is generated. The obtained results demonstrate that synthetic catalase-like nanozymes can functionally compensate for the deficiency of a natural antioxidant enzyme in living microbial cells. The restoration of growth under methanol-containing conditions, together with the simultaneous decrease in intracellular H_2O_2 levels, indicates that the nanoparticles can at least partially substitute for the missing catalase function. These findings provide experimental evidence that nanozyme catalytic activity can be expressed functionally within living cells rather than being restricted to cell-free systems.

In conclusion, platinum and platinum–gold nanozymes were able to compensate for deficient catalase activity in *O. polymorpha* C-105 cells. The nanozyme-mediated decrease in intracellular hydrogen peroxide levels was associated with the restoration of yeast growth under methanol-containing cultivation conditions. These findings highlight the potential of catalase-like nanozymes as intracellular artificial enzyme substitutes and provide a basis for their further application in microbial biotechnology, the screening of novel nanozymes, and the development of cell-based biosensing systems.

References

1. Behzadi, S., Serpooshan, V., Tao, W., Hamaly, M. A., Alkawareek, M. Y., Dreaden, E. C., Brown, D., Alkilany, A. M., Farokhzad, O. C., & Mahmoudi, M. (2017). Cellular uptake of nanoparticles: Journey inside the cell. *Chemical Society Reviews, 46*(14), 4218–4244.
2. Gonchar, M. V., Ksheminska, G. P., Hladarevska, N. M., & Sibirny, A. A. (1990). Catalase-minus mutants of methylotrophic yeast *Hansenula polymorpha* impaired in regulation of alcohol oxidase synthesis. In T. M. Lachowicz (Ed.), *Genetics of respiratory enzymes in yeasts* (pp. 222–228). Wroclaw University Press.
3. He, S. B., Yang, L., Lin, M. T., Balasubramanian, P., Peng, H. P., Kuang, Y., Deng, H. H., Chen, W. (2021). Platinum group element-based nanozymes for biomedical applications: An overview. *Biomedical Materials, 16*(3).

4. Murphy, M. P., Bayir, H., Belousov, V. V., Chang, C. J., Davies, K. J. A., Davies, M. J., Dick, T. P., Finkel, T., Forman, H. J., Janssen-Heininger, Y., & Gems, D. (2022). Guidelines for measuring reactive oxygen species and oxidative damage in cells and in vivo. *Nature Metabolism, 4*, 651–662.
5. Prokopiv, T., Stasyuk, N., & Gonchar, M. (2023). Nanozyme can substitute a natural *Ogataea polymorpha* catalase enzyme in vivo. *Microchimica Acta, 190*, 174. https://doi.org/ 10.1007/ s00604-023-05753-8
6. Wu, J., Wang, X., Wang, Q., Lou, Z., Li, S., Zhu, Y., Qin, L., & Wei, H. (2019). Nanomaterials with enzyme-like characteristics (nanozymes): Next-generation artificial enzymes (II). *Chemical Society Reviews, 48*, 1004–1020.
7. Xu, D., Wu, L., Yao, H., & Zhao, L. (2022). Catalase-like nanozymes: Classification, catalytic mechanisms, and their applications. *Small, 18*(37), 2203400.
8. Yang, W., Yang, X., Zhu, L., Chu, H., Li, X., & Xu, W. (2021). Nanozymes: Activity origin, catalytic mechanism, and biological application. *Coordination Chemistry Reviews, 448*, 214170.

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METABOLIC REMODELING DURING THE EVOLUTIONARY TRANSITION TO MACROSCOPIC MULTICELLULARITY

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Abstract. The evolution of macroscopic body size in multicellular organisms is a key but poorly understood step in the history of life. Using the yeast long-term evolution experiment, in which selection for larger size drove clonal clusters of the budding yeast *Saccharomyces cerevisiae* to become macroscopic over more than 1,000 daily rounds of selection, we combined steady-state metabolomics, ^{13}C metabolic flux analysis, and transcriptomics to characterize the accompanying metabolic changes. We found a system-wide metabolic reallocation from growth toward maintenance, marked by depletion of amino acids, lipids, nucleotides, and central-carbon metabolites, reduced growth rate, and a sharp rise in the protective sugar trehalose. These results indicate that metabolic remodeling, and trehalose accumulation in particular, might be a major driver of the evolutionary transition to macroscopic multicellularity in this system.
Keywords: multicellularity; snowflake yeast; metabolic remodeling; trehalose; proteostasis

The evolution of multicellularity was a major step in the rise of complex life on Earth, yet our understanding of this transition is still incomplete. Progress has been hindered by the ancient origins of extant multicellular lineages, making it difficult to reconstruct the changes that accompanied the emergence of multicellularity. In particular, it remains largely unknown whether metabolic changes facilitated early multicellular evolution.

To test whether metabolism reorganizes during the transition to multicellularity, we used yeast evolved in the Multicellularity Long-Term Evolution Experiment (MuLTEE), an experimental evolution system that lets us examine this transition as it unfolds. In the MuLTEE, replicate populations of *Saccharomyces cerevisiae* founded from a common ancestor undergo daily selection for larger size through rapid settling, driving the evolution

of increasingly large and complex multicellular clusters. Over 1,000 daily rounds of selection, this yeast evolved from microscopic snowflake clusters into macroscopic groups containing hundreds of thousands of clonally related cells (Bozdag et al., 2023). Using steady-state metabolomics, metabolic flux analysis, and transcriptomics, we mapped metabolic changes across this evolutionary course to determine how metabolism is reorganized as multicellular size and complexity evolve.

Macroscopic snowflake yeast shows a broad metabolic transition from growth toward maintenance and structural reinforcement: amino acids, lipids, and TCA intermediates decline while trehalose accumulates massively alongside NAD^+ biosynthesis and cell wall precursors, with the serine-glycine-one-carbon axis showing the strongest evolutionary signal. This transition is highly convergent across independently evolved populations, with metabolic change concentrated around the point at which macroscopic size is reached and then largely plateauing. This global metabolic downshift correlated with a reduced growth rate in the macroscopic multicellular state. Interestingly, we found that trehalose, a storage sugar and chemical chaperone, rose sharply during the transition. Our prior work found that chaperones, particularly Hsp90, declined (Montrose et al., 2024), facilitating the transition toward macroscopic multicellularity and suggesting that trehalose upregulation may compensate for proteostasis. Transcriptomics supports these shifts, and upregulation of the NAD^+/kynurenine pathway reflects the added fermentative burden of larger anaerobic clusters.

Together, our results show that metabolism is broadly reoriented from growth toward maintenance and structural reinforcement in evolved macroscopic clusters, supporting the progression of an evolutionary transition in individuality from small to macroscopic group sizes. Our results also suggest that trehalose – potentially by stabilizing protein folding and modulating cytoplasmic viscosity – is a major driver of this shift.

References

1. Bozdag, G. O., Zamani-Dahaj, S. A., Day, T. C., Kahn, P. C., Burnetti, A. J., Lac, D. T., Tong, K., Conlin, P. L., Balwani, A. H., Dyer, E. L., Yunker, P. J., & Ratcliff, W. C. (2023). De novo evolution of macroscopic multicellularity. *Nature*, *617*(7962), 747–754. https://doi.org/10.1038/s41586-023-06052-1
2. Montrose, K., Lac, D. T., Burnetti, A. J., Tong, K., Bozdag, G. O., Hukkanen, M., Ratcliff, W. C., & Saarikangas, J. (2024). Proteostatic tuning underpins the evolution of novel multicellular traits. Science Advances, 10, eadn2706. https://doi.org/10.1126/sciadv.adn2706

GENETICS OF MICROORGANISMS

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COMPARATIVE ANALYSIS OF ANTIMICROBIAL BIOSYNTHETIC GENE CLUSTERS IN A PUTATIVE NOVEL *PAENIBACILLUS* SPECIES AND ITS PHYLOGENETIC RELATIVES

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Abstract. Three *Paenibacillus* strains isolated from forest litter showed antagonistic activity against fungal phytopathogens, as well as Gram-positive and Gram-negative bacterial pathogens. Whole-genome analysis indicated that these strains represent the same genomic species. Their genomic divergence from the closest characterized *Paenibacillus* species suggests that these isolates may represent a previously undescribed species. Multiple biosynthetic gene clusters showed similarity to those associated with fusaricidins, tridecaptins, paenicidins, brevicidine-like compounds, and icosalides, providing genomic support for the potential production of antimicrobial metabolites.
Keywords: forest-liter-derived *Paenibacillus* strains, microbial antagonism, whole-genome sequencing, comparative genome mining, secondary metabolites.

Forest litter represents a complex microbial habitat characterized by intense competition for nutrients and space. *Paenibacillus* species inhabiting soil and plant-associated environments are known to produce antimicrobial metabolites and extracellular hydrolytic enzymes that may contribute to pathogen suppression (Yuan et al., 2022). The aim of this study was to characterize antimicrobial *Paenibacillus* strains isolated from forest litter and compare their predicted antimicrobial biosynthetic potential with that of closely related *Paenibacillus* species.

Three antimicrobial isolates, B21–B23, were obtained from oak–hornbeam forest litter and identified as members of the genus *Paenibacillus* based on 16S rRNA gene sequencing. Antagonistic activity was evaluated against phytopathogenic fungi, an oomycete, and Gram-positive and Gram-negative bacterial phytopathogens using dual-culture and agar well diffusion assays. Whole-genome sequencing was performed for B21–B23, and biosynthetic gene clusters (BGCs) associated with the production of antimicrobial metabolites were identified using antiSMASH (Blin et al., 2019). The BGC profiles of the isolates were compared with those reported or predicted in closely related species, including *P. terrae, P. peoriae, P. kribbensis, P. polymyxa*, and *P. brasilensis*.

The forest-litter-derived isolates B21–B23 exhibited a broad-spectrum antagonistic profile against the tested fungal and bacterial pathogens. Strain B23 showed the strongest inhibitory activity against *Fusarium verticillioides* and pronounced antibacterial activity against *Xanthomonas campestris, Pectobacterium carotovorum*, and *Salmonella enteritidis*.

Genome mining with antiSMASH identified multiple BGCs associated with antimicrobial metabolite biosynthesis, including clusters showing high similarity to the biosynthetic gene clusters for fusaricidin B, tridecaptin, paenicidin B, and icosalide A/B. In addition, clusters showing lower similarity to BGCs associated with brevicidine, paenilipoheptin, and paeninodin were detected, suggesting additional, potentially unexplored biosynthetic capacity within this lineage. Comparative genome mining of *Paenibacillus* genomes has similarly revealed substantial diversity of antimicrobial BGCs, including both conserved and potentially novel biosynthetic systems (Kim et al., 2024).

Comparative analysis with closely related *Paenibacillus* species placed these findings within the broader genomic context of the genus. BGCs associated with fusaricidin- and tridecaptin-like compounds appeared to be relatively conserved and were also detected in related species, including *P. terrae, P. kribbensis, P. peoriae*, and *P. polymyxa* (Machushynets et al., 2024*)*. Experimental studies have demonstrated the antimicrobial activity of tridecaptin-related compounds, supporting their relevance as important antimicrobial metabolites in *Paenibacillus* (Machushynets et al., 2024). In contrast, several other detected BGCs showed a more restricted distribution or lower similarity to characterized reference clusters. This pattern is consistent with the substantial biosynthetic diversity reported in *Paenibacillus*, including the identification of previously uncharacterized BGCs and novel antimicrobial compounds (Kim et al., 2024).

Overall, the genomic architecture of strains B21–B23 combines conserved biosynthetic features characteristic of the genus with potentially distinctive BGCs. The presence of multiple antimicrobial-associated clusters provides a plausible genomic basis for the broad antagonistic phenotype observed *in vitro*. However, the observed inhibition cannot be directly attributed to individual BGCs based on genome mining alone. The co-occurrence of fusaricidin- and tridecaptin-like clusters with strong antifungal and antibacterial activities, respectively, supports these pathways as candidates for further functional investigation, while the less-characterized clusters may contribute to the expanded antagonistic potential of the isolates.

Taken together, *Paenibacillus* strains isolated from forest litter possess a diverse predicted antimicrobial biosynthetic repertoire that combines conserved pathways with potentially distinctive biosynthetic features. The correspondence between multiple antimicrobial-associated BGCs and experimentally demonstrated broad-spectrum antagonism highlights these strains as promising candidates for further metabolomic and functional characterrization. Comparative genome mining may help distinguish conserved antimicrobial biosynthetic mechanisms from lineage-specific traits potentially associated with adaptation to the forest-litter environment.

References

1. Blin, K., Pascal Andreu, V., de los Santos, E. L. C., Del Carratore, F., Lee, S. Y., Medema, M. H., & Weber, T. (2019). The antiSMASH database version 2: A comprehensive resource on secondary metabolite biosynthetic gene clusters. *Nucleic Acids Research, 47*(D1), D625–D630. https://doi.org/10.1093/nar/gky1060
2. Kim, M. S., Jeong, D.-E., Jang, J.-P., Jang, J.-H., & Choi, S.-K. (2024). Mining biosynthetic gene clusters in *Paenibacillus* genomes to discover novel antibiotics. *BMC Microbiology, 24*, 226. https://doi.org/10.1186/s12866-024-03375-5
3. Machushynets, N. V., Al Ayed, K., Terlouw, B. R., Du, C., & van Wezel, G. P. (2024). Discovery and derivatization of tridecaptin antibiotics with altered host specificity and enhanced bioactivity. *ACS Chemical Biology, 19*(5), 1106–1115. https://doi.org/10.1021/acschembio.4c00034
4. Yuan, L., Jiang, H., Jiang, X., Li, T., Lu, P., Yin, X., & Wei, Y. (2022). Comparative genomic and functional analyses of *Paenibacillus peoriae* ZBSF16 with biocontrol potential against grapevine diseases, provide insights into its genes related to plant growth-promoting and biocontrol mechanisms. *Frontiers in Microbiology, 13*, 975344. https://doi.org/10.3389/fmicb.2022.975344

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A LINK BETWEEN THE MITOCHONDRIAL AND CYTOSOLIC QUALITY CONTROL REVEALED THROUGH ANALYSIS OF ALS-ASSOCIATED MUTATION IN OMA1 PROTEASE USING YEAST MODEL

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Mitochondrial dysfunction is widely recognized as a key causative factor in a gamut of neurodegenerative diseases (Suomalainen & Nunnari, 2024), but molecular underpinnings of its contribution to these maladies remain far from clear. Analysis of samples from patients afflicted with Amyotrophic Lateral Sclerosis (ALS) identified several missense mutations in the conserved metallopeptidase Oma1 (Daoud et al., 2011). Oma1 has emerged as a crucial controller of mitochondrial physiology, signaling, and metabolism (Pernas & Scorrano, 2016; Deshwal et al., 2020; Purohit & Khalimonchuk, 2024); however, the mechanism behind its contribution towards ALS pathogenesis is unclear.

Here, we investigated one such ALS-linked Oma1 mutation using baker's yeast model. Our genetic, biochemical, and multi-omics analyses reveal that the mutation in question does not impair Oma1 topogenesis but affects the enzyme's basal proteolytic activity and stability of the Oma1 high-mass complex. Moreover, cells expressing mutant Oma1 variants show reduced tolerance to homeostatic insults and exhibit profound hallmarks of oxidative damage and increased mitochondria-specific ubiquitination and accumulation of ubiquitinated mitochondrial surface proteins. Remarkably, these effects do not influence the general autophagy or mitophagy flux, suggesting that other facets of mitochondrial clearance are affected, which is in line with the metabolic and transcriptomic changes observed in the Oma1-deficient cells. Our data provide new mechanistic insights into the role of Oma1 and mitochondria in etiology of ALS and unravel a novel link between the mitochondrial and cytosolic quality control systems.

Keywords: yeast model, Oma1, Mitochondria, Proteases, Ubiquitination, ALS.

References:

1. Suomalainen, A. & Nunnari, J. (2024). Mitochondria at the crossroads of health and disease. Cell 1 187, 2601–2627. Doi: 10.1016/j.cell.2024.04.037.
2. Daoud, H., Valdmanis, P.N., Gros-Louis, F., et al. (2011). Resequencing of 29 candidate genes in patients with familial and sporadic Amyotrophic Lateral Sclerosis. JAMA Neurology 68, 587–593. Doi: 10.1001/archneurol.2010.351.
3. Pernas, L. & Scorrano, L. (2016). Mito-morphosis: Mitochondrial fusion, fission, and cristae remodeling as key mediators of cellular function. Annual Reviews on Physiology 78, 505–531. Doi: 10.1146/annurev-physiol-021115-105011.
4. Deshwal, S., Fiedler, K.U., Langer, T. (2020). Mitochondrial proteases: Multifaceted regulators of mitochondrial plasticity. Annual Reviews in Biochemistry 89, 501–528. Doi: 10.1146/annurev-biochem-062917-012739.
5. Purohit, G., Ghosh, P., Khalimonchuk, O. (2024) Mitochondrial metallopeptidase OMA1 in cancer. Advances in Cancer Research 162, 75–97. Doi: 10.1016/bs.acr.2024.05.001.

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STREPTOMYCES SPP. FROM WAR-IMPACTED SOILS: WHOLE-GENOME ANALYSIS, METABOLITE PROFILING, AND ANTAGONISTIC ACTIVITY AGAINST PHYTOPATHOGENS

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Abstract. Fifty streptomycete isolates were recovered from war-impacted soils, of which nine of the most antagonistically active strains were selected for further study based on dual-culture screening against fungi and bacteria. Strains Y15, SK4, SK14 and SK16 displayed the strongest and broadest inhibitory activity, with Y15 producing the largest inhibition zones overall, notably against *Bipolaris sorokiniana* (27.9 ± 0.2 mm) and *Xanthomonas campestris* (15.8 ± 0.5 mm). Whole-genome sequencing and orthoANI analysis identified *Streptomyces* sp. SK4 as *S. lidicus* (100 % identity), while Y15, SK16 and SK14 showed at most 88–92 % identity to their closest relatives. Genome mining with antiSMASH revealed a conserved biosynthetic core shared by Y15 and SK4 alongside seven strain-specific biosynthetic gene clusters in each, and LC-HRMS profiling confirmed production of reedsmycin F and youssoufene A1 by Y15 and streptolydigin by SK4. These findings highlight war-impacted soils as a valuable, underexplored source of biotechnologically promising streptomycetes.

Keywords: *Streptomyces,* war-impacted soil, whole-genome sequencing, biosynthetic gene clusters, antagonistic activity, antibiotics.

Streptomyces spp. are well known as a prolific biofactory of secondary metabolites (Alam et al., 2022). The isolation of novel strains from unfavourable environments, including war-impacted soils, is a valuable source of bioactive representatives, since such strains may combine the adaptive potential required for survival under stress with a unique, biotechnologically valuable metabolism. The aim of this study was to screen *Streptomyces* spp. isolated from war-impacted soils, perform whole-genome sequencing, identification and genome annotation, determine their antifungal and antibacterial activity, and carry out metabolite profiling of the most active strains.

Streptomycete strains were cultivated on GYM medium without prior chemical treatment. Whole-genome sequencing (Illumina NovaSeq) was performed at Explogen LLC (Lviv, Ukraine); Average Nucleotide Identity (ANI) was calculated using the FastANI algorithm implemented in DFAST_QC (Elmanzalawi et al., 2025), and biosynthetic gene clusters (BGCs) were identified using antiSMASH 8.0 (Blin et al., 2025). Antifungal activity was assessed by a modified dual-culture confrontation method, and antibacterial activity by lawn seeding of test bacteria followed by agar-block application and measurement of the inhibition zone. Metabolite profiling of the most promising strains was performed by LC-HRMS (Dionex Ultimate 3000 RSLC coupled to a Bruker maXis 4G hr-ToF mass spectrometer).

In total, 50 isolates were recovered, of which 9 were selected for further study based on their antagonistic activity; strains Y15, SK4, SK14 and SK16 proved most promising. Y15 strain exhibited the largest inhibition zones overall, notably against *Bipolaris*

sorokiniana TR2 (27.9 ± 0.2 mm), *Alternaria arborescens* TR1 (17.5 ± 0.6 mm) and *Fusarium oxysporum* DSM 21731 (15.8 ± 1.7 mm), and the highest antibacterial activity, against *Xanthomonas campestris* IMV 80036 (15.8 ± 0.5 mm). SK16 showed the strongest bacterial suppression after Y15 strain (*Agrobacterium tumefaciens* IMV 8628 and *X. campestris*, 11.8 ± 0.5 mm each) with moderate-to-strong fungal inhibition (up to 11.3 ± 1.2 mm). SK4 train produced large zones against both fungal (*A. arborescens* TR1, 15.7 ± 0.6 mm) and bacterial (*X. campestris* IMV 80036, 10.6 ± 1.3 mm) agents, while SK14 generated the single largest zone against *F. graminearum* DSM 21727 (16.0 ± 0.0 mm), though its antibacterial effect remained low.

By orthoANI analysis, Y15 strain showed the highest similarity to *S. youssoufiensis* JCM 18307 (91.51 %), while SK4 showed 100 % identity with *S. lidicus* ATCC 25470; SK16 was most similar to *S. aurantiacus* JCM 4677 (88.16 %) and SK14 to *S. coriariae* CMB-FB (89.35 %), indicating that Y15, SK16 and SK14 may potentially represent novel species. Genome mining with antiSMASH 8.0 revealed a conserved core of three shared BGCs in Y15 strain and *S. lidicus* SK4 – for isorenieratene, ectoine, and a piericidin-related T1PKS – alongside seven strain-specific clusters in each. *Streptomyces* sp. Y15 was enriched in arylpolyene/polyketide clusters (youssoufene A1 and analogues B1–B4, reedsmycins, echosides A–E, the diisonitrile antibiotics SF2768, nigericin, naseseazine C/C3-arylperroindolines, geosmin), whereas *S. lidicus* SK4 carried predominantly peptide- and siderophore-associated clusters (streptolydigin, the lanthipeptide SapB, the lassopeptide citrulassin D, antipain, desferrioxamine E, a spore-pigment T2PKS, and mannopeptimycin).

LC/MS analysis confirmed that *Streptomyces* sp. Y15 produces reedsmycin F ($[M+H]^+$ m/z 667.4013, RT 7.1 min), a non-glycosylated polyene that binds fungal ergosterol and disrupts membrane integrity, and youssoufene A1 ($[M+H]^+$ m/z 537.2969, RT 14.2 min), a cinnamoyl lipid antibiotic active against Gram-positive bacteria such as *S. aureus*, while *S. lidicus* SK4 produces streptolydigin ($[M+H]^+$ m/z 601.3094, RT 10.8 min), an RNA-polymerase-targeting antibiotic active against *S. aureus* and *B. subtilis*. These results experimentally validate the BGCs predicted by antiSMASH genome mining.

Thus, war-impacted soils proved to be a valuable, underexplored reservoir of biotechnologically promising streptomycetes. Strain Y15 combines the broadest antagonistic spectrum with biosynthetic capacity for both antifungal (reedsmycin F) and antibacterial (youssoufene A1) compounds, making it the most promising candidate for biotechnologies. *S. lidicus* SK4 is a source of the antibiotic streptolydigin, of interest for agricultural and biomedical use, while SK16 and SK14 are additional candidates for targeted biocontrol of bacterial and *Fusarium*-associated phytopathogens, respectively.

References

1. Alam, K., Mazumder, A., Sikdar, S., Zhao, Y.-M., Hao, J., Song, C., Wang, Y., Sarkar, R., Islam, S., Zhang, Y., & Li, A. (2022). *Streptomyces*: The biofactory of secondary metabolites. Frontiers in Microbiology, 13, 968053. https://doi.org/10.3389/fmicb.2022.968053
2. Blin, K., Shaw, S., Vader, L., Szenei, J., Reitz, Z. L., Augustijn, H. E., Cediel-Becerra, J. D. D., de Crécy-Lagard, V., Koetsier, R. A., Williams, S. E., Cruz-Morales, P., Wongwas, S., Segurado Luchsinger, A. E., Biermann, F., Korenskaia, A., Zdouc, M. M., Meijer, D., Terlouw, B. R., van der Hooft, J. J. J., ... Weber, T. (2025). antiSMASH 8.0: Extended gene cluster detection capabilities and analyses of chemistry, enzymology, and regulation. Nucleic Acids Research, 53(W1), W32–W38. https://doi.org/10.1093/nar/gkaf334

3. Elmanzalawi, M., Fujisawa, T., Mori, H., Nakamura, Y., & Tanizawa, Y. (2025). DFAST_QC: Quality assessment and taxonomic identification tool for prokaryotic genomes. BMC Bioinformatics, 26(1), 3. https://doi.org/10.1186/s12859-024-06030-y

ECOLOGY OF MICROORGANISMS

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FUNGAL COMMUNITY PROFILING OF TECHNOGENICALLY IMPACTED SOILS BY ITS2 AMPLICON SEQUENCING

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Abstract. The structure of fungal communities in meadow-brown soils exposed to the impact of the Perechyn TIMVer and Chemical Plant and railway transport was investigated. ITS2 amplicon sequencing was used to characterize the taxonomic composition from phylum to species level. *Ascomycota* dominated all samples; however, the technogenically impacted sites differed in the relative abundance of major taxa. The results indicate a specific response of soil mycobiota to different types of technogenic pressure.
Keywords: soil, mycobiota, ITS2, amplicon sequencing, technogenic pressure.

Anthropogenic pressure transforms soil ecosystems and their microbial communities. Previous studies of Transcarpathian soils demonstrated changes in microbiocenosis in areas affected by railway transport and industrial facilities (Bobryk et al., 2012; Kryvtsova et al., 2017). Similar changes have been reported for other technogenically transformed substrates (Kuzmishyna et al., 2015). ITS2 sequencing enables detailed characterization of fungal communities and their responses to environmental contamination (Bengtsson-Palme et al., 2013; Okrasińska et al., 2022).

The aim of the study was to determine the taxonomic structure of fungal communities in soils affected by the Perechyn TIMVer and Chemical Plant separately and in combination with railway transport, compared with a conditionally control site.

Materials and methods. Meadow-brown soils were sampled in June 2025 in Perechyn from the 0–10 cm layer (DSTU ISO 10381-2:2004). S1 represented the zone of combined impact of the Perechyn TIMVer and Chemical Plant and railway tracks (50 m); S2 – the zone affected by the plant (50 m); S3 – a conditionally control site without sources of technogenic pollution or substantial traffic within a radius of 250 m. Each sample consisted of four individual subsamples. ITS2 amplicon sequencing was performed; denoising and ASV inference were carried out using DADA2 (Callahan et al., 2016), and taxonomic annotation was performed using the UNITE database. The ten most abundant taxa from phylum to species level were compared.

Results and discussion. ITS2 amplicon sequencing revealed clear differences in the taxonomic structure of fungal communities in technogenically impacted soils S1 and S2 compared with the conditionally control site S3. *Ascomycota* dominated all samples, but their relative abundance was higher in S1 (80.63 %) and S2 (83.19 %) than in S3 (69.14 %). In contrast, *Basidiomycota* accounted for only 2.39 % in S2 compared with 15.90 % in S3, whereas their abundance in S1 was close to control (14.25 %). The predominance of

Ascomycota is characteristic of many contaminated post-industrial soils and may reflect selection of stress-tolerant representatives capable of colonizing transformed environments (Okrasińska et al., 2022).

At the class level, the abundance of dominant *Sordariomycetes* remained relatively stable (36.94–39.70 %). However, *Dothideomycetes* were approximately three times more abundant in S1 than in S3 (14.44 vs 4.68 %), while their proportion in S2 was 8.25 %. *Tremellomycetes* showed the opposite pattern: 9.12 % in S1 and 12.31 % in S3, but only 0.68 % in S2. Thus, the strongest shift in the proportions of major phyla and classes was observed in the zone affected by the industrial plant.

At the family level, *Aspergillaceae* increased in S1 to 14.80 % compared with 8.76 % in S3, while *Nectriaceae* increased to 13.69 % in S1 and 14.35 % in S2 versus 4.89 % in S3. The most pronounced feature of S2 was an increase in *Microascaceae* to 11.65 % compared with only 0.55 % in control. In contrast, *Chaetomiaceae* were approximately twice less abundant in the impacted soils: 4.22 % in S1 and 4.01 % in S2 compared with 9.98 % in S3. These changes indicate redistribution of the fungal community under technogenically altered conditions.

At the genus level, S1 and S2 showed different deviation patterns. S1 was characterized by an increase in *Penicillium* to 14.43 % compared with 8.54 % in S3. In S2, the most pronounced changes involved *Fusarium* and *Aspergillus*: *Fusarium* reached 13.82 % compared with 3.64 % in control, while *Aspergillus* increased to 11.35 % versus only 0.11 %. Representatives of *Penicillium*, *Aspergillus* and other *Ascomycota* are frequently detected in technogenically contaminated soils, and their increased abundance may be associated with stress tolerance and metabolic plasticity (Okrasińska et al., 2022). Conversely, *Saitozyma*, which accounted for 7.99 % in S3, decreased to 0.03 % in S1 and was not among the ten most abundant genera in S2. A similar contrast was observed for *Saitozyma podzolica*. Thus, S1 was characterized mainly by increased *Dothideomycetes*, *Nectriaceae* and *Penicillium*, whereas S2 showed a marked decrease in *Basidiomycota* and *Tremellomycetes* together with increases in *Microascaceae*, *Fusarium* and *Aspergillus*. This indicates different patterns of fungal community restructuring depending on the type of technogenic pressure.

Conclusions. Technogenically impacted soils showed distinct differences in fungal taxonomic structure compared with the conditionally control site. Despite the relatively stable dominance of *Sordariomycetes*, S1 and S2 demonstrated different directions of mycobiota restructuring. S1 was characterized mainly by increases in *Dothideomycetes*, *Nectriaceae* and *Penicillium*, whereas S2 showed decreases in *Basidiomycota* and *Tremellomycetes* and increases in *Microascaceae*, *Fusarium* and *Aspergillus*. The results demonstrate the sensitivity of fungal community structure to the type of technogenic impact and support the use of ITS2 amplicon sequencing for biomonitoring technogenically transformed soils.

References

1. Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., De Wit, P., Sánchez-García, M., Ebersberger, I., de Sousa, F., Amend, A. S., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y. J. K., Sanli, K., Eriksson, K. M., Vik, U., Nilsson, R. H. (2013). Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods in Ecology and Evolution, 4(10), 914–919. https://doi.org/10.1111/2041-210X.12073
2. Bobryk, N. J., Kryvtsova, M. V., & Nikolaychuk, V. I. (2012). Meadow soil microbiocoenosis under influence of railway transport. Biosystems Diversity, 20(2), 3–9. https://doi.org/10.15421/011221
3. Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, 13(7), 581–583. https://doi.org/10.1038/nmeth.3869

4. Kryvtsova, M., Bobrik, N., Sabov, M., & Sabov, M. (2017). Microbiological indication of soils in zone of influence of Perechin TIMVer and Chemical Plant. Agrobiodiversity for Improving Nutrition, Health and Life Quality, 1, 258–263. https://doi.org/10.15414/agrobiodiversity. 2017. 2585-8246.258-263
5. Kuzmishyna, S. V., Hnatush, S. O., & Halushka, A. A. (2015). Microbiota of coal pit waste heaps of Chervonograd Mining Region after coal ash application. Visnyk of Dnipropetrovsk University. Biology, Ecology, 23(1), 33–38. https://doi.org/10.15421/011506
6. Okrasińska, A., Decewicz, P., Majchrowska, M., Dziewit, L., Muszewska, A., Dolatabadi, S., Kruszewski, Ł., Błocka, Z., & Pawłowska, J. (2022). Marginal lands and fungi – linking the type of soil contamination with fungal community composition. Environmental Microbiology, 24(8), 3809–3825. https://doi.org/10.1111/1462–2920.16007

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METHODOLOGICAL ASPECTS OF ASSESSING THE STATE OF THE SOIL MICROBIAL COMPLEX IN AGROCENOSIS

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Abstract. This study justifies the selection of a set of diagnostic indicators for assessing the state of the soil microbial complex by combining the quantitative counts of microorganisms from key physiological and taxonomic groups with the calculation of an integral indicator of soil biogenicity and four ecological coefficients that reflect the direction of microbiological processes. The scale for assessing microbial abundance in soils has been standardized and expanded. Additionally, a new scale has been proposed to evaluate soil biogenicity and the intensity of microbial organic matter transformation.
Keywords: soil microbiome, physiological groups of microorganisms, soil biogenicity, ecological coefficients.

The state of the soil microbial community is an integral indicator of its biological activity, fertility, and ecological stability, since the microbiota plays a leading role in organic matter transformation, the cycling of biogenic elements, humus profile formation, and the maintenance of soil homeostasis. Given the diversity of microbiological parameters, it is essential to employ a comprehensive system of diagnostic indicators that combine both quantitative and functional-ecological characteristics of the microbiota. However, the high labor intensity of the full range of analyses requires the use of a representative minimum set of indicators with high analytical value, reproducibility, and informativeness.

Based on long-term research on soil samples collected from agrocenoses across various agro-climatic zones in Ukraine and Europe, conducted at LLC "INSTITUTE OF APPLIED BIOTECHNOLOGY", we have substantiated the selection of the following basic microbiological indicators of abundance for major taxonomic and physiological groups. These include micromycetes, actinomycetes, ammonifying, amylolytic, oligonitrophilous, oligotrophic, and pedotrophic microorganisms, as well as phosphate-mobilizing microorganisms

(mobilizing mineral /calcium, aluminum, iron/ and organic phosphorus compounds), cellulolytic, and humate-transforming microorganisms. We selected these groups as indicators because of their strategic role in biogeochemical cycles and the energetics of soil formation. Each of them serves as a specific functional link that determines the direction of matter and energy transformation.

Based on classical assessment scales (D.G. Zvyagintsev, V.I. Titova, A.V. Kozlov) and empirical microbiological soil studies (natural /virgin and anthropogenically altered), we propose a unified scale for evaluating soil biological richness. Unlike previous frameworks, it includes highly specialized functional groups: phosphate-mobilizing, cellulolytic, and humate-transforming microorganisms, as well as micromycetes and actinomycetes. Furthermore, phosphate-mobilizing bacteria that transform organic and mineral compounds are classified under the same gradation as oligonitrophilic and amylolytic bacteria. Actinomycetes should be assessed using a soil enrichment scale (million CFU/g of soil): very poor < 0.15; poor – 0.15–0.5; moderate – 0.5–1.5; rich – 1.5–2.5; very rich > 2.5. Micromycetes (thousands CFU/g of soil): very poor < 20; poor – 20–40; moderate – 40–80; rich – 80–160; very rich > 160. Cellulolytic (thousands CFU/g of soil): very poor < 20; poor – 20–40; moderate – 40–80; rich – 80–160; very rich > 160.

The soil biogenicity index (the total count of microorganisms across all studied groups) is included in the soil fertility assessment scale as an integral measure of its biological activity. Based on this indicator, the degree of soil enrichment is categorized as follows (million CFU/g of soil): very poor < 25; poor: 25–40; moderate: 40–80; rich: 80–180; and very rich > 180.

To integrally assess the direction of microbiological processes in agrocenoses, the evaluation focuses on calculating four ecological coefficients: mineralization–immobilization ($K_{M\text{-}I}$), microbial transformation of soil organic matter (K_{TOM}), oligotrophicity (K_O), and pedotrophicity (K_P).

Assessing K_{M-I} allows for determining the balance between the synthesis and degradation of organic matter, the availability of nutrients for plants, and the risk of dehumification at early stages –before changes in soil agrochemical properties become detectable. A K_{M-I} value > 1 indicates a predominance of nitrogen immobilization processes, where nitrogen accumulates in microbial biomass; this prevents leaching but may cause temporary competition for available nitrogen between microorganisms and plants. A K_{M-I} value < 1 indicates intensified mineralization, leading to the decomposition of organic matter and the accumulation of mineral nitrogen forms in the soil. A K_{M-I} value of 1 (±0.1) indicates equilibrium between organic matter synthesis and decomposition processes. The K_{TOM} of the soil reflects the intensity of microbial organic compound transformation. Its evaluation is carried out according to the following scale: very slow: K_{TOM} < 15; slow: K_{TOM} = 15–30; moderate: K_{TOM} = 30–80; intense: K_{TOM} = 80–140; and very intense: K_{TOM} > 140.

The K_O index allows us to determine the soil's supply of readily available nutrients essential for microbial functioning. An increase in the K_O value represents an adaptive response of the microbial community to deteriorating environmental conditions and serves as a diagnostic indicator of soil fatigue or the depletion of readily available organic matter. Specifically, a K_O value > 1 indicates a decline in readily available nutrients and the onset of adverse degradation processes, whereas a K_O value < 1 suggests that the soil microbiota is sufficiently supplied with these substrates. A K_O value of 1 reflects a balanced soil microbial community, characterized by an equal proportion of oligotrophic and saprotrophic microorganisms.

The K_P reflects the direction of metabolic activity within the microbial community, differentiating between the utilization of fresh organic matter and the breakdown and mineralization of the soil humus matrix. A K_P value < 1 indicates minimal involvement of

soil humus in transformation processes within agroecosystems, favoring organic matter conservation. Conversely, a K_P value > 1 indicates active development of the autochthonous soil microbiota and accelerated decomposition of mobile organic compounds (leading to a decrease in their content), as well as – depending on accompanying coefficients and agrochemical parameters – an intensification of either humification or dehumification. A K_P value of 1 reflects an equilibrium between the decomposition of fresh organic matter and the transformation of soil humus.

Thus, the selected set of indicators comprehensively reflects the architecture and functional potential of the soil microbial complex, determining the direction of microbiological processes. This ensures objective and detailed monitoring of the soil microbiocenosis while achieving an optimal balance among informational value, methodological relevance, and labor efficiency – which is particularly crucial for large-scale agroecosystem analysis.

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AGENT OF CROWN GALLS – *AGROBACTERIUM TUMEFACIENS* OF BLUEBERRIES (*VACCINIUM CORYMBOSUM* L.)

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Abstract. As a result of monitoring epiphytotia on the territory of the blueberry nursery, damage was detected in 60-80 % of planting material. Bacterial strains were isolated in pure cultures from the affected blueberry tissues and characterized for their complex of morphological-cultural and physiological-biochemical and pathogenic properties. It was established that the *Agrobacterium* spp. strains isolated by us are related to typical representatives of the genus *Agrobacterium*, in particular the species *A. tumefaciens*.
Keywords: blueberry, crown galls, *A. tumefaciens*.

Blueberry (*Vaccinium corymbosum* L.) is a low-calorie berry of the heather family, which contains many antioxidants, vitamins C and K, and also improves brain and heart function. It is believed that due to the high content of anthocyanins, it reduces the number of free radicals in the body and slows down the aging and tumor formation processes, which is why, in recent years, it has gained real popularity among consumers. According to the International Blueberry Organization (IBO), over the past 5 years, the world's volume of cultivated blueberries has doubled − to almost 2 million tons. The volume in the countries of South and North America did not increase significantly (about 20 %), while steady growth was observed in Asia, the countries of the European region and Africa (over 100 %). According to the literature, in 2026 Ukraine ranked 10th in the world and 1st in Eastern Europe in terms of blueberry area. The total area is about 6 thousand hectares (of which 5 thousand hectares are already bearing fruit). In 2025, the gross harvest was 14.33 thousand tons. However, the average yield of this berry still lags behind the world average and is only 2.87 t/ha, while the world average is 9.05 t/ha. One of the factors that prevents increasing the yield of this crop and achieving world values is damage by pathogens of various etiologies, including bacterial ones (Patyka V. P., 2017). According to world literature, blueberries can be affected by the following types of phytopathogenic bacteria: *Agrobacterium tumefaciens* − the

causative agent of crown galls (Alippi et al., 2012), *Xylella fastidiosa* − the causative agent of bacterial leaf blight (Chang et al., 2009), *Pseudomonas syringae pv. syringae* − the causative agent of bacterial canker (Kałużna et al., 2013), *Xanthomonas arboricola* − the causative agent of irregular leaf spot – (Kałużna et al., 2022). According to some data, blueberries can also be affected by *Ralstonia solanacearum* (Erarslan et al., 2026) causing wilt. Despite the widespread cultivation of blueberries, the causative agents of bacterial diseases of blueberries in Ukraine have been almost unstudied. That is why the purpose of our research was to establish the cause of epiphytotia in a blueberry nursery in Ukraine.

In 2026, the researchers of the Department of Phytopathogenic Bacteria, D. R. Zabolotny Institute of Microbiology and Virology the National Academy of Sciences of Ukraine received an appeal from a private enterprise regarding the establishment of epiphytotia of blueberry seedlings in a nursery in Ukraine. The seedlings were previously obtained from a Ukrainian producer by microclonal propagation. The researchers selected samples of affected blueberry plants and conducted their phytopathological and bacteriological analysis. The cultural characteristics of bacterial strains isolated from affected blueberry plants were evaluated by colony morphology and growth rate on nutrient media. Cell morphology was examined after Gram staining. Artificial infection was performed on host plants and indicator plants with a bacterial suspension at a concentration of 10^8 CFU/ml. The tumor-inducing ability of strains isolated from blueberries and collection strains was tested on carrot explants and Kalanchoe leaves. A bacterial suspension was applied to the surface of previously damaged tissues and the material was incubated in a humid chamber at a temperature of 22–25 °C. The results were evaluated after 14–28 days for the presence and intensity of tumor formation. The assessment of tumor development was carried out according to the following scale: 0 – no tumors; 1– single small tumors are formed at the inoculation site; 2 – medium-sized tumors are formed; 3 – intensive tumor formation is observed at the inoculation site; 4 – massive tumor formation or fusion of tumors is noted (Patyka, 2017). For identification by biochemical activity associated with the fermentation of some carbohydrates, amino acids and proteins, API 20E strips of a commercial test system (bioMérieux, France) were used as an express method. The counting was carried out for 20–24 hours. The results were evaluated for the presence or absence of a reaction according to the manufacturer's recommendations.

As a result of monitoring the blueberry nursery, the total percentage of damage ranged from 60–80 %. Visual inspection of blueberry samples revealed that most of them had the following symptoms of damage: large hard brown growths formed on the root neck. This symptom indicates the long-standing development of the tumor formation process, since young tumors have a yellow-white color and are small. Bacterial strains were isolated from the tumors described above in pure culture. On solid agar nutrient media, they formed raised, moist, shiny cream colonies with even edges and a smooth surface. Under microscopy, the cells had the appearance of gram-negative motile rods, which is consistent with the typical morphological features of representatives of the genus *Agrobacterium* (Patyka, 2017). Physiological and biochemical studies showed that the isolated strains produced catalase and oxidase, coagulated milk, diluted gelatin and metabolized certain carbohydrates. At the same time, indole and hydrogen sulfide were not formed. The general biochemical profile of the isolated strains, determined by API testing, was similar to the collection strains of *A. tumefaciens*. The pathogenicity of *Agrobacterium* spp. isolates was confirmed in experiments on artificial infection, which was accompanied by the formation of typical tumors on Kalanchoe leaves and carrot explants. Therefore, according to the complex of pathogenic, morphological-cultural and physiological-biochemical properties, the strains isolated by us are related to typical representatives of the genus *Agrobacterium*, in particular the species

A. tumefaciens. The systematic position of the *Agrobacterium* spp. strains isolated by us from blueberries requires further clarification based on the analysis of genotype characteristics, which we plan to do in the future.

References

1. Alippi, A. M, López, A. C. & Balatti, P. A. (2012). Diversity among agrobacteria isolated from diseased plants of blueberry (*Vaccinium corymbosum*) in Argentina. Eur J Plant Pathol, 134, 415–https://doi.org/430 10.1007/s10658-012-0001-x
2. Erarslan, G., Karakaya A. (2026) Bacterial threats to blueberry (*Vaccinium* spp.) production: A synthesis of pathogen diversity, epidemiology and control strategies. Microbial Pathogenesis, 216, 108494 https://doi.org/10.1016/j.micpath.2026.108494
3. Kałużna, M. & Pothier J (2022). Complete genome sequence data of two *Xanthomonas arboricola* strains isolated from blueberry plants displaying bacterial leaf blight in Poland. Phytopathology, 112(8), 1814–1818 https://doi.org/10.1094/PHYTO-11-21-0484-A
4. Kałużna, M. Puławska, J., & Meszka, B. A. (2013) A new bacterial disease on blueberry (*Vaccinium corymbosum*) caused by *Pseudomonas* spp. Journal of Plant Protection Research, 53(1), 32–36 https://doi.org/10.2478/jppr-2013-0004
5. Oliver, J. E.,. Cobine, P. A., & La Fuente, L. De (2015). *Xylella fastidiosa* isolates from both subsp. multiplex and fastidiosa cause disease on southern highbush blueberry (*Vaccinium* sp.) under greenhouse conditions. Phytopathology, 105(7), 855–862 https://doi.org/10.1094/PHYTO-11-14-0322-FI
6. Patyka V. P., Pasichnyk L. A., Gvozdyak R. I. et al. (2017). Phytopathogenic bacteria. Research methods: monograph. Vinnytsia: Vindruk.

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Diakiv S.[1], Komovych L.[1], Kuzmishyna I.[2], Kalinkin V.[1]

MICROBIOTA OF HIGHBUSH BLUEBERRY RHIZOSPHERE ON GREY SANDY LOAM SOILS

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Abstract. The local growth conditions were analyzed and the number of agronomically valuable groups of microorganisms in the rhizosphere of high-growing blueberries Duke variety was investigated. Blueberry bushes were planted on a typical substrate and gray sandy soil as well. The most optimal conditions for the development of oligotrophic microorganisms ($52.27 \cdot 10^6$ CFU/g ADS) and microscopic fungi ($2.07 \cdot 10^6$ CFU /g ADS) were formed in the experimental gray sandy soil.
Keywords: rhizosphere microbiota, highbush blueberry, *Vaccinium corymbosum* L. cultivar Duke, ecological-trophic groups of microorganisms.

High-growing blueberry (*Vaccinium corymbosum* L.) is one of the most promising berry crops due to its high content of antioxidants, anthocyanins and vitamins. However, its growing is associated with significant difficulties: the main problem – is the need for acidic soil with a pH level 3.5–5.0. Blueberry root system is devoid of root hairs and feeds exclusively on symbiosis with ericoid mycorrhiza, which is formed only in an acidic environment.

Therefore, the study of blueberry rhizosphere microbiota on soils atypical for this culture is a current scientific and practical task.

The purpose of the work was to investigate the soil microbiota composition from rhizosphere of blueberries Duke variety. Soil samples from three areas of blueberry rhizosphere were taken: control 1 – peat substrate with sulfur application of 180 kg/ha (Dubno, Rivne region); control 2 – peat substrate without sulfur application (Dubno), experimental sample – gray sandy loam soil with the application of sawdust and sulfur 180kg/ha (Lutsk).

The rhizospheric soil sampling was carried out after start of vegetation period. Soil pH level (potentiometric method) and humidity (thermostatic-weight method), as well as definition of the number of different microorganisms groups were conducted in laboratory conditions. While sowing the soil suspension we used nutrient media: Nutrient Agar – for eutrophs (ammonifiers); Hungry Agar – for oligotrophs; Chapeka Medium – for micromycetes; Ashby Medium – for nitrogen fixers; Muromtseva Medium – for phosphomobilizers. All of the measurements were three times repeated.

Despite numerous studies of the plant qualitative parameters (Yavorska et al., 2016), the blueberry rhizosphere microbiota data are presented in small quantities, and almost all sources are foreign (Kasurinen & Holopainen, 2001; Kelvin et al., 2025). Meanwhile microbiota practically forms the growth conditions and affects the yield of the crop. Therefore we investigated the number of some ecological-trophic microorganisms groups with agronomic value in the blueberry rhizosphere soil under the different growing conditions.

In the experimental gray sandy soil sample the most optimal conditions for the development of oligotrophic microorganisms ($52.27 \cdot 10^6$ CFU /g absolutely dry soil (ADS)) and microscopic fungi ($2.07 \cdot 10^6$ CFU /g ADS) were formed. While other investigated microorganisms' groups dominate in peat substrates due to typical local conditions for blueberry growth. The highest number of microbiota, except oligotrophs were represented in control 2. Among the three studied samples, control 1 was distinguished by the smallest number of microorganisms of all studied groups, which is probably associated with pH level 5.34.

The determination of microbiological processes direction in the soils showed the high oligotrophic coefficients for all of the studied samples, significantly higher than 0.25–0.30. This indicates the nutrient deficiency in the soil or depletion of humus reserves.

Therefore, the conducted research confirm that successful blueberries cultivation is possible in acidic, organic-rich environment, which supports well balanced microbiota number with a high proportion of eutrophs and nitrogen fixers microbes. On gray sandy loam soils without radical preparation, a suppressed microbiocenosis is formed, which is unable to provide the plant nutrition.

References

1. Kasurinen, A. & Holopainen, T. (2001). Mycorrhizal colonisation of highbush blueberry andits native relativesin central Finland. *Agricultural and Food Science in Finland.* 10, 113–119. https://journal.fi/afs/article/view/5683/4882
2. Kelvin, K., Esther, M., Clabe, W., Tavasi, N., Jonh, M., Dennis O., Dennsi O., Hamond, M., Sipho, M., Janet, T. (2025). Unveiling the roles, mechanisms and prospects of soil microbial communities in sustainable agriculture. *Discover Soil*. 2(10). https://doi.org/10.1007/s44378-025-00037-4
3. Yavorska, N. Y., Lobachevska, O. V., Khorkavtsiv, Ya. D., Kyyak, N. Ya (2016). Microclonal propagation of the varieties of highbush blueberry *Vaccinium corymbosum* L. *Biotechnologia Acta*. 9(5), 30–37. https://jnas.nbuv.gov.ua/article/UJRN-0000663271

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ANTIBIOTIC RESISTANCE GENES IN ENVIRONMENTAL RESISTOMES: ECOLOGICAL AND EPIDEMIOLOGICAL SIGNIFICANCE

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Abstract. The resistomes of hospital wastewater may include antibiotic-resistant bacteria – including multidrug-resistant and pandrug-resistant variants – as well as antibiotic resistance genes; this poses environmental and epidemiological risks due to the formation of the chain: "hospital resistomes – sewage – treatment plants – treated effluent – natural water bodies".
Keywords: resistomes, antibiotic-resistant bacteria, antibiotic resistance genes, ecological and epidemiological significance.

Relevance of the topic. The spread of antibiotic-resistant bacteria (ARB) as causative agents of infections that are difficult to treat has reached a global scale. Epidemiologically, these infections are characterized primarily by transmission within healthcare settings, though they may also manifest as sporadic community-acquired cases. Their spread is considered a key element of the "One Health" concept, given the rising global incidence of bacterial infections that do not respond to antibiotic treatment.

Materials and methods. A search for scientific publications was conducted using the PubMed, Google Scholar, Scopus, Web of Science, CARD, and NDARO systems, followed by an analysis of the information, the significance and reliability of the results, and the formulation of conclusions.

Research objective. Assessment of the eco-epidemiological risks of antibiotic-resistant bacteria and antibiotic resistance genes.

Research results. Environmental issues may arise from the detection of ARB and antibiotic resistance genes (ARGs) in the environment – specifically in sewage, drinking water, and airborne aerosols. The aggregate of such genes constitutes the "resistome", which determines the genetic potential for antibiotic resistance within an ecosystem's ARB population. The resistome encompasses ARGs from both pathogenic and non-pathogenic bacteria; these genes can be transferred through intercellular interactions within microbial communities – such as the intestinal tract and organism-associated biofilms – as well as in external microbial communities like activated sludge or bottom sediments. In this context, intraspecies and interspecies gene transfer is possible involving plasmids, transposons, integrons, and bacteriophages, as well as free DNA molecules (eDNA); furthermore, ARGs can exist as extracellular genetic structures. In populations of humans and animals associated with human activity, the emergence and spread of ARGs depend directly on the use of antibiotics as therapeutic agents or in agricultural practices. However, ARGs are evidently a component of natural biocenoses, conferring a competitive advantage on their carriers when coexisting with antibiotic-producing microorganisms; indeed, ARB have been detected in individuals from "uncontacted tribes" where antibiotics had never been used.

From an ecological perspective, the existence and spread of ARGs align with the "selfish gene" concept – a metaphor introduced by Richard Dokinz in 1976 – which views organisms as systems utilized by genes for their own survival (Dokinz, 2020).

The epidemiological significance of sewage is a fundamental concept in epidemiology. The detection of antibiotic resistance such water presents an additional epidemiological challenge, particularly regarding the potential transmission of poly-resistant, multi-resistant, and pan-resistant bacterial variants and the associated clinical consequences. The simultaneous detection of ARGs – both as components of cellular genomes and within extracellular genetic structures – has shifted the focus of this issue to the genetic level.

It is understood that the presence of ARGs in the environment should not be considered a direct epidemiological threat. However, the horizontal transfer of ARGs cannot be ruled out within the resistomes present in hospital, agricultural, and pharmaceutical facilities – specifically in sewage and the biofilms of sanitary fixtures (Li et al., 2025).

Of course, the detection of ARGs does not in itself indicate an immediate epidemiological hazard. However, biofilms formed during the circulation of sewage may harbor an epidemiologically hazardous microsystem comprising ARGs, pathogenic bacteria, and mobile genetic elements that facilitate the acquisition of antibiotic resistance by bacteria. For instance, this process might unfold as follows: hospital → hospital antibiotics → multidrug-resistant bacteria → biofilm → bacterial lysis → multidrug-resistance genes (eARGs) → gene transfer → new resistant bacteria → internal plumbing system → hospital effluent → municipal sewer system → wastewater treatment plant → treated water → natural water body.

To date, the existence of such a scheme has been identified only at certain stages. However, modern technologies in metagenomics, proteomics, and volatilomics are capable of confirming the reality of this scheme, which will serve to predict the onset of epidemic outbreaks and epidemics.

References

1. Dokinz R. (2020). The selfish gene. KSD Publishing. 544 pp. ISBN 9786171225237
2. Li, X., Lin, L., & Liu, Q. (2025). Fate of extracellular antibiotic resistant genes in wastewater treatment plants: Characteristics, persistence, transformation, removal and potential risk. Energy & Environmental Sustainability, 1(2), Article 100022. https://doi.org/10.1016/j.eesus.2025.100022

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Halushka A.[1], Meniv N.[1,2], Skoptsova V.[1], Hnatush S.[1]

GROWTH OF BACTERIA ISOLATED FROM THE SPOIL HEAP OF JSC "LVIV COAL COMPANY" UNDER THE INFLUENCE OF HEAVY METAL COMPOUNDS

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Abstract. The effect of lead nitrate and nickel(II) chloride on the growth of *Streptomyces* sp. CEF-12 bacteria isolated from black rock from the spoil heap of JSC "Lviv Coal Company" was studied. Lead nitrate caused a decrease in bacterial biomass at concentrations of 0.0035 mM and 0.2 mM. Nickel chloride promoted an inhibitory effect on the studied bacteria at concentrations of 0.25 mM and above.
Keywords: spoil heap, lead nitrate, nickel(II) chloride, *Streptomyces* sp.

The mining industry within the Lviv-Volyn coal basin has a negative impact on the environment. Subsidence and flooding occur above fake mining operations, landslides occur, soils, groundwater and air, and precipitation are polluted. This poses a threat to human life and health. Rivers into which waste is discharged lose their ability to self-purify due to overloading with toxic substances (Poberezhsky et al., 2019). River effluents have high acidity (pH 2.7–3.5) due to the formation of sulfuric acid (Kuzmishyna et al., 2014). Changes in humidity occur in the aerobic zone of the soil, which can cause the complete disappearance of some plants and animals (Poberezhsky et al., 2019).

Around industrial facilities, peculiar local geochemical anomalies with an increased content of harmful elements are formed. According to the list of the American Environmental Protection Agency, the most dangerous environmental pollutants are cadmium, chromium, copper, lead, nickel, zinc (Moroz et al., 2009).

The effect of lead nitrate and nickel(II) chloride on the growth of *Streptomyces* sp. CEF-12 bacteria isolated from black rock from the spoil heap of JSC "Lviv Coal Company" was studied. The bacteria were grown in a nutrient broth (Farmaktiv, Ukraine) with glass beads under aeration conditions. The seeding density was 0.1 g/L. Biomass was determined photoelectrocolorimetrically.

Lead nitrate at concentrations of 0.035 mM, 0.35 mM and 1.6 mM did not affect the growth of bacteria. At concentrations of 0.0035 mM and 0.2 mM, a decrease in biomass of 56 and 46 %, respectively, compared to the control, was observed only on the first day of cultivation. Nickel chloride did not affect bacterial growth at a concentration of 0.025 mM and caused a decrease in biomass, compared to the control, at 0.25–6.25 mM.

In bacteria of the genus *Streptomyces*, nickel and lead have a predominantly toxic effect at elevated concentrations, but they differ mechanistically. Ni is a physiologically essential trace element: in *S. coelicolor*, it participates in the regulation of Ni-dependent enzymes and antioxidant defense systems, and its intracellular level is controlled by the regulators Nur and NmtR. Excess Ni^{2+} can disrupt the functioning of metal-containing enzymes, cause oxidative stress and inhibit the production of secondary metabolites (Manley et al., 2020).

Unlike Ni, Pb has no known physiological function in *Streptomyces* and is mainly considered as a toxic xenobiotic metal. Its toxicity is largely due to the ability of Pb^{2+} to bind to functional groups of cellular components and displace physiologically important metals. *Streptomyces* has been shown to have a significant capacity to bind and accumulate Pb^{2+}: for example, a Pb-binding protein identified as Fe/Zn superoxide dismutase was isolated from *S. subrutilus*, which bound large amounts of Pb^{2+} ions (So et al., 2001).

The effects of nickel are not limited to toxicity. In *Streptomyces pratensis* NA-ZhouS1, the addition of 100 μM Ni^{2+} activated a previously inactive biosynthetic pathway and led to the discovery of novel angucycline antibiotics, stremycin A and stremycin B (Akhter et al., 2018). For lead, there is much less convincing evidence of the activation of new antibiotic biosynthetic clusters in *Streptomyces*. However, the ability of various *Streptomyces* to resist, biosorb and bioaccumulate Pb is well documented, which is important for survival in contaminated environments. For example, a strain capable of accumulating up to 600 mg Pb per g biomass has been described among *Streptomyces* isolates from mining areas (El Baz et al., 2015).

Streptomyces sp. CEF-12 bacteria showed greater resistance to the presence of lead ions in the environment than bacteria of the *Actinomycetota* division isolated from abandoned mining areas in Morocco (El Baz et al., 2015). This makes them promising for use in technologies for the remediation of environments contaminated with lead.

References

1. Akhter, N., Liu, Y., Auckloo, B. N., Shi, Y., Wang, K., Chen, J., Wu, X., & Wu, B. (2018). Stress-driven discovery of new angucycline-type antibiotics from a marine *Streptomyces pratensis* NA-ZhouS1. *Marine Drugs, 16*(9), 331. Doi: 10.3390/md16090331.

2. El Baz, S., Baz, M., Barakate, M., Hassani, L., El Gharmali, A., & Imziln, B. (2015). Resistance to and accumulation of heavy metals by actinobacteria isolated from abandoned mining areas. *The Scientific World Journal, 2015*, 761834. Doi: 10.1155/2015/761834.
3. Kuzmishyna, S., Hnatush, S., Moroz, O., et al. (2014). Microbiota of the coal pit waste heaps of Chervonograd mining region. *Visnyk of the Lviv University. Series Biology, 67*, 234–242.
4. Moroz, O. M., Gudz, S. P., Podopryhora, O. I., Klym, I. R., Borsukevych, B. M., Derkach, M. B., Parasiuk, O. V., & Hnatush, S. O. (2009). Hard methals influence on growth and sulfate reducing by *Desulfovibrio desulfuricans*. *Scientific Bulletin of Uzhhorod University. Series Biology, 26*, 206–215. [In Ukrainian].
5. Poberezhsky, A., Buchynska, I., Shevchuk, O., & Mukan, T. (2019). Mining complex of the Lviv-Volyn coal basin and its impact on the ecosystem of the region. *Geology & Geochemistry of Combustible Minerals, 3*(180), 52–59. Doi: 10.15407/ggcm2019.03.052. [In Ukrainian].
6. Manley, O. M., Myers, P. D., & Toney, D. J. (2020). Evaluation of the regulatory model for Ni^{2+} sensing by Nur from *Streptomyces coelicolor*. *Journal of Inorganic Biochemistry, 203*, 110859. Doi: 10.1016/j.jinorgbio.2019.110859.
7. So, N.-W., Rho, J.-Y., Lee, S.-Y., Hancock, I. C., & Kim, J.-H. (2001). A lead-absorbing protein with superoxide dismutase activity from *Streptomyces subrutilus*. *FEMS Microbiology Letters, 194*(1), 93–98. Doi: 10.1111/j.1574-6968.2001.tb09452.x.

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PHYSIOLOGICAL AND BIOCHEMICAL PROPERTIES OF MYXOBACTERIA ISOLATED FROM NATURAL BIOTOPES OF THE BLACK SEA

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Abstract. The physiological and biochemical properties of 35 myxobacterial strains isolated from natural biotopes in the Black Sea coastal zone near Odesa were studied. The strains showed variable growth responses to temperature, pH, and NaCl concentration, with 25–30 °C, pH 7.0, and 0.5–1.5 % NaCl being the most favorable conditions for most strains. Strain-dependent variability was also observed in oxidase and catalase activities and enzymatic profiles. Overall, the strains exhibited a limited spectrum of low-molecular-weight substrate assimilation, with the most restricted spectrum among representatives of the genus *Nannocystis*. Taken together, these findings demonstrate substantial physiological and biochemical heterogeneity among the investigated myxobacteria and support their potential for further investigation as producers of biologically active compounds and hydrolytic enzymes.
Keywords: myxobacteria, Black Sea, physiological and biochemical properties.

Myxobacteria are Gram-negative prokaryotes with a unique life cycle, social behavior, large genomes, and high biosynthetic potential. They are active producers of secondary metabolites, which motivates the search for new natural sources for their isolation and investigation (Saggu et al., 2023). The specific physicochemical conditions of marine ecosystems differ substantially from those of terrestrial ecosystems, particularly soil ecosystems, promoting physiological and biochemical adaptations and metabolic characteristics in microorganisms and making marine microbiota, including myxobacteria, a promising source of biologically active compounds and enzymes (Barzkar et al., 2024; Saggu et al., 2023).

The microbial diversity of natural biotopes of the Black Sea remains insufficiently studied, particularly with regard to representatives of *Myxococcota*. Therefore, the aim of

this study was to investigate the physiological and biochemical properties of myxobacterial strains isolated from Black Sea biotopes.

Thirty-five myxobacterial strains isolated from natural substrates of the Black Sea coastal zone near Odesa were investigated. The strains are maintained in the culture collection of microorganisms at Odesa I. I. Mechnikov National University at −80 °C. Physiological properties were assessed based on the ability of the strains to grow on VY/2 agar at temperatures of 10–40 °C, pH values of 4.0–9.0, and NaCl concentrations of 0.0–4.0 % (Zhang et al., 2024). The strains were incubated for 21 days, and swarm diameters were measured on days 7, 14, and 21. Growth intensity was assessed based on the dynamics of swarm diameter expansion over the incubation period (Ahearne et al., 2023). Biochemical properties were determined using the API ZYM and API 20NE test systems (bioMérieux, France) according to the manufacturer's instructions, while oxidase and catalase activities were determined according to Zang et al. (2024).

The studied strains demonstrated variable physiological properties. Growth occurred at 10–35 °C, with most strains showing the highest growth intensity at 25–30 °C; 30 °C was optimal based on the overall dynamics of swarm expansion. Eight strains grew at 15 and 20 °C, two of which also grew at 10 °C, although growth was slower at lower temperatures. At 35 °C, approximately 70.0 % of strains exhibited weak growth, while no growth occurred above 35 °C. All strains formed fruiting bodies, whose coloration and timing of formation depended on temperature: at 25–30 °C, some strains formed pale yellow, lemon-yellow, or orange fruiting bodies, whereas colorless fruiting bodies predominated under suboptimal condition.

Growth occurred at pH 6.0–9.0, with pH 7.0 being optimal for all strains. At pH 6.0, five strains exhibited weak growth, three moderate growth, and one intensive growth, while the remaining strains did not grow. More than 74.0 % of strains grew at pH 7.5–8.5. At pH 9.0, only three strains showed weak growth, with the first signs appearing after 14 days, followed by the formation of colorless fruiting bodies 1–2 days later.

Growth was observed at NaCl concentrations of 0.0–3.0 %. At 0.0–2.5 %, growth intensity varied among strains, with approximately 92.0 % exhibiting intensive growth at 0.5–1.5 % NaCl and approximately 8.0 % showing less intensive growth. These concentrations were the most favorable for growth. Increasing NaCl concentrations resulted in gradual decreases in swarm expansion and growth intensity and, in most cases, earlier formation of colorless fruiting bodies. One strain grew at 3.0 % NaCl, whereas no growth occurred above 3.0 %. No myxobacterial strains unable to grow in the absence of NaCl (0.0 %) were detected, which could indicate an obligate marine origin.

The biochemical properties of the strains showed substantial variability in oxidase and catalase activities, enzymatic profiles, and the spectrum of assimilated substrates. API ZYM revealed activities of phosphatases, esterases, lipases, selected proteases (trypsin, α-chymotrypsin, leucine arylamidase, valine arylamidase, and cysteine arylamidase), and glycosidases, with specific reaction intensities varying substantially among strains. *Nannocystis* representatives showed relatively high activities of selected proteolytic enzymes (trypsin and α-chymotrypsin), lipolytic enzymes, and phosphatases, accompanied by weak or absent glycosidase activity, whereas *Myxococcus* representatives exhibited pronounced activities of alkaline phosphatase, esterase-lipase C8, lipase C14, trypsin, and acid phosphatase, with weak reactions for esterase C4, leucine arylamidase, valine arylamidase, and cysteine arylamidase, α-chymotrypsin, and naphthol-AS-BI-phosphohydrolase. The observed spectrum of enzymatic activities indicates the potential of the studied strains to hydrolyze complex organic compounds. API 20NE revealed an overall limited spectrum of low-molecular-weight substrate assimilation with pronounced strain-dependent variability. *Nannocystis* representatives had the most limited assimilation spectrum among the substrates

included in the API 20NE panel, as evidenced by the absence of or only occasional positive reactions to individual carbon substrates.

Taken together, the results obtained indicate substantial physiological and biochemical heterogeneity among the studied marine myxobacteria, manifested in variability in their temperature, pH, and salt tolerance, enzymatic profiles, and the spectrum of substrates they assimilate. The combination of physiological plasticity and the potential ability to hydrolyze complex organic compounds characterizes the studied strains as promising candidates for further investigation of their biotechnological potential.

References

1. Ahearne, A., Phillips, K.E., Knehans, T., Hoing, M., Dowd, S.E., & Stevens, D.C. (2023). Chromosomal organization of biosynthetic gene clusters, including those of nine novel species, suggests plasticity of myxobacterial specialized metabolism. *Front Microbiol.*, *14*, 1227206. https://doi.org/10.3389/fmicb.2023.1227206
2. Barzkar, N., Sukhikh, S., & Babich, O. (2024). Study of marine microorganism metabolites: new resources for bioactive natural products. *Front. Microbiol*. *14*, 1285902. https://doi.org/10.3389/fmicb.2023.1285902
3. Saggu, S.K., Nath, A., & Kumar, S. (2023). Myxobacteria: biology and bioactive secondary metabolites. *Research in Microbiology*, 174, 104079. https://doi.org/10.1016/j.resmic.2023. 104079
4. Zang, Y., Zhang, X., Wang, Z., Tong, Q., Zhou, Y., Yao, Q., & Zhu, H. (2024). *Hyalangium ruber* sp. nov, characterization of a novel myxobacterium strain s54d21 and their secondary metabolites. *Front Microbiol.*, *15*, 1369499. https://doi.org/10.3389/fmicb.2024.1369499

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POLYHYDROXYALKANOATES IN SYNTHETIC MICROBIAL COMMUNITIES OF ANTARCTIC ENDOPHYTIC BACTERIA

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Abstract. This study investigates the accumulation dynamics of polyhydroxyalkanoates (PHA) in synthetic microbial communities formed by Antarctic endophytic bacteria isolated from *Deschampsia antarctica* and *Colobanthus quitensis*. We evaluated the influence of biofilm architecture (air-liquid-solid [ALS] vs. liquid-solid [LS]) and wide temperature range (4, 15, 25, 37, and 42 °C) on PHA biosynthesis. Quantitative gravimetric analysis and confocal laser scanning microscopy (CLSM) demonstrated that PHA accumulation is significantly enhanced in ALS multi-species biofilms compared to monocultures, peaking at 4 °C. Higher temperatures led to rapid depletion of energy reserves. Oxygen availability and spatial organization within the biofilm matrix are critical drivers of carbon allocation toward PHA storage in cold-adapted microbial communities.
Keywords: Polyhydroxyalkanoates, Antarctic endophytes, synthetic microbial communities, biofilm architecture, psychrotolerant bacteria, confocal microscopy.

The growing global contamination by synthetic plastics has accelerated the search for eco-friendly, biocompatible alternatives. Polyhydroxyalkanoates (PHA) produced by

microorganisms represent one of the most promising bio-based substitutes. However, the efficiency of microbial PHA biosynthesis heavily depends on environmental factors, including nutrient composition, temperature, pH, and trace element availability. Antarctic endophytic bacteria, adapted to extreme cold and low nutrient availability, possess unique physiological strategies for energy storage. Understanding how multi-species cooperation and biofilm architecture regulate PHA accumulation under temperature stress provides critical insights into cold-adaptation mechanisms and offers novel biotechnological avenues for low-temperature bioplastic production.

The objective of the study was to investigate the characteristics of polyhydroxyalkanoate accumulation in synthetic microbial communities formed by Antarctic endophytic bacteria and establish the effects of biofilm architecture and temperature regimes on PHA biosynthesis intensity.

Materials and Methods. Endophytic bacterial strains were previously isolated from vascular tissues of Antarctic plants (*Deschampsia antarctica* and *Colobanthus quitensis*) during the 24th Ukrainian Antarctic Expedition (2020) and identified via 16S rRNA gene sequencing. Synthetic microbial communities (SMCs) were assembled based on biofilm formation capacity of ALS (Air-Liquid-Solid) community included *Siminovitchia terrae* 9.1, *Pseudomonas salomonii* 10.1, *Pseudomonas yamanorum* 24.4, *Hafnia psychrotolerans* 25.2, *Pseudomonas* sp. 26.2, and *Pseudarthrobacter* sp. 26.7. And LS (Liquid-Solid) community included *Psychrobacter* arcticus 10.4, *Arthrobacter psychrochitiniphilus* 15.6, *Agreia* sp. 23.2, *Brachybacterium* sp. 39.12, and *Kocuria salsicia* 40.1. Standardized microbial suspensions (McFarland Standard 1, ~$3 \cdot 10^8$ CFU/cm³) were inoculated in minimal salts medium (MSM) and incubated under static conditions at 4, 15, 25, 37, and 42 °C for 3 and 6 days. Primary screening for intracellular PHA granules was performed using Sudan Black B staining under light microscopy (×1000). Total quantitative yield (% of cell dry weight) was determined gravimetrically following lyophilization and chloroform extraction. Biofilm architecture and spatial localization of PHA granules were visualized via Confocal Laser Scanning Microscopy (CLSM, Leica TCS SPE) using triple fluorescent staining: SYBR Green I (cellular DNA), Nile Red (lipophilic PHA granules), and Calcofluor White (exopolysaccharide matrix). Statistical significance was assessed using one-way ANOVA followed by post-hoc tests relative to the 4 °C reference group ($p < 0.05$).

Results and Discussion. Individual bacterial isolates in monocultures exhibited limited PHA biosynthetic capacity. In contrast, their assembly into complex ALS biofilms resulted in a substantial enhancement and stable deposition of PHA under low temperatures. Quantitative fluorescence normalization (Nile Red/SYBR Green I signal ratio) and CLSM imaging confirmed that intracellular PHA accumulation peaked at 4 °C. As incubation temperature increased (15–42 °C), a progressive inhibition of PHA synthesis and rapid reserve mobilization were observed. Long-term retention of polymeric reserves was characteristic exclusively of cold-cultivated biofilms. Oxygen availability and spatial stratification within the ALS biofilm structure proved to be critical determinants for carbon distribution between cellular growth and energy reserve storage. Integrating experimental data with comparative metagenomic analysis supports the hypothesis that aerobic metabolism provides the key ATP yield necessary for efficient PHA polymerization in extreme cold.

Conclusions. Synthetic microbial consortia of Antarctic endophytes display clear temperature- and architecture-dependent patterns of PHA biosynthesis. Cooperation within ALS biofilms significantly enhances PHA storage at 4 °C, reflecting a specialized cold-adaptation survival strategy. These findings deepen our understanding of polar microbial ecology and highlight cold-adapted synthetic consortia as promising platforms for sustainable, energy-efficient bioplastic production.

References

1. Auman, A. J., & Breezee, J. (2020). Polyhydroxyalkanoate production by psychrotolerant bacteria from polar environments. Applied Microbiology and Biotechnology, 104(12), 5211–5223.
2. Moshynets, E. V., & Karpenko, O. V. (2023). Biofilm matrix architecture and carbon allocation in extreme environments. Frontiers in Microbiology, 14, Article 1098234.
3. Subhash, G. V., & Rajvanshi, M. (2021). Microbial polyhydroxyalkanoates: Bio-based polymers for sustainable development. Bioresource Technology, 320, Article 124385.
4. Yungin, O. S., & Suslova, O. O. (2025). Endophytic microbial consortia of Antarctic vascular plants: Biofilm formation and metabolic potential. Ukrainian Antarctic Journal, 23(1), 45–58.

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THE ROLE OF MOSS COVER IN THE MICROBIOLOGICAL TRANSFORMATION OF NITROGEN COMPOUNDS IN SOILS OF ANTHROPOGENICALLY ALTERED FOREST ECOSYSTEMS

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Abstract. In felling forest areas, the moss stimulates soil microbial metabolism and intensifies the conversion of N compounds in the soil, maintaining higher levels of mineral forms of the biogenic element throughout the growing season compared to bare substrate. By buffering the hydrothermal regime of the soil, accumulating dead shoot residue, and enhancing the infiltration of moisture enriched with labile nutrient compounds from the turf, moss promotes a spring increase in the activity of N compound transformation by microorganisms, with a summer peak in ammonification and an autumn maximum in nitrification.
Keywords: ammonifiers, nitrifiers, ammonium, nitrates, disturbed forest areas.

Bryophytes are a key functional component of many ecosystems. By modulating soil microconditions, they influence the dynamics of microbial biomass, as well as the abundance and structure of microbiocenoses (Siwach et al., 2024). Mosses stimulate ammonification and nitrification processes as basic stages of the N cycle, thereby determining ecosystem productivity (Glime, 2024). The removal of moss cover leads to soil nutrient depletion and a decline in microbial diversity (Cheng et al., 2021). At the same time, moss turfs contribute to the deposition of organic C and biogenic elements (in particular, N) in the surface substrate horizon (Karpinets et al., 2014; Wei et al., 2025) and to the accumulation of mineral N in the rhizoid layer of forest soils across varying gradients of anthropogenic pressure (Karpinets et al., 2025). Under the conditions of a transformed forest site – a felling area of beech-oak tree stands – it is relevant to study the impact of moss on the functional capacity of the ammonifying and nitrifying microbiota, which determines the intensity and specificity of N mineralization and the level of accumulation of its available forms (ammonium and nitrates) in the upper soil horizon. The aim of this study was to evaluate the characteristics of changes in NH_4^+ and NO_3^- contents, as well as the abundance of ammonifiers and nitrifiers in the soil beneath *Atrichum undulatum* (Hedw.) P. Beauv. under felling forest conditions.

Soil samples (under the *A. undulatum* moss and without it) were collected in April, July, and October 2024 at designated plots within the felling area of the Stradch Training and Research Forest Enterprise. As a result of felling and the construction of forest roads, extensive areas of open sites with increased insolation and wind exposure were formed here, causing soil temperature destabilization and a decrease in soil moisture.

Pure cultures of microorganisms were isolated from the study samples using classical microbiological methods of cultivation on solid (agarized) and liquid nutrient media. Specifically, ammonifying bacteria were grown on Tryptic Soy Agar medium, while nitrite-oxidizing microorganisms (Phase II nitrification) were cultivated on Vinogradsky medium (Hudz et al., 2014). Soil nitrate content was determined using the disulfophenolic acid method, and ammonium content was measured using Nessler's reagent (Nikolaychuk & Bilyk, 1997).

It was established that in April, July, and October, the activity of nitrifying and ammonifying bacteria under the moss cover was higher than in the soil without moss turfs. In April, the abundance of nitrifiers and ammonifiers in the rhizoid soil layer exceeded the corresponding values in the bare substrate to the greatest extent – by 1.8 and 3.4 times, respectively. The high physiological activity of ammonifiers was presumably driven by the degradation of organic matter from dead turf residues accumulated over the winter period, which ensured the deposition of NH_4^+ and stimulated the development of chemolithotrophic nitrifying bacteria. The maximum abundance of ammonifying microbiota under the moss cover was recorded under extreme summer microclimatic conditions, which was driven by its ability to stabilize the hydrothermal regime and saturate the surface soil layer with accumulated moisture infiltrated from the turf. Nitrite-oxidizing bacteria exhibited a trend toward a sequential increase in their abundance within the rhizoid soil layer, reaching maximum values in autumn. The active development of nitrifiers under the lowest soil temperatures in October was presumably stimulated by the precipitation-driven leaching of labile N compounds from the moss turf.

Analysis of the dynamics of mineral N forms indicates their sequential increase under the moss cover throughout the entire growing season, reaching a maximum in October, whereas in bare soil, their content decreased in autumn. At the same time, the greatest difference between the variants was recorded in July, when the content of NH_4^+ and NO_3^- under moss – primarily due to the sequestration capacity of the turf – exceeded the values in soil without moss turfs by 1.7 and 1.5 times, respectively.

Thus, moss cover serves as an important biological regulator of N compound transformation in forest soils. It supports the functional state of disturbed soils and stabilizes the biogenic element cycle under conditions of microclimatic changes and anthropogenic pressure.

References

1. Cheng, C., Gao, M., Zhang, Y., Long, M., Wu, Y., & Li, X. (2021). Effects of disturbance to moss biocrusts on soil nutrients, enzyme activities, and microbial communities in degraded karst landscapes in southwest China. Soil Biology & Biochemistry, 152, 108065. https://doi.org/10.1016/j.soilbio.2020.108065
2. Glime, J. M. (2024). Roles of bryophytes in forest sustainability–positive or negative? Sustainability, 16(6), 2359. https://doi.org/10.3390/su16062359
3. Hudz, S. P., Hnatush, S. O., Yavorska, H. V., Bilinska, I. S., & Borsukevych, B. M. (2014). Praktykum z mikrobiolohii [Guidebook on microbiology]. Vydavnychyi Tsentr Lvivskoho Natsionalnoho Universytetu imeni Ivana Franka, Lviv (in Ukrainian).
4. Karpinets, L., Lobachevska, O., & Baranov, V. (2014). Vplyv briofitiv na vmist makroelementiv ta orhanichnoho vuhletsiu u tekhnozemakh vidvaliv Chervonohradskoho hirnychopromyslovoho

kompleksu [The influence of the bryophytes on the content of macroelements and organic carbon in technozems of the dumps of the Chervonohrad mining industrial complex]. Bulletin of Kharkiv National Agricultural University, Biological Series, 33, 52–59 (in Ukrainian). http://nbuv.gov.ua/UJRN/Vkhnau_biol_2014_3_9
5. Karpinets, L. I., Lobachevska, O. V., & Yavorska, H. V. (2025). Vplyv mokhovoho pokryvu na vmist mineralnykh spoluk nitrohenu zalezhno vid mikroklimatychnykh ta edafichnykh chynnykiv seredovyshcha u lisovykh ekosystemakh Ukrains'koho Roztochchya [Influence of moss cover on the nitrogen mineral compounds content depending on microclimatic and edaphic factors of the environment in forest ecosystems of the Ukrainian Roztochchya]. Chornomorski Botanical journal, 21(4), 354–364 (in Ukrainian). https://doi.org/10.32999/ksu1990-553X/2025-21-4-4
6. Nikolaichuk, V. I., & Bilyk, P. P. (1997). Laboratorni ta praktychni roboty z hruntoznavstva: navchalnyi posibnyk [Laboratory and practical work on soil science: a textbook]. Uzhhorod: Patent. 112 p. (in Ukrainian)
7. Siwach, A., Zhuang, Q., & Baishya, R. (2024). Impact of seasonality, moss cover, and forest types on soil microbial biomass and enzymatic activity: an environmental prospective from the Himalaya. Catena, 244, 108269. https://doi.org/10.1016/j.catena.2024.108269
8. Wei, M., Sun, Q., Liu, D. (2025). Moss cover modulates soil fungal functional communities and nutrient cycling in alpine forests. Forests, 16(1), 138. https://doi.org/10.3390/f16010138

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CHALLENGES AT SIMULATION OF FUNGAL BIODETERIORATION OF MEDIEVAL FRESCOES IN EXPERIMENTAL LABORATORY MODELS

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Abstract. The unique medieval frescoes in Saint Sophia cathedral in Kyiv have suffered from dark-spotted biodeterioration caused by xerophilic aspergilli. This work is aimed at the development of experimental models adequately simulating this type of biodeterioration under laboratory conditions to be able to test antifungal treatments prior to their application *in situ*. A microcosm was developed based on ground medieval plaster (2000 g/L), which reproduced calcite-rich mineral matrix, extreme low-water activity required for xerophilic aspergilli, and the original dark-spotted biodeterioration along with general properties of authentic plaster. The developed model advantages, emerged challenges and mitigation measures for future research were identified.
Keywords: fungi, cultural heritage, frescoes biodeterioration, xerophilic aspergilli, experimental modelling.

It is well known that fungi are major contributors to the biodeterioration of cultural heritage, displaying remarkable adaptability and the capacity to thrive under unfavourable conditions, including extreme xerophilic behaviour within indoor heritage environments. Cultural heritage materials vary widely in composition and structure, and fungi are able to adapt successfully to each substrate, often in unpredictable ways, exhibiting puzzling patterns of material colonisation. This is particularly true of xerophilic aspergilli, which are

typical biodeteriogens of museum environments. Combating fungal colonisation effectively demands a thorough understanding of the multiple factors influencing this process, along with the design of tailored experimental strategies suited to each individual case, with special emphasis on the creation of suitable controlled laboratory systems that accurately replicate cultural heritage microenvironments.

The current work was dedicated to the development of adequate experimental laboratory models which can correctly simulate the process of dark-spotted biodeterioration of medieval mineral frescoes of Saint Sophia cathedral (Kyiv, Ukraine) in order to develop specific antifungal treatment against xerophilic aspergilli causing this biodeterioration.

The experimental system was based on a microcosm of sterile plastic 6-well cell culture plates. *Aspergillus vitricola* strain, isolated from biodeteriorated frescoes of Saint Sophia cathedral, was used as a model deteriogenic microorganism. Fungi were cultivated on PDA +15 % NaCl medium mixed with ground medieval plaster. Light microscopy, scanning electron microscopy (SEM), image analysis (ImageJ/Fiji), spectrometry for analysis of phenolic compounds, and one-way ANOVA (MINITAB) were used. Also, total DNA was extracted from the samples of microcosm plaster using a QIAamp DNA Mini Kit (QIAGEN) (Fomina et al., 2022).

Plaster concentration was found to strongly affect fungal colonisation of the microcosm: colony area increased sharply at 15–120 g/L (due to sparse marginal mycelium and guerrilla-type growth (Fomina et al., 2024)), then declined at 250–2000 g/L. SEM showed endolithic growth within mineral matrix, biogeochemical activity, and versatile morphology of *A vitricola*. Colonies darkened with increasing plaster content (1.3-fold lower brightness at 2000 g/L vs control), and content of total phenolic compounds decreased. The 2000 g/L plaster medium was the best to reproduce the dark-spotted biodeterioration on cathedral walls and it was chosen for further studies where the effect of ethanol treatment on fungi was tested. Ethanol treatment was observed to cause increased conidiogenesis and hyphal aggregation during fungal recovery in the affected areas.

Advantages of the developed model:

The microcosm was established as a scaled-down model of a painted wall, using ground medieval plaster to reproduce a calcium-rich mineral matrix dominated by calcite, together with extreme low-water-activity conditions (achieved via 15 % NaCl) required for xerophilic aspergilli.

The 2000 g/L plaster variant successfully reproduces both the original dark-spot damage on the cathedral walls and the general characteristics of authentic plaster.

The model is well suited to microscopic examination by both stereomicroscopy and SEM, allowing colonisation of the internal plaster space to be observed and endolithic mycelial behaviour to be documented.

This variant was successfully applied in preliminary antifungal treatment trials using ethanol, validating the model as a platform for testing antimicrobial agents in vitro prior to their application on actual heritage surfaces.

Challenges and mitigation measures for future research:

The nutrient-rich conditions used to accelerate colonisation in this model were highly exaggerated compared with the oligotrophic conditions found on the actual cathedral walls. The next step would be experiments with reduced nutrient content.

The dense mineral matrix formed at high plaster concentrations protects fungal biomass so effectively that it hinders homogenisation and the extraction of nucleic acids and other metabolites using standard methods. The next step would be to explore alternative homogenisation approaches (e.g., a bead-mill homogeniser with glass-bead lysis tubes) or develop an alternative model based on a different substrate, such as glass.

The optimal time frame for treating biodeteriorated plaster remains unclear. The next step would be further experiments focused on antifungal treatment trials.

References

1. Fomina, M., Cuadros, J., Pinzari, F., Hryshchenko, N., Najorka, J., Gavrilenko, M., Hong, J. W. & Gadd, G. M. (2022). Fungal transformation of mineral substrata of biodeteriorated medieval murals in Saint Sophia's cathedral, Kyiv, Ukraine. *International Biodeterioration & Biodegradation*, *175*, 105486.
2. Fomina, M., Gromozova, O., & Gadd, G. M. (2024). Morphological responses of filamentous fungi to stressful environmental conditions. *Advances in applied microbiology*, *129*, 115–169.
3. Gadd, G. M., Fomina, M., & Pinzari, F. (2024). Fungal biodeterioration and preservation of cultural heritage, artwork, and historical artifacts: extremophily and adaptation. *Microbiology and Molecular Biology Reviews*, *88*(1), e00200-22.

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MICROBIAL DYNAMICS AND BIOLOGICAL ACTIVITY IN SHELL-EXPLOSION-DAMAGED SOILS

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Abstract. The full-scale Russian invasion of Ukraine has severely disrupted soil ecosystems and microbial communities. This study evaluated phytobacterial remediation of shell-explosion-damaged soil near Bakhmut using microbial bioformulations, white clover (*Trifolium repens*), and *Rhizobium leguminosarum* bv. *trifolii* 320b. After 60 days, nitrogen-fixing, phosphate-mobilising, amylolytic, ammonifying, and pedotrophic microorganisms increased, whereas oligotrophic and cellulolytic populations declined. The strongest increases were observed for phosphate-mobilising microorganisms (2.2–5.2-fold). Overall, remediation promoted functional restructuring of the soil microbiome and recovery of nutrient cycling and biological soil fertility.
Keywords: soil microbiota, military impact, microbial abundance, bioformulations.

The full-scale Russian invasion of Ukraine has resulted in substantial degradation of soil ecosystems through mechanical disturbance, chemical contamination, and disruption of microbial communities (Solokha et al., 2024). Explosive residues, including TNT, RDX, and HMX, may persist in soils, exert toxic effects, and alter the structure and functional activity of soil microbial communities (Corredor et al., 2024). While the physicochemical impacts of warfare are increasingly recognised, alterations in soil microbiome structure and function remain insufficiently understood. Given the essential role of microorganisms in nutrient cycling, soil organic matter turnover, fertility, and ecosystem functioning (Sokol et al., 2022), restoration of microbial communities represents an important component of post-war soil rehabilitation. Therefore, this study aimed to assess microbial dynamics and biological activity in soils affected by shell explosions and to evaluate microbial bioformulations as a biotechnological approach for accelerating their ecological recovery.

The study was conducted under vegetation conditions using soil collected from an artillery shell crater near Bakhmut (Donetsk region, Ukraine); unaffected soil sampled 50 m

from the crater served as the control. Remediation treatments included microbial bioformulations developed at the D. K. Zabolotny Institute of Microbiology and Virology, NAS of Ukraine, BioStymix Standard®, and white clover (*Trifolium repens* cv. Rivendell) used as a phytoremediator. Clover seeds were inoculated with *Rhizobium leguminosarum* bv. *trifolii* 320b. Soil samples were collected after 60 days of plant growth. The abundance of major ecological-functional microbial groups and microbial ecological indices were determined as described previously (Biliavska et al., 2024). Microbial abundance was expressed as CFU g^{-1} of absolutely dry soil, while ecological indices were calculated from ratios of microbial counts on selective culture media.

Combined application of microbial bioformulations and white-clover-based phytoremediation induced a pronounced restructuring of the soil microbial community, with the strongest effects observed in functional groups involved in nitrogen cycling, phosphorus mobilisation, and organic matter transformation.

Oligonitrotrophic and nitrogen-fixing microorganisms increased by approximately 20 % after Ultrachyst® + *Streptomyces violaceus* IMV Ac-5027 treatment and by up to 1.7-fold when combined with white clover and additional bioformulations. This response likely reflects improved rhizosphere conditions and greater availability of root-derived carbon substrates, while *Rhizobium leguminosarum* bv. *trifolii* 320b may have further promoted recovery of biological nitrogen cycling.

Phosphate-mobilising microorganisms increased 2.2–5.2-fold following bioformulation treatments, indicating enhanced microbial phosphorus solubilisation. Amylolytic microorganisms increased 1.5–2.3-fold, likely reflecting intensified utilisation of root-derived carbohydrates. These changes suggest activation of phosphorus mobilisation and carbon turnover, representing an early stage of functional recovery of nutrient cycling in the remediated soil.

Ammonifying microorganisms increased by 2.5- to 3.3-fold, with the highest abundance observed in soil planted with inoculated white clover. This response may reflect intensified decomposition of organic nitrogen compounds generated by root turnover, microbial biomass, and rhizodeposition.

Pedotrophic microorganisms increased after 60 days, indicating enhanced nutrient availability and rhizosphere activation. In contrast, oligotrophic populations generally declined, except after seed inoculation alone, where their abundance increased by 37.6 %. This shift likely reflects the development of more copiotrophic conditions and a transition from a stress-affected microbial community toward a more metabolically active system. Under such conditions, slow-growing oligotrophs may have been competitively displaced by rapidly proliferating pedotrophic and ammonifying populations. Cellulolytic microorganisms decreased 2.0–4.8-fold after combined remediation, likely reflecting a functional shift toward microbial groups utilising more readily available carbon substrates.

The mineralisation–immobilisation index remained low across all treatments (0.10–0.23), indicating a predominance of nitrogen immobilisation. We hypothesise that active microbial growth following remediation increased microbial demand for available nitrogen, resulting in temporary incorporation of mineral nitrogen into microbial biomass. Such immobilisation may represent a transitional stage of soil recovery in which nutrients are retained within the microbial pool and subsequently become available through biomass turnover.

Overall, the microbial response suggests that phytobacterial remediation didn`t merely increase total microbial abundance but induced a functional reorganisation of the soil microbiome. The observed enrichment of nitrogen-fixing, phosphate-mobilising, ammonifying, amylolytic, and pedotrophic microorganisms, together with the decline of oligotrophic, fungal, and cellulolytic groups, supports the hypothesis that remediation shifted the

microbial community toward a more nutrient-responsive and rhizosphere-driven functional state. This restructuring may constitute an important mechanism underlying the restoration of nutrient cycling and biological fertility in contaminated soils.

References

1. Biliavska, L., Iutynska, G., Loboda, M., Ropotilov, B., & Skrotskyi, S. (2024). Diagnostics and bioremediation of soils affected by military operations in Ukraine. Biological Systems: Theory and Innovation, 15(3), 67–78. https://doi.org/10.31548/biologiya/3.2024.67
2. Corredor, D., Duchicela, J., Flores, F. J., Maya, M., & Guerron, E. (2024). Review of explosive contamination and bioremediation: insights from microbial and bio-omic approaches. Toxics, 12(4), 249. https://doi.org/10.3390/toxics12040249
3. Sokol, N. W., Slessarev, E., Marschmann, G. L., Nicolas, A., Blazewicz, S. J., Brodie, E. L., Firestone, M. K., Foley, M. M., Hestrin, R., Hungate, B. A., Koch, B. J., Stone, B. W., Sullivan, M. B., Zablocki, O., Trubl, G., McFarlane, K., Stuart, R., Nuccio, E., Weber, P., Pett-Ridge, J. (2022). Life and death in the soil microbiome: how ecological processes influence biogeochemistry. Nature Reviews Microbiology, 20, 415–430. https://doi.org/10.1038/s41579-022-00695-z
4. Solokha, M., Demyanyuk, O., Symochko, L., Mazur, S., Vynokurova, N., Sementsova, K., & Mariychuk, R. (2024). Soil degradation and contamination due to armed conflict in Ukraine. Land, 13(10), 1614. https://doi.org/10.3390/land13101614

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FUNCTIONAL PROPERTIES OF *ARTHROBACTER* SP. E35 – AN ENDOPHYTE OF THE *DESCHAMPSIA ANTARCTICA*: PSYCHROTOLERANCE, METAL RESISTANCE, AND SYNTHESIS OF AUXIN-LIKE COMPOUNDS

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Abstract. A psychrotolerant strain of the bacterium *Arthrobacter* sp. E35 was isolated from the endosphere of the Antarctic plant *Deschampsia antarctica* (King George Island). Based on the nucleotide sequence of the 16S rRNA gene (GenBank: OR899264.1), the strain is most closely related to *Arthrobacter agilis* DSM 20550 (99.92 % identity). These bacteria are Gram-positive, aerobic, catalase-positive, nonmotile cocci that grow at temperatures ranges from +4 to +30 °C. The bacteria are resistant to heavy metal salts (Co^{2+}, Cu^{2+}, Fe^{2+} up to 2.0 mM; $Cr_2O_7^{2-}$, Mn^{2+} up to 1.0 mM; Cd^{2+} up to 0.4 mM) and can tolerate up to 2.5 % NaCl. The strain was found to effectively synthesize auxin-like compounds in a medium containing L-tryptophan; at 22 °C, phytohormone production was 5 times higher than at 28 °C (9.6 mg/g dry weight).
Keywords: auxins, *Arthrobacter* sp., *Deschampsia antarctica*, endophytes, heavy metals, wheat.

Introduction. Modern agriculture requires the development of environmentally safe biotechnological solutions to increase crop yields amid increasing abiotic stresses and global climate change. Of particular interest in this context are endophytic microorganisms, which colonize the internal tissues of plants and form close symbiotic relationships with them (Wippel, 2023). A unique source of stress-resistant bioagents is the endosphere of Antarctic vascular plants, which survive in extreme ecological niches characterized by low temperatures, high ultraviolet radiation, and a constant nutrient shortage (Iungin et al., 2024;

Rodriguez et al., 2025). In particular, Antarctic hairgrass (*Deschampsia antarctica* Desv.) possesses a high adaptive potential due to its specific microbiota, which is capable not only of withstanding extreme environmental factors but also of stimulating the host plant's growth through the production of biologically active substances (including phytohormones) (Podolich et al., 2021; Znój et al., 2022). The study and utilization of such cold-adapted and stress-tolerant endophytic bacteria with plant growth-promoting activity represent a promising avenue for developing effective next-generation biological products for agriculture in temperate and cold climates.

The aim of this study was to characterize the psychrotolerant endophytic strain *Arthrobacter* sp. E35, isolated from *D. antarctica*, in terms of its taxonomic relationship, morphological, physiological, and biochemical properties, resistance to heavy metal salts, and ability to synthesize auxin-like compounds.

Materials and Methods. Bacterial isolate E35 was obtained from the endosphere of *D. antarctica*, collected by members of the 27th Ukrainian Antarctic Expedition at Oasis Point Thomas (King George Island, South Shetland Islands; –62.163491, –58.468756; 90 m above sea level). A phylogenetic analysis was performed by sequencing the 16S rRNA gene (GenBank: OR899264.1). Morphological, physiological, and biochemical properties were assessed using standard microbiological methods. Resistance to heavy metal salts was determined by growth on tryptic soy agar containing various concentrations of the salts $CoCl_2 \cdot 6H_2O$, $K_2Cr_2O_7$, $CdCl_2 \cdot 2.5H_2O$, $MnCl_2 \cdot 4H_2O$, $CuCl_2 \cdot 2H_2O$, or $FeSO_4 \cdot 7H_2O$. The ability to synthesize auxin-like compounds was detected in the culture supernatant using Salkowski's reagent.

Results. On tryptic soy agar, these bacteria form small (2–3 mm), pink, round, shiny colonies with a drop-shaped profile and smooth edges. The cells of these bacteria are small, Gram-positive, nonmotile cocci. The bacteria are aerobic, catalase-positive, oxidase-negative, facultative psychrophiles (growth temperature range: +4…+30 °C; optimal pH: 6.8–7.2), and halotolerant (tolerant to 2.5 % NaCl). The strain utilizes D-galactose, D-fructose, and D-mannose; it does not utilize citrate, does not metabolize urea, does not solubilize gelatin, and does not reduce nitrates.

According to the NCBI GenBank database, the 16S rRNA gene sequence of isolate E35 (GenBank: OR899264.1) shows a high degree of identity with the 16S rRNA gene sequence of *Arthrobacter agilis* DSM 20550 (99.92 %, 100 % query cover), *Arthrobacter bussei* KR3265004 (99.77 %, 100 % query cover), *Arthrobacter cheniae* Hz2 (99.62 %, 100 % query cover), and *Arthrobacter ruber* MDB1-42 (99.17 %, 100 % query cover). The 16S rRNA gene sequence of isolate E35 belongs to the same clade as that of *Arthrobacter agilis* DSM 20550, confirming the phylogenetic relationship between these two strains.

Bacteria *Arthrobacter* sp. E35 are resistant to 2.0 мМ $CoCl_2 \cdot 6H_2O$, 1.0 мМ $K_2Cr_2O_7$, 0.4 мМ $CdCl_2 \cdot 2.5H_2O$, 1.0 мМ $MnCl_2 \cdot 4H_2O$, 2.0 мМ $CuCl_2 \cdot 2H_2O$, 2.0 мМ $FeSO_4 \cdot 7H_2O$.

During growth in a nutrient broth containing 0.15 % tryptophan at a temperature of 28 °C, the *Arthrobacter* sp. E35 strain synthesizes up to 9.6 mg/g of dry weight of auxin-like compounds. At 22 °C, these bacteria produce 5 times as much auxin-like compounds as at 28 °C.

Conclusions. The Antarctic endophytic strain *Arthrobacter* sp. E35 shows promise for the development of biological products designed to increase crop yields under adverse conditions.

This study was conducted under the State Target Scientific and Technical Research Program in Antarctica for 2011–2025 and within the project "Development of an approach to restore soil fertility in soils affected by hostilities using microbial biological preparations", state registration No. 0124U000931.

References

1. Iungin, O., Prekrasna-Kviatkovska, Y., Kalinichenko, O., Moshynets, O., Potters, G., Sidorenko, M., Savchuk, Y., & Mickevičius, S. (2024). Endophytic Bacterial Biofilm-Formers Associated with Antarctic Vascular Plants. Microorganisms, 12(10), 1938. https://doi.org/10.3390/microorganisms12101938
2. Podolich, O., Prekrasna, I., Parnikoza, I., Voznyuk, T., Zubova, G., Zaets, I., Miryuta, N., Myryuta, G., Poronnik, O., Kozeretska, I., Kunakh, V., Pirttila, A. M., Dykyi, E., & Kozyrovska, N. (2021). First record of the endophytic bacteria of *Deschampsia antarctica* Ė. Desv. from two distant localities of the maritime Antarctic. Czech Polar Reports, 11(1), 134–153. https://doi.org/10.5817/cpr2021-1-10
3. Rodriguez, R., Barra, P. J., Saldivar-Diaz, M., Larama, G., Alvarado, R., López, D., Delgado, M., Orlando, J., Oses, R., Merino, C., Tortella, G., & Duran, P. (2025). Holobiome Structure and Microbial Core Assemblages of *Deschampsia antarctica* Across the South Shetland Islands. Plants, 14(23), 3657. https://doi.org/10.3390/plants14233657
4. Wippel, K. (2023). Plant and microbial features governing an endophytic lifestyle. Current Opinion in Plant Biology, 76, 102483. https://doi.org/10.1016/j.pbi.2023.102483
5. Znój, A., Grzesiak, J., Gawor, J., Gromadka, R., & Chwedorzewska, K. J. (2022). Highly specialized bacterial communities within three distinct rhizocompartments of Antarctic hairgrass (*Deschampsia antarctica* Desv.). Polar Biology, 45(5), 833–844. https://doi.org/10.1007/s00300-022-03027-2

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THE EFFECT OF ZINC SULPHATE AND COBALT CHLORIDE ON THE GROWTH OF BACTERIA OF THE GENUS *STREPTOMYCES* ISOLATED FROM THE CHERVONOGRAD MINING DISTRICT

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Abstract. The effect of heavy metal compounds on the growth of *Streptomyces* sp. CEF-13 bacteria isolated from the Chervonohrad mining region was studied. Zinc sulfate at a concentration of 0.07 mM accelerated the transition of the culture to the death phase, and in the presence of 0.7–4.5 mM of this compound, growth inhibition was observed. Cobalt(II) chloride inhibited growth at concentrations of 0.35 mM and higher.
Keywords: Chervonohrad mining region, zinc sulfate, cobalt(II) chloride, *Streptomyces* sp.

Heavy metals are one of the most dangerous environmental pollutants, as they are not subject to biodegradation and therefore accumulate in soils, water bodies and living organisms. Heavy metal ions, in particular zinc, cobalt, copper and lead, even at relatively low concentrations have a toxic effect on microorganisms – they damage cell membranes, disrupt the functioning of enzymes and inhibit the synthesis of nucleic acids (Cimermanova et al., 2021). Industrial regions of Ukraine, in particular the Chervonohrad mining region of Lviv region, are characterized by an increased content of heavy metals in the soil due to long-

term coal and mining activities. This necessitates the search for effective and environmentally safe methods of neutralizing these metals.

Microbiological remediation is considered one of the most promising approaches to restoring polluted ecosystems. Its effectiveness is determined primarily by the presence of microorganisms with natural resistance to specific pollutants. Among soil microorganisms, actinobacteria of the genus *Streptomyces* occupy a special place: they are characterized by high metabolic diversity and produce a wide range of extracellular enzymes, are the dominant cultivated group in the soils of most terrestrial ecosystems, and constitute about 20 % of the cultivated bacterial biomass (Norte et al., 2025; Chater, 2016). They are also capable of producing specific mechanisms of resistance to metal ions – biosorption, active efflux, siderophore production and intracellular sequestration. It is significant that strains of the genus *Streptomyces* isolated from technogenically contaminated substrates often demonstrate a significantly higher level of resistance to metals compared to isolates from uncontaminated environments. This indicates the adaptive potential of these microorganisms and their prospects in bioremediation processes (Cimermanova et al., 2021).

The effect of zinc sulfate and cobalt(II) chloride on the growth of *Streptomyces* sp. CEF-13 bacteria isolated from the Chervonohrad mining region was studied. The bacteria were grown in nutrient broth (Farmaktiv, Ukraine) with glass beads under aeration conditions. The seeding density was 0.1 g/L. Biomass was determined photoelectrocolorimetrically.

In the control, the biomass of bacteria reached a maximum on the first or second day. Zinc sulfate at a concentration of 0.07 mM did not affect bacterial growth during the first two days, but accelerated the transition of the culture to the death phase. In the presence of 0.7–4.5 mM of this compound, growth inhibition was observed during all six days of cultivation.

Zinc is an important trace element for members of the genus *Streptomyces*, as it is involved in the functioning of metal-dependent enzymes and the regulation of gene expression related to the maintenance of metal homeostasis. Under conditions of limited Zn^{2+} availability, the activity of the zinc-sensitive regulator Zur changes, which is accompanied by the induction of zinc capture systems, the reorganization of the ribosomal apparatus and the redistribution of intracellular Zn^{2+}. Thus, zinc deficiency can cause significant metabolic restructuring of the cell (Owen et al., 2007; Choi et al., 2017). At the same time, the availability of Zn^{2+} directly affects secondary metabolism and morphological differentiation in members of the genus *Streptomyces*. In *Streptomyces coelicolor*, actinorhodin production was maximal in a certain range of Zn^{2+} concentrations, while in the absence of added zinc or its excess, it was significantly reduced; at high concentrations of Zn^{2+} growth inhibition was also observed (Hesketh et al., 2009).

Cobalt chloride at a concentration of 0.035 mM had no effect on biomass accumulation. At 0.35 mM, a decrease in biomass of 83 % was observed, compared with the control, on the first day and by 44 % on the sixth. At 3 mM, complete growth inhibition was observed.

Cobalt is an important trace element for representatives of the genus *Streptomyces*, in particular as the central atom of cobalamin (vitamin B_{12}), which is involved in a number of metabolic processes. Violation of cobalamin biosynthesis in *Streptomyces coelicolor* leads to changes in primary metabolism and impaired morphological development, including the formation of aerial mycelium and spores, which indicates the important role of B_{12}-dependent processes in the life cycle of the bacterium. In the case of insufficient availability of Co^{2+}, the limitation of its use for cobalamin biosynthesis could potentially exacerbate such metabolic and physiological limitations (Locatelli et al., 2016).

Compared with the literature data (El Baz et al., 2015), it can be stated that *Streptomyces* sp. CEF-13 bacteria are very resistant to compounds of this metal. This makes them promising for use in remediation technologies for environments contaminated with zinc.

References

1. El Baz, S., Baz, M., Barakate, M., Hassani, L., El Gharmali, A., & Imziln, B. (2015). Resistance to and accumulation of heavy metals by actinobacteria isolated from abandoned mining areas. The Scientific World Journal, 2015, 761834. Doi: 10.1155/2015/761834.
2. Chater, K. F. (2016). Recent advances in understanding *Streptomyces*. F1000Research, 5, 2795. Doi: 10.12688/f1000research.9534.1.
3. Cimermanova, M., Pristas, P., & Piknova, M. (2021). Biodiversity of actinomycetes from heavy metal contaminated technosols. Microorganisms, 9(8), 1635. Doi: 10.3390/ microorganisms 9081635.
4. Choi, S.-H., Lee, K.-L., Shin, J.-H., Cho, Y.-B., Cha, S.-S., & Roe, J.-H. (2017). Zinc-dependent regulation of zinc import and export genes by Zur. Nature Communications, 8, 15812. Doi: 10.1038/ncomms15812.
5. Hesketh, A., Kock, H., Mootien, S., & Bibb, M. (2009). The role of absC, a novel regulatory gene for secondary metabolism, in zinc-dependent antibiotic production in *Streptomyces coelicolor* A3(2). Molecular Microbiology, 74(6), 1427–1444. Doi: 10.1111/j.1365-2958.2009. 06941.x.
6. Locatelli, F. M., Goo, K.-S., & Ulanova, D. (2016). Effects of trace metal ions on secondary metabolism and the morphological development of streptomycetes. Metallomics, 8(5), 469–480. Doi: 10.1039/C5MT00324E.
7. Norte, D. M., Avitia-Dominguez, L. A., & Rozen, D. E. (2025). Evolution and ecology of *Streptomyces*. Annual Review of Microbiology, 79, 383–403. Doi: 10.1146/annurev-micro-051524-032254.
8. Owen, G. A., Pascoe, B., Kallifidas, D., & Paget, M. S. B. (2007). Zinc-responsive regulation of alternative ribosomal protein genes in *Streptomyces coelicolor* involves Zur and σR. Journal of Bacteriology, 189(11), 4078–4086. Doi: 10.1128/JB.01901-06.

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NICKEL CHLORIDE INFLUENCE ON ELEMENTAL SULFUR, NITRATE IONS AND CHROMIUM (VI) REDUCTION BY BACTERIA OF GENUS *DESULFUROMONAS*

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Abstract. $NiCl_2$ at concentrations of 1.0–4.0 mM in the incubation mixture inhibited the growth and dissimilatory reduction of S^0, NO_3^- and Cr(VI) by bacteria *Desulfuromonas acetoxidans* IMV B-7384 in the medium with three electron acceptors (3.5 mM). In this medium the efficiency of nitrates reduction by bacteria was higher than that of S^0 and Cr(VI), possibly due to the higher redox potential of nitrates than elemental sulfur and the high toxicity of hexavalent chromium to cells.
Keywords: *Desulfuromonas*, elemental sulfur, nitrates, Cr(VI).

The level of environment pollution by heavy metal compounds, in particular, chromium, ferrum, lead, nickel, cobalt, cadmium, etc., is increasing every year. The genotoxicity and toxicity of Cr(VI) is due to damage to DNA and proteins by oxygen radicals formed in cells as a result of its reduction (Viti et al., 2014). Various species of fungi and bacteria have shown promise for the removal of Cr(VI) from industrial wastewater (Sharma et al., 2022). Sulfidogenic bacteria isolated from polluted environments and adapted to stress factors are suitable for use in environment bioremediation technologies from chromium compounds and other toxicants (Moroz et al., 2022). Bacteria of genus *Desulfuromonas* use elemental or

polysulfide sulfur, nitrates, nitrites, L-malate, fumarate, three- or tetrachloroethylene, and oxidized forms of heavy metals (Cr(VI), Fe(III), Mn(IV), Cu(II)) as electron acceptors (An & Picarda, 2015), oxidizing H_2 or organic compounds to CO_2. Technogenically altered ecotopes, such as Yavorivske Lake, are characterized by complex pollution. The influence of nickel ions, the content of which in the deep layers of the lake significantly exceeds the maximum permissible concentrations, on the physiological and biochemical properties of microorganisms is insufficiently studied (Tarabas et al., 2017). Therefore, the aim of the work was to study the influence of nickel ions on the reduction of elemental sulfur, nitrate ions, and hexavalent chromium by bacteria of genus *Desulfuromonas*, isolated from Yavorivske Lake, when they are simultaneously present in the medium.

Bacteria *Desulfuromonas acetoxidans* IMV B-7384 were grown in Kravtsov-Sorokin medium without SO_4^{2-} and Mohr's salt (sowing density – 0.1 g/L) under anaerobic conditions for 10 days at temperature of 28–30 °C (Gudz et al., 2014). Weights of S^0 and sterile 1 M $NaNO_3$ and $K_2Cr_2O_7$ solutions were added to the medium at electron acceptors concentration of 3.5 mM. To study the ability of bacteria to reduce S^0, NO_3^- and Cr(VI) under the influence of Ni^{2+}, cells were precipitated by centrifugation (15 min, 4025 g), resuspended in 0.9 % NaCl and incubated for 1 h with different volumes of sterile 1 M $NiCl_2$ solution at concentrations: 0 (control); 1.0; 2.0; 2.5; 3.0; 4.0 mM, precipitated, washed twice with 0.9 % NaCl and sown in medium with different electron acceptors. Biomass was determined turbidimetrically, HS^-, Cr(VI) and Cr(III) concentrations were determined spectrophotometrically (Gudz et al., 2014). The content of NO_3^- and NH_4^+ in the cultural liquid was measured using ion-selective electrodes and the ionometer "I-160Mi". The results were statistically processed (Petrovska et al., 2022).

In the medium with S^0, NO_3^- and Cr(VI) the biomass of cells, incubated with 1.0–4.0 mM $NiCl_2$, was 1.1–1.5 times lower than in the control. Cells, incubated with Ni^{2+}, during growth in the medium with S^0, NO_3^- and Cr(VI) produced HS^- 1.3–4.2 times less than in the control. The efficiency of NO_3^- reduction by cells, incubated with $NiCl_2$, in the medium with S^0, NO_3^- and Cr(VI) decreased by 6.3–28.0 %, while the NH_4^+ content in the medium was 1.1–1.8 times lower than in the control. The efficiency of Cr(VI) reduction by bacteria under the action of $NiCl_2$ in the medium with S^0, NO_3^- and Cr(VI) decreased by 6.6–21.0 %, and the bacteria produced Cr(III) 1.1–1.6 times less than in the control. Thus, we found that nickel ions (1.0–4.0 mM) inhibit the growth and dissimilatory reduction of S^0, NO_3^- and Cr(VI) by sulfur-reducing bacteria in the medium with three electron acceptors, added simultaneously.

At higher concentrations nickel is toxic to microorganisms, it disrupts crucial cellular processes and enzymatic functions. After penetration into the cells, nickel interacts with cellular metabolites, changing their properties, affects DNA reparation. Nickel can replace essential metals in composition of metalloproteins, interact with catalytic residues of non-metalloenzymes or bind outside their catalytic sites, causing allosteric inhibition. In addition, nickel induces oxidative stress, which can negatively affect proteins, DNA and lipids (Darwiche et al., 2025). Therefore, with increasing Ni^{2+} concentration during incubation of cells, an increase in the inhibition level of growth and reduction of all studied electron acceptors by bacteria of genus *Desulfuromonas* was observed. Under the conditions of simultaneous presence of S^0 and $K_2Cr_2O_7$ in the medium, bacteria reduced more Cr(VI) than S^0 (at pH 7.0 the standard redox potential of the Cr(VI)/Cr(III) pair (E_0' = +1.33 V) is significantly higher than that of the S^0/HS^- pair (E_0' = –0.27 V)) (Richter et al., 2012), since the content of H_2S produced by bacteria was lower than the content of Cr(III). Although the standard redox potential at pH 7.0 of the Cr(VI)/Cr(III) pair is significantly higher than that of the NO_3^-/NO_2^- and NO_2^-/NH_4^+ pairs (E_0' = +0.43 and E_0' = +0.34 V, respectively) (Richter et al., 2012), bacteria reduced more nitrates than Cr(VI). However, $K_2Cr_2O_7$ inhibited nitrate

reduction carried out by bacteria. Although S^0 and NO_3^- are less powerful electron acceptors of anaerobic respiration for bacteria than oxidized forms of metals, they used a thermodynamically more favourable electron acceptor, such as Cr(VI), less efficiently, possibly due to its high toxicity.

References

1. An, T. T., & Picarda, F. W. (2015). *Desulfuromonas carbonis* sp. nov., an Fe (III)-, S^0 and Mn (IV)-reducing bacterium isolated from an active coalbed methane gas well. International Journal of Systematic and Evolutionary Microbiology, 65(5), 1686–1693. Doi:10.1099/ijs.0.000159.
2. Darwiche, L., Rodriguez-Bornot, C. A., Ingrassia, R. A., Loccisano, M. J., Waldschmidt, G., & Goff, J. L. (2025). The molecular basis of the synergistic toxicity of nickel and copper, common environmental co-contaminants. Applied and Environmental Microbiology, 91, e01627–25. Doi:10.1128/aem.01627-25.
3. Gudz, S. P., Hnatush, S. O., Yavorska, G. V., Bilinska, I. S., & Borsukevych, B. M. (2014). Workshop on microbiology: textbook. Lviv: Ivan Franko National University of Lviv.
4. Moroz, O. M., Hnatush, S. O., Yavorska, H. V., Zvir, G. I., & Tarabas O. V. (2022). Influence of potassium dichromate on the reduction of sulfur, nitrate and nitrite ions by bacteria *Desulfuromonas* sp. Regulatory Mechanisms in Biosystems, 13(2), 153–167. Doi:10.15421/022220.
5. Petrovska, I. R., Salyha, Y. T., & Vudmaska, I. V. (2022). Statistical methods in biological research: a teaching and methodological manual. Kyiv: Agrarna nauka.
6. Richter, K., Schicklberger, M., & Gescher, J. (2012). Dissimilatory reduction of extracellular electron acceptors in anaerobic respiration. Applied and Environmental Microbiology, 78(4), 913–921. Doi:10.1128/AEM.06803-11.
7. Sharma, P., Singh, S. P., Parakh, S. K., & Tong Y. W. (2022). Health hazards of hexavalent chromium (Cr (VI)) and its microbial reduction. Bioengineered, 13(3), 4923–4938. Doi:10.1080/21655979.2022.2037273.
8. Tarabas, O., Moroz, O., Hnatush, S., Yavorska, G., Zvir, G., & Kovalchuk, M. (2017). Ecological trophic groups of microorganisms of Yavorivske Lake water. Visnyk of Lviv University. Biological Series, 76, 166−178.
9. Viti, C., Marchi, E., Decorosi, F., & Giovannetti, L. (2014). Molecular mechanisms of Cr (VI) resistance in bacteria and fungi. FEMS Microbiology Reviews, 38(4), 633–659. Doi:10.1111/1574-6976.12051.

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PHYTOPATHOGENIC BACTERIA OF THE GENUS *CURTOBACTERIUM* – A NEW PATHOGEN OF LENTIL DISEASE IN UKRAINE

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Abstract. Lentils are gradually making a comeback in Ukrainian agriculture, which is not surprising, as they are rich in nutrients, high in protein, drought-tolerant, and undemanding to grow. Despite this, their yield can be significantly reduced by diseases. There is insufficient information worldwide about bacterial diseases of lentils; in particular, no such studies have been conducted in Ukraine before. We were able to isolate bacterial pathogens from infected lentils and describe them.
Keywords: phytopathogenic bacteria, *Curtobacterium,* lentil.

Phytopathogenic bacteria are constant companions of both cultivated and wild plants. They cause significant economic losses in crop production, forestry, and floriculture, and noticeably reduce crop yields and product quality.

Among plants of the legume family, lentils are being used more and more widely in agriculture as both a forage and an edible crop, as they are economically and environmentally beneficial (Sichkar, 2020). Moreover, Ukraine's climate is suitable for growing lentils (Petkevych, 2016). They are resistant to drought and high humidity, and given that temperatures in Ukraine are gradually rising each year (for example, over the past 60 years, they have increased by 1.5 degrees (Kyrylenko, 2020)), it can be predicted that lentils will be quite promising in the future. In 2025, the area planted with red lentils increased to 25,000–26,000 ha, and the average yield was 2–2.5 t/ha. The price of lentils is currently stable and high (25,000 UAH/t), making it one of the most profitable crops of the season (Basanets, 2025).

According to the literature, it is known that lentils can be affected by *Pseudomonas syringae* pv. *syringae* and *Xanthomonas axonopodis* (Adhikari, 2018; Kulshrestha, 2015); however, there have been no such reports in Ukraine, which demonstrates how little research has been done on this issue.

The purpose of this study is to isolate the pathogens responsible for bacterial diseases from various lentil varieties and to identify them based on their physiological and biochemical properties.

Methods and materials. During the 2022–2025 period, samples of seeds, leaves, and pods of the "Linza" and "Chervona" lentil varieties exhibiting disease symptoms were collected. Symptoms were described, and isolates were obtained from the samples. Isolation was performed on LB solid medium and incubated at 27 °C for 3–5 days. Bacterial colonies resembling phytopathogenic bacteria were then selected. To determine the virulence of the isolates, artificial inoculation of "Linza" lentils was performed under greenhouse conditions. After 7 days, the degree of infection was assessed, and virulent isolates were selected. Morphology was described, and microscopy was performed. To determine the physiological and biochemical properties, an O/F test was performed and oxidase activity was measured. For one of the isolates, fatty acids were also isolated, and their composition was then determined using the Agilent 6800N/5973 Inert chromatography-mass spectrometry system.

Results and discussion. Five isolates were selected that were found to be virulent toward lentils. All caused chlorosis and the appearance of necrotic spots at the site of infection on the leaves and pods. No signs of infection were observed on the stems. Microscopic examination revealed that all 5 isolates were Gram-positive, small rods, occurring singly or occasionally in short chains of 2 rods arranged in the shape of the letter "v." Based on the appearance of the colonies, the isolates were light pink (5k, 7k, and 11k), yellow (2k), and cream-colored (1k). Based on biochemical characteristics, the isolates were found to be low in activity; they did not ferment glucose or lactose and were oxidase-negative. Based on morphological, cultural, and physiological-biochemical characteristics, the isolates are similar to the plant pathogen *Curtobacterium* spp. (specifically the species *flaccumfaciens*). Additionally, the fatty acid composition of the cell lipids was determined for isolate 1k, revealing the presence of C15:0, iC16:0, aiC17:0, and C16:1; of these, aiC17:0 and iC16:0 are marker fatty acids for *C. flaccumfaciens* (Tegli, 2011).

It is known that *C. flaccumfaciens* infects legumes (particularly soybeans and beans) and causes bacterial wilt and rusty-brown leaf spot. The isolation of such strains from infected lentils may indicate that this pathogen is expanding its host range. This pathogen is dangerous because it can be transmitted through seeds and plant debris, is resistant to

desiccation, and can persist for long periods of time; it is also listed on the A-2 quarantine list in Ukraine and many other countries around the world (CORBFL, 2021).

Conclusions. In Ukraine, a description was conducted of lesions on the seeds and green parts of lentil plants of the "Linza" and "Chervona" varieties caused by bacterial pathogens. Five isolates were obtained, all of which were found to be virulent toward lentils. Based on their morphological and physiological-biochemical properties, strains 1k, 2k, 5k, 7k, and 11k were identified as belonging to the genus *Curtobacterium*.

References

1. Adhikari, A. D., Nielsen, K. A., Harveson, R. M., Graham, C., Beck, R., & Mathew, F. M. (2018). Bacterial blight of lentil (*Lens culinaris*) caused by *Pseudomonas syringae* pv. *syringae*. *Plant Health Progress*, *19*(1), 54–55.
2. Basanets, O. (2025). Red lentil production is a promising and profitable venture, given the crop's specific characteristics. https://superagronom.com/news/21371-virobnitstvo-chervonoyi-sochevitsi-ye-perspektivnim-ta-marjinalnim-yakscho-vrahuvati-osoblivosti-kulturi
3. European and Mediterranean Plant Protection Organization. (2021). *Curtobacterium flaccumfaciens pv. flaccumfaciens (CORBFL)*. EPPO Global Database. https://gd.eppo.int/taxon/CORBFL/datasheet
4. Kulshrestha, S., Chaturvedi, S., Jangir, R., & Agrawal, K. (2015). In vitro Evaluation of antibacterial activity of some plant leaf extracts against *Xanthomonas axonopodis* pv. *phaseoli* isolated from seeds of lentil (Lens culinaris Medik.). *Int. Res. J. Biol. Sci*, *4*(7), 59–64.
5. Kyrylenko, O. V., Basok, B. I., Bazieiev, Y. T., & Blinov, I. V. (2020). Ukraine's energy sector and the realities of global warming. Technical Electrodynamics (Tekhnichna Elektrodynamika), (3).
6. Petkevych, Z. Z., & Melnichenko, H. V. (2016). Chickpea and lentil as promising leguminous crops for cultivation in southern Ukraine. Irrigated Agriculture, 65, 104–107.
7. Sichkar, V. I., Kryvenko, A. I., & Solomonov, R. V. (2020). Lentils in the World and in Ukraine: Current Status and Prospects. *Journal of Native and Alien Plant Studies*, *16*, 178–193.
8. Tegli, S. (2011). *Curtobacterium flaccumfaciens pv. flaccumfaciens*. *BULLETIN OEPP*, *41*, 320–328.

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SOIL MYCOBIOME OF FOREST ECOSYSTEMS OF WESTERN POLISSIA OF UKRAINE: BIODIVERSITY AND STRUCTURAL ORGANISATION

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Abstract. The research dedicated to the study of biodiversity and structural organisation of soil mycobiomes in various forest ecosystems of Western Polissia of Ukraine demonstrated a high species diversity of microscopic fungi. The representatives of *the Ascomycota* shaped a stable taxonomic core, with the genus *Penicillium* being the dominant one. Different forest edaphic habitats demonstrated a high degree of species similarity in their mycobiomes, indicating ecological stability and the presence of a shared regional fungal pool. The results obtained provide a basis for future mycological monitoring and forest restoration.

Keywords: fungal community; fungal diversity; mycobiome structure; forest ecosystem; forest type; edatope; Western Polissia.

Relevance. Forest ecosystems are among the most complex biomes on Earth and play a key role in the conservation of biodiversity, the regulation of water resources, climate stability and the provision of ecosystem services (Melnyk-Shamrai & Ivaniuk, 2026). Soil microbiomes play a central role in maintaining the viability of forests, ensuring the cycling of nutrients, the transformation of organic matter and energy exchange (Marín, et al., 2023). In view of human impact, climate change and the degradation of forest ecosystems as a result of military action, the study of mycobiomes of climax ecosystems remains a topical area.

Materials and Methods. The study was carried out in the Zhytomyr, Rivne and Volyn regions. Twenty monitoring plots were surveyed, representing a range of forest edaphic types: fresh and moist pine bor forests; fresh and moist pine subor forests; moist pine subor forests; moist and damp sugrud forests; fresh and moist sugrud forests; and moist and damp pine subor forests. The predominant soils were sod-podzolic soils with varying degrees of podzolisation, soil moisturising and gleying.

Soil samples were collected from the 0–10 cm layer in accordance with international standards from three sampling plots on each site. The quantitative and qualitative composition of microfungi was determined using classical microbiological methods. Isolates were identified on the basis of macro- and micromorphological characteristics, utilising modern taxonomic keys and the MycoBank and Outline of Fungi databases. The taxonomic composition and α- and β-diversity indices (Shannon, Simpson, Pielou, Berger–Parker, Margalef, Menhinick, Sørensen, Jaccard, Ochiai, Kulczynski, Whittaker) were assessed, and principal component analysis (PCA) and cluster analysis were performed. Statistical analysis was performed using Statistica 10, Mothur v.1.30.2 and Cluster 3.0. The significance level was set at $P < 0.05$.

Results. A high level of species richness of soil fungi was recorded in the forest ecosystems of Western Polissya. The phylum *Ascomycota* dominated in all edaphic sites (87.12–91.22 per cent of the species composition). The proportion of *Mucoromycota* was 8.29–11.04 per cent, and that of *Entomophthoromycota* was 0.47–0.61 per cent. The leading genus in all groups was *Penicillium* (26–27 species; approximately 13–16 per cent of the species composition). The taxonomic core was also formed by *Aspergillus*, *Cephalotrichum*, *Fusarium*, *Trichoderma*, *Mucor*, *Alternaria*, *Talaromyces* and *Colletotrichum*. The ten most abundant genera accounted for approximately 46–53 per cent of the total species diversity. All the communities studied exhibited exceptionally high biodiversity, characteristic of mature natural ecosystems: Shannon index $H' = 4.39–4.66$; Simpson's 1–D index = 0.983–0.989; Piel's evenness index J = 0.859–0.870. Differences between edatops were determined mainly by rare and associated taxa, whilst the core was formed by widespread saprotrophic genera.

Cluster analysis revealed gradual changes in the composition of the mycobiome along the ecological gradient of pine bor forests → pine subor forests → sugrud forests, without any sharp floristic barriers. The structure of the communities corresponded to a nested organisation: the mycobiota of oligotrophic pine bor forests was largely incorporated into the richer communities of pine subor forests and sugrud forests. The increase in diversity occurred mainly through the addition of new species, rather than the replacement of dominant taxa. Functionally, the clusters differed in their range of life strategies: K-strategists predominated in plots 1–4; a complex of K–V-strategists in plots 5–8; an r–K spectrum in plots 9–12; V-strategists in plots 13–16; and r-strategists in plots 17–20

Conclusions.

1. Soil mycobiomes in the forest ecosystems of Western Polissya are characterised by high taxonomic richness (163–212 species of microscopic fungi) and structural stability.

2. The phylum *Ascomycota* dominates in all edaphic communities (87–91 per cent), whilst the genus *Penicillium* is the key component of the taxonomic core.

3. The high similarity of the mycobiota between edaphic sites (Sørensen = 0.800–0.930) indicates the presence of a shared regional pool of soil fungi characteristic of the forests of Western Polissya.

4. The structure of the soil mycobiota is determined by a complex of factors: soil trophic level, moisture content and acidity, the composition of the tree stand, and the quality of litter.

5. For the first time, a nested type of mycobiome organisation has been established for the main forest edaphic types of Western Polissya: the mycobiota of oligotrophic pine bor forests is largely encompassed by the richer communities of pine subor forests and sugrud forest communities.

6. The combination of high similarity among communities and significant taxonomic richness indicates the ecological stability of Polissya's natural forest ecosystems and the relatively high level of conservation of their soil mycobiomes. The identified taxonomic core, together with edaphically and topically specific rare taxa, can serve as a basis for further monitoring of changes in forest ecosystems.

References

1. Melnyk-Shamrai, V. V., & Ivaniuk, R. O. (2026). Impact of environmental factors on forest ecosystems and the provision of ecosystem services. *Environmental Sciences*, (1(64)), 161–167. https://doi.org/10.32846/2306-9716/2026.eco.1-64.23
2. Marín, C., Godoy, R., Boy, J., & Öpik, M**.** (2023). Geological history and forest mycorrhizal dominance effects on soil fungal communities. *New Phytologist*, 239(2), 743–756. https://doi.org/10.1111/nph.18950

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BACTERIAL COMMUNITIES OF MICROBIAL MATS IN FRESHWATER LAKES OF THE MARITIME ANTARCTIC: TAXONOMIC STRUCTURE, ABUNDANCE, AND PHYSIOLOGICAL PROPERTIES

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Abstract. The taxonomic structure, abundance, and physiological properties of bacteria inhabiting microbial mats in lakes on Irizar and Uruguay Islands in the Maritime Antarctic were investigated. Differences in the structure of bacterial communities and the abundance of culturable bacteria were identified. Bacterial isolates differed in their ability to grow on various culture media. The results indicate taxonomic, quantitative, and physiological and biochemical heterogeneity among the microbial mats studied.
Keywords: microbial mats, Maritime Antarctic, taxonomic structure, bacterial isolates, freshwater lakes.

Antarctic freshwater ecosystems are characterized by low temperatures, pronounced seasonality, and limited nutrient availability. Despite these extreme conditions, microbial mats develop in Antarctic water bodies and play important roles in polar ecosystem functioning and biogeochemical cycles. Microbial activity within mats drives key processes, particularly carbon and nitrogen fixation, promoting organic carbon accumulation and the formation of bioavailable nitrogen compounds under nutrient-limited conditions (Sohm et

al., 2020). Antarctic bacteria are also of considerable interest as a source of metabolically active microorganisms adapted to extreme environment and as promising subjects for fundamental and applied research (Medeiros et al., 2025). Bacteria isolated from Antarctic freshwater microbial mats exhibit diverse physiological and biochemical properties, including the ability to utilize various carbon sources, proteolytic and lipolytic activities, tolerance to a wide pH range, antagonistic activity, and resistance to antibacterial compounds (Górniak et al., 2025; Górniak et al., 2026).

The aim of this study was to investigate the taxonomic structure and abundance of bacteria inhabiting microbial mats in freshwater lakes on Irizar and Uruguay Islands (Argentine Islands, Maritime Antarctic), compare these characteristics among the studied water bodies, and determine the physiological properties of the bacterial isolates obtained.

The taxonomic structure of bacterial communities in microbial mats from freshwater lakes on Irizar and Uruguay Islands in the Maritime Antarctic was investigated using metagenomic analysis. In the microbial mats of the lake on Irizar Island, the predominant bacterial phyla were *Cyanobacteria* (34.0–47.0 %), *Pseudomonadota* (18.7–26.7 %), and *Bacillota* (17.5–17.8 %). In contrast, the microbial mats of the lake on Uruguay Island were characterized by the predominance of *Pseudomonadota* (54.2–66.2 %), *Bacillota* (2.03–20.1 %), and *Bacteroidota* (2.27–20.5 %).

The bacterial communities of the microbial mats studied included representatives of the families *Cytophagaceae*, *Chitinophagaceae*, *Sphingobacteriaceae*, and *Flavobacteriaceae*. Representatives of the phyla *Deinococcota*, *Myxococcota*, *Planctomycetota*, and *Bdellovibrionota* were also detected in all samples, indicating substantial taxonomic diversity within the bacterial communities. These results are consistent with findings from a study of microbial mats in Antarctic Lake Enigma, where *Myxococcota* and *Deinococcota* were also detected among the rare community members, with a relative abundance of less than 1 % (Smedile et al., 2024).

The abundance of culturable bacteria inhabiting microbial mats in Lakes Irizar and Uruguay was assessed using 15 samples collected during the 28th and 30th Ukrainian Antarctic Expeditions. Samples of microbial mats were suspended, serially diluted, and plated on TSA, R2A, Pikovskaya's medium, MSM supplemented with ZnO, and carboxymethyl cellulose-containing medium. After 5–7 days of cultivation, bacterial abundance was determined as CFU/g dry weight, adjusted for sample moisture. In the microbial mats of Lake Irizar sampled during the 28th UAE, phosphate-solubilizing bacteria were most abundant ($4.52 \cdot 10^5$ CFU/g dry weight), whereas their abundance in Lake Uruguay was substantially lower ($8.31 \cdot 10^3$ CFU/g dry weight). During the 30th UAE, oligotrophic bacteria predominated within the microbial mats of both lakes, reaching $1.93 \cdot 10^8$ and $4.18 \cdot 10^6$ CFU/g dry weight in Lakes Irizar and Uruguay, respectively. In Lake Irizar, the abundances of phosphate-solubilizing, zinc-solubilizing, and cellulolytic bacteria were lower than during the 28th UAE, whereas cellulolytic bacteria in Lake Uruguay reached $1.97 \cdot 10^6$ CFU/g dry weight.

The physiological and biochemical properties of 185 bacterial isolates obtained from microbial mats in Lakes Irizar and Uruguay were investigated. The isolates differed in their ability to grow on different culture media. Growth on Pikovskaya's medium was observed for 149 isolates (80.5 %), while 100 isolates (54.1 %) grew on MSM supplemented with ZnO and 91 (49.2 %) on medium containing carboxymethyl cellulose. Among isolates obtained from the microbial mats of the lake on Irizar Island, 86.2 % grew on Pikovskaya's medium, 61.2 % on MSM supplemented with ZnO, and 46.0 % on medium containing carboxymethyl cellulose. For isolates from the microbial mats of the lake on Uruguay Island, these proportions were 84.7 %, 45.5 %, and 59.1 %, respectively.

The results revealed differences in the taxonomic structure of bacterial communities in the microbial mats of the lakes on Irizar and Uruguay Islands. Culturable bacterial abundance

varied with sampling location and period, with the highest values recorded in the microbial mats of the lake on Irizar Island. Differences in growth on various culture media, together with molecular and quantitative analyses, indicate taxonomic, quantitative, physiological and biochemical heterogeneity of bacterial communities in freshwater microbial mats of the Maritime Antarctic.

References

1. Górniak, D., Świątecki, A., Kowalik, J., Grzesiak, J., Możejko-Ciesielska, J., & Zdanowski, M. K. (2026). Ecological differentiation at the polar frontier: Metabolic strategies of endemic and cosmopolitan bacteria in Antarctic microbial mats. *Frontiers in Microbiology, 17*, 1835163. https://doi.org/10.3389/fmicb.2026.1835163
2. Górniak, D., Świątecki, A., Kowalik, J., Grzesiak, J., Jastrzębski, J., & Zdanowski, M. K. (2025). High antagonistic activity and antibiotic resistance of flavobacteria of polar microbial freshwater mats on King George Island in maritime Antarctica. *Scientific Reports, 15*, 13615. https://doi.org/10.1038/s41598-025-97205-x
3. Medeiros, W., Kralova, S., Oliveira, V., Ziemert, N., & Sehnal, L. (2025). Antarctic bacterial natural products: From genomic insights to drug discovery. *Natural Product Reports, 42(5)*, 774–787. https://doi.org/10.1039/D4NP00045E
4. Sohm, J. A., Niederberger, T. D., Parker, A. E., Tirindelli, J., Gunderson, T., Cary, S. C., Capone, D. G., & Carpenter, E. J. (2020). Microbial mats of the McMurdo Dry Valleys, Antarctica: Oases of biological activity in a very cold desert. *Frontiers in Microbiology, 11*, 537960. https://doi.org/10.3389/fmicb.2020.537960
5. Smedile, F., La Cono, V., Urbini, S., Benedetti, G., La Spada, G., Crisafi, F., Azzaro, M., Porcino, N., Fazi, S., Amalfitano, S., Tassi, F., Vaselli, O., Venturi, S., Madigan, M. T., Hallsworth, J. E., & Yakimov, M. M. (2024). The perennially ice-covered Lake Enigma, Antarctica supports unique microbial communities. *Communications Earth & Environment, 5*, 741. https://doi.org/10.1038/s43247-024-01842-5

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REPRESENTATIVES OF THE GENUS *TRICHODERMA* COLONIZING ROTTING WOOD IN FOREST-PARK ZONES OF THE WESTERN FOREST-STEP OF UKRAINE

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Abstract. In this work we aimed to study mycobiome composition of species inhabiting decomposing wood of forest steppe area. Material was collected in several parts of deciduous forest. We obtained 115 Trichoderma strains by isolating fungi on PDA medium from decaying trees. Next Sanger sequencing of ITS region as well as TEF1 gene was performed for each strain in order to identify their species belonging.
Keywords: *Trichoderma*, Western Forest-Step of Ukraine.

Members of the genus *Trichoderma* are dominant xylotrophic and soil ascomycetes that play a key role in the degradation of lignocellulosic substrates and the maintenance of microbiological balance in forest ecosystems. In the mosaic ecosystems of the Western Forest-Steppe of Ukraine (particularly the Podillia and Opillia regions), the colonization of decaying wood by this group of microorganisms is determined by biotopic factors and the specifics of the mycocenosis.

In the decaying wood of deciduous (*Quercus robur*, *Fagus sylvatica*, *Carpinus betulus*) and mixed stands of the Western Forest-Steppe, species such as *Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma koningii*, *Trichoderma atroviride*, and *Trichoderma citrinum* (teleomorph *Hypocrea citrina*) are most frequently identified. They act as secondary colonizers and saprotrophs, displacing primary wood-decaying basidiomycetes (Błaszczyk et al., 2015).

Colonization of dead wood is carried out through a powerful cellulolytic and hemicellulolytic enzymatic complex (endoglucanases, cellobiohydrolases, β-glucosidases). This enables *Trichoderma* to break down complex biopolymers of plant cell walls into simple sugars, accelerating humification and the carbon cycle in parkland and natural forest stands (Druzhinina et al., 2011).

In degraded wood, *Trichoderma* strains actively suppress the development of phytopathogenic and wood-decaying fungi through a threefold action: mycoparasitism: direct coiling around pathogen hyphae and secretion of lytic enzymes (chitinases, β-1,3-glucanases); antibiosis: secretion of volatile and non-volatile metabolites (leptomycins, trichocin-like compounds); competition: rapid substrate acquisition and nutrient uptake (Chan et al., 2023).

Preserving fallen logs and dead wood in the forest parks of the Western Forest-Steppe is a critical factor in supporting the species diversity of *Trichoderma*. These microorganisms not only accelerate the utilization of woody debris but also serve as a natural reservoir of antagonists against pathogens causing root and stem rots (*Armillaria* spp., *Heterobasidion* spp.).

The forest-park territory of the Western Forest-Steppe zone of Ukraine has previously attracted the attention of Ukrainian mycologists (Heluta et al., 2014), but its mycobiota has not been investigated using molecular methods. Therefore, the goal of this study is the identification and collection of species inhabiting decaying wood.

The research material – specifically fallen dead wood – was collected in forest-park zones belonging to the Western Forest-Steppe region of Ukraine.

Samples were placed on Petri dishes with PDA medium for fungal isolation. The germinating mycelium was subsequently subcultured onto fresh dishes until pure cultures were obtained. DNA extraction was performed on mycelium harvested from agar plates. PCR was carried out using two primer pairs (ITS1 and ITS4, ef1 and ef2) targeting the ITS region and the *TEF1-α* gene. Strain identification to the species level was conducted by comparing sequences with those available in the BLASTn database. Phylogenetic analysis was performed in MEGA11.

A total of 115 strains of the genus *Trichoderma* were isolated from the wood. Molecular analysis identified 9 species: *T. atrobrunneum*, *T. atroviride*, *T. citrinoviride*, *T. hamatum*, *T. harzianum*, *T. longipile*, *T. pararogersonii*, *T. pleuroticola*, and *T. simmonsii*. Out of the 115 strains, only 58 were identified to the species level. Based on *TEF* sequences, a Neighbor-Joining tree was constructed, with the majority of strains belonging to the Harzianum clade/group.

It is known that *Trichoderma* species are difficult to identify via molecular analysis (Jaklitsch & Voglmayr, 2015). Analyzing strains at the species level usually requires the amplification of a larger number of genomic fragments. In our study, the ITS region demonstrated poor utility as a species-level marker for *Trichoderma* strains from the studied area, whereas *TEF* exhibited higher specificity in identification.

Within the Hamatum species complex, ITS is not a sufficiently informative marker, whereas phylogenetic analyses based on the *TEF* gene fragment allow assigning *T. atrobrunneum* to the *T. pleuroticola* group in a sister clade to the Hamatum species group. All strains identified to the species level according to Cai and Druzhinina (2021) are amenable to molecular identification, which adds significance to our results.

Overall, nine species were identified, with *T. atroviride* being dominant. All strains not assigned to a specific species belong to the polyphyletic *T. hamatum* species complex, the identification of which requires further molecular and ecological analysis. *T. pararogersonii* is reported for the first time in Ukraine. The strains obtained in this study will be used for subsequent ecological analysis.

References

1. Błaszczyk, L., Strakowska, J., Chełkowski, J., Gąbka-Buszek, A., & Kaczmarek, J. (2015). *Trichoderma* species occurring on wood with decay symptoms in mountain forests in Central Europe: Genetic and enzymatic characterization. Journal of Applied Genetics, 57(3), 397–407. https://doi.org/10.1007/s13353-015-0326-1
2. Cai, F., & Druzhinina, I. S. (2021). In honor of John Bissett: Authoritative guidelines on molecular identification of *Trichoderma*. Fungal Diversity, 107(1), 1–69. https://doi.org/10.1007/s13225-020-00464-4
3. Chan, M. E., Tan, J. Y., Lee, Y. Y., Lee, D., Fong, Y. K., Mutwil, M., Wong, J. Y., & Hong, Y. (2023). Locally isolated Trichoderma harzianum species have broad spectrum biocontrol activities against the wood rot fungal species through both volatile inhibition and mycoparasitism. Journal of Fungi, 9(6), Article 675. https://doi.org/10.3390/jof9060675
4. Druzhinina, I. S., Seidl-Seiboth, V., Herrera-Estrella, A., Horwitz, B. A., Kenerley, C. M., Mendoza-Mendoza, A., Rey, M., Scazzocchio, C., Atanasova, L., Yang, D. M., & Kubicek, C. P. (2011). Trichoderma: The genomics of opportunistic success. Nature Reviews Microbiology, 9(10), 749–759. https://doi.org/10.1038/nrmicro2637
5. Heluta V., Haiova V., & Tikhonenko, Y. (2014) Species diversity of fungi in the National Park "Podilski Tovtry". Regional Aspects of Floristic and Faunistic Researches. Materials of The First International Scientific and Practical Conference 10–12 April 2014, Khotyn. https://www.researchgate.net/publication/286640500_Species_diversity_of_fungi_in_the_National_Park_Podilski_Tovtry_In_Ukrainian_Vidova_riznomanitnist_gribiv_Nacionalnogo_prirodnogo_parku_Podilski_Tovtri
6. Jaklitsch, W. M., & Voglmayr, H. (2015). Biodiversity of *Trichoderma* (*Hypocreaceae*) in southern Europe and macaronesia. Studies in Mycology, 80, 1–87. https://doi.org/10.1016/j.simyco.2014.11.001

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COMPARATIVE CHARACTERISTICS OF SOME THERMAL WATER MICROBIOTA SAMPLES OF WESTERN UKRAINE

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Abstract. The physicochemical characteristics of thermal waters, particularly temperature, mineralization, and concentrations of major ions and dissolved gases, represent major environmental

determinants of microbial composition. The aim of this study was to compare the microbiota of three thermal water samples from western Ukraine. The results indicate that increasing mineralization and temperature are associated with a decrease of many heterotrophic and nutrient-transforming microorganisms.
Keywords: thermal water, total mineralization, temperature, microbial functional group

The physicochemical characteristics of thermal waters, such as temperature and mineral content, are key environmental factors that determine the composition and activity of microbial communities (Çelik et al., 2022). Temperature acts as a fundamental limiting factor: higher thermal stress usually reduces microbial diversity and favors thermophilic or thermotolerant microorganisms. Mineral content and ionic composition affect the microbiota through osmotic stress, specific ion effects, and changes in nutrient and electron acceptor availability, promoting the growth of microorganisms with corresponding adaptive mechanisms (Barbosa et al., 2023). So, the quality of thermal waters should be seen as a mix of physical, chemical and microbiological traits that significantly impact diversity, community structure and the ecological roles of microorganisms.

The composition of the microbiota of three thermal water wells in western Ukraine and its dependence on some physicochemical parameters, including temperature and mineralization, was determined.

The level of mineralization, temperature was determined according to DSTU 878-93 Packaged mineral waters. Technical specifications. With Changes and Amendments. Qualitative and quantitative indicators of the composition of the microbiota of the studied water samples were carried out according to Nikolenko S.I.

In the water from well I, characterized by the lowest mineralization (3–4 g/L) and moderate temperature (36–38 °C), the microbial community demonstrated comparatively high abundances of ammonifying aerobes and anaerobes (10^6–10^8 CFU/mL), oligocarbothrophs ($1.6 \cdot 10^3$ CFU/mL), microorganisms assimilating organic N-containing compounds ($2.1 \cdot 10^3$ CFU/mL), and lipid-degrading microorganisms ($4.2 \cdot 10^2$ CFU/mL), indicating the availability and active transformation of organic substrates. The relatively high abundance of heterotrophic and ammonifying microorganisms in this water type may reflect the more favorable combination of moderate salinity and temperature, which imposes less osmotic and thermal stress on metabolically versatile microorganisms (Nishiyama et al., 2018). In contrast, the water from well II, with a higher mineralization of 7–8 g/L and a broader temperature range of 30–45 °C, characterized by a marked reduction in several functional groups, including microorganisms assimilating organic N-containing compounds and oligocarbothrophs, while ammonifying anaerobes remained abundant (10^8 CFU/mL). This pattern suggests that increasing mineralization may act as an environmental filter, selectively suppressing less salt-tolerant microorganisms while preserving or favoring physiological groups capable of maintaining cellular homeostasis under increased osmotic pressure (Narsing Rao et al., 2021).

The most extreme physicochemical conditions were recorded for well III, where mineralization reached 18–22 g/L and temperature 56–60 °C; under these conditions, the total abundance and functional diversity of culturable microorganisms were substantially reduced, while oligocarbothrophs, amylolytic microorganisms, and methanogens remained detectable. The persistence of selected microbial groups under these conditions indicates that the combined effects of elevated salinity and temperature constitute a strong selective pressure, favoring microorganisms possessing specific osmotic and thermal adaptation mechanism (Barbosa et al., 2023).

A particularly important relationship was observed for sulfur-transforming microorganisms: sulfur-oxidizing *Thiobacillus thioparus* was detected at 10^3 CFU/mL in well I but

was virtually absent from the more mineralized waters, whereas sulfate-reducing *Desulfovibrio desulfuricans* was detected only at a low abundance (2 CFU/mL) in well I. The occurrence of *T. thioparus* is consistent with its capacity for chemo-lithotrophic sulfur oxidation, whereas sulfate-reducing *D. desulfuricans* participates in the reductive branch of the sulfur cycle; therefore, their distribution may reflect differences in the availability of reduced sulfur compounds, electron donors, oxygen conditions, and other geochemical parameters rather than mineralization alone (Çelik et al., 2022).

The relatively high abundance of methanogenic microorganisms (10^2–10^3 CFU/mL) in all three water types, despite pronounced differences in mineralization and temperature, suggests that methanogenesis may represent an important anaerobic terminal process within these microbial communities. The simultaneous reduction of several heterotrophic functional groups and persistence of selected anaerobic and oligotrophic microorganisms in the most mineralized water indicates a shift from metabolically diverse communities toward physiologically specialized assemblages adapted to increased environmental stress (Narsing Rao et al., 2021).

Overall, the results indicate that increasing mineralization and temperature are associated with a decrease in the abundance of many heterotrophic and nutrient-transforming microorganisms and with the relative persistence of oligotrophic, anaerobic, methanogenic, and other physiologically specialized groups, demonstrating the strong role of physicochemical conditions in structuring the microbial communities of the studied mineral waters. These findings are consistent with contemporary microbial ecology, which considers salinity, temperature, pH, redox conditions, nutrient availability, and geochemical composition as interacting environmental filters that determine microbial community composition and the functioning of carbon, nitrogen, and sulfur biogeochemical cycles (Barbosa et al., 2023).

References

1. Nikolenko, S. I., Glukhovska, S. M., Khmelevska, O. M., & Petrovska, V. B. (2011). Posibnyk z metodiv kontrolyu pryrodnykh mineralnykh vod, shtuchno-mineralizovanykh vod, napoyiv na yikh osnovi ta preformovanykh zasobiv. CH. 2. Mikrobiolohichni doslidzhennya (Manual on methods of control of natural mineral waters, artificially mineralized waters, beverages based on them and preformed products. Part 2. Microbiological research). KIM. (In Ukrainian)
2. Çelik, I., & Keskin, E. (2022). Revealing the Microbiome of Four Different Thermal Springs in Turkey with Environmental DNA Metabarcoding. Biology, 11(7), 998. https://doi.org/10.3390/biology11070998
3. Narsing Rao, M. P., Dong, Z. Y., Luo, Z. H., Li, M. M., Liu, B. B., Guo, S. X., Hozzein, W. N., Xiao, M., & Li, W. J. (2021). Physicochemical and Microbial Diversity Analyses of Indian Hot Springs. Frontiers in microbiology, 12, 627200. https://doi.org/10.3389/fmicb.2021.627200
4. Barbosa, C., Tamayo-Leiva, J., Alcorta, J., Salgado, O., Daniele, L., Morata, D., & Díez, B. (2023). Effects of hydrogeochemistry on the microbial ecology of terrestrial hot springs. Microbiology spectrum, 11(5), e0024923. https://doi.org/10.1128/spectrum.00249-23
5. Nishiyama, E., Higashi, K., Mori, H., Suda, K., Nakamura, H., Omori, S., Maruyama, S., Hongoh, Y., & Kurokawa, K. (2018). The Relationship Between Microbial Community Structures and Environmental Parameters Revealed by Metagenomic Analysis of Hot Spring Water in the Kirishima Area, Japan. Frontiers in bioengineering and biotechnology, 6, 202. https://doi.org/10.3389/fbioe.2018.00202

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MACHINE LEARNING-ASSISTED PREDICTION OF BACTERIAL INACTIVATION BY COBALT FERRITE-ACTIVATED HYDROGEN PEROXIDE

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Abstract. Hydrogen peroxide activated on cobalt ferrite is a promising non-chlorine option for continuous-flow water disinfection, but bacterial survival depends nonlinearly on oxidant dose, initial load, and contact time. This study compared Weibull and Gompertz equations with ten supervised machine-learning algorithms using 208 individual *Escherichia coli* observations collected at 3, 6, and 10 mM H_2O_2. Gradient boosting and random forest performed best; their averaged model reached a group-based R^2 of 0.963. Independent experiments at 4 mM H_2O_2 confirmed good interpolation, supporting dose-contact-time selection within the tested range.
Keywords: *Escherichia coli*; hydrogen peroxide; cobalt ferrite; continuous-flow disinfection; machine learning.

Reliable control of flow-through water disinfection requires a model that links the initial microbial load, oxidant dose, and residence time to the number of viable cells remaining after treatment. Cobalt ferrite is attractive as a heterogeneous Fenton-like catalyst because it activates H_2O_2 and can be used as a stable fixed bed in continuous flow (Danyliuk et al., 2025; Tatarchuk et al., 2024). Recent studies also demonstrate the broader potential of ferrite-assisted peroxide treatment and machine-learning approaches for predicting disinfection performance (Huang et al., 2025; Tran et al., 2024). The aim of this study was to determine whether conventional kinetic equations or data-driven models can more reliably predict *E. coli* inactivation across a variable operating window.

Granulated $CoFe_2O_4$ (0.8–1.0 mm) was placed in a 100 cm × 12 mm stainless-steel packed-bed reactor. *E. coli* ATCC 35218 suspensions were treated with 3, 6, or 10 mM H_2O_2 at initial bacterial loads of $1.0 \cdot 10^3$–$5.0 \cdot 10^4$ CFU/L and six nominal contact times from 0.46 to 27.64 min. Viable bacteria were enumerated on Endo agar after 24 h at 37 °C. Replicates were retained as individual observations, giving 208 data points. Weibull and Gompertz models and ten supervised machine-learning algorithms were evaluated. Random forest (RF) and gradient boosting (GB) were selected for detailed analysis, including group-based five-fold cross-validation in which each of 28 complete kinetic curves was kept within a single fold. An averaged RF + GB model was then tested against an independent 4 mM H_2O_2 dataset that had not been used for training.

The classical models captured only parts of the inactivation trajectory. The Weibull model described the initial decline most closely at 3 mM H_2O_2, but its error increased with peroxide concentration and contact time. The fitted Weibull exponent n = 0.42 indicated substantial heterogeneity in bacterial resistance and the presence of a more resistant

subpopulation. The Gompertz model also performed satisfactorily at 3 mM in the early stage but systematically overestimated survival at 6–10 mM, with mid-phase relative errors exceeding 400 % under the strongest oxidative conditions. Its accuracy improved at the longest exposure time (27.6 min).

The ensemble algorithms were substantially more robust. In group-based validation, R^2 values were 0.915 for RF, 0.961 for GB, and 0.963 for the averaged RF + GB model; the corresponding MAE values were 3214, 2229, and 2018 CFU/L, while RMSE decreased from 4635 for RF to 3055 CFU/L for the combined model. RF tended to overestimate and GB to underestimate residual bacterial numbers at longer contact times, so averaging their predictions reduced directional bias. At 3 and 6 mM H_2O_2, errors of the combined model were mostly within ±10% during the first 10 min. Even at 10 mM, late-stage errors remained within ±18 %, lower than those of the individual models. Independent validation at 4 mM confirmed good interpolation: at an initial load of $1.62 \cdot 10^4$ CFU/L, errors were ≤ +10 % at 0.46–1.7 min and reached about −15 % at longer contact times. Larger percentage errors at the lower starting load occurred mainly when residual bacterial counts were very low, so small absolute deviations produced large relative errors.

Thus, cobalt ferrite-activated H_2O_2 produces distinctly nonlinear bacterial inactivation kinetics that are not adequately represented by a single classical equation over the full operating range. GB showed stronger generalization, while the RF + GB average provided the most balanced prediction. Within the tested domain, the ensemble approach offers a practical basis for estimating the H_2O_2 dose and contact time required to reach a target residual bacterial count. Further validation is needed before extrapolating the model to other reactor geometries, water matrices, catalyst batches, or peroxide ranges.

References

1. Danyliuk, N., Husak, V., Boichuk, V., Ziółkowska, D., Danyliuk, I., & Shyichuk, A. (2025). Kinetics of H_2O_2 decomposition and bacteria inactivation in a continuous-flow reactor with a fixed bed of cobalt ferrite catalyst. *Applied Sciences, 15*(15), 8195. https://doi.org/10.3390/app15158195
2. Huang, R., Ma, C., Huangfu, X., & Ma, J. (2025). A framework predicting removal efficacy of antibiotic resistance genes during disinfection processes with machine learning. *Journal of Hazardous Materials, 492*, 138048. https://doi.org/10.1016/j.jhazmat.2025.138048
3. Tatarchuk, T., Shyichuk, A., Danyliuk, N., Lapchuk, I., Husak, V., & Macyk, W. (2024). Fenton-like water disinfection using fixed-bed reactor filled with a $CoFe_2O_4$ catalyst: Mechanisms, the impact of anions, electromagnetic heating, and toxicity evaluation. *Separation and Purification Technology, 348*, 127748. https://doi.org/10.1016/j.seppur.2024.127748
4. Tran, D. T., Nguyen, T. P., Khuat, T. T. T., Vu, T. P. T., & Pham, Q. T. (2024). Accelerating water disinfection by integrating $ZnFe_2O_4$/$BiVO_4$ heterojunction with H_2O_2/air-nanobubbles. *Journal of Water Process Engineering, 65*, 105870. https://doi.org/10.1016/j.jwpe.2024.105870

MEDICAL AND VETERINARY MICROBIOLOGY

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ELIMINATION OF CEFTRIAXONE-INDUCED HORMESIS IN *STAPHYLOCOCCUS AUREUS* BY METABOLITES OF PROBIOTIC BACTERIA OF THE GENUS *BACILLUS*

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Abstract. The study investigates the elimination of ceftriaxone-induced hormesis in *Staphylococcus aureus* 209 using probiotic metabolites. Subinhibitory concentrations of ceftriaxone (0.125–0.0075 μg/mL) were found to stimulate pathogen growth, significantly shortening the lag phase. To counteract this effect, the cell-free culture supernatant (CFCS) of the probiotic "Biosporin" (*Bacillus subtilis* and *B. licheniformis*) was applied. The combined use of ceftriaxone and bacillary CFCS completely neutralized the hormetic effect, ensuring 100 % inhibition of *S. aureus* viability across all tested subinhibitory points. Thus, *Bacillus* cell-free metabolites represent promising enhancers for overcoming antibiotic-induced bacterial stress.
Keywords: *Staphylococcus aureus*, *Bacillus* spp., ceftriaxone, subinhibitory concentrations, hormesis, elimination.

Subinhibitory antibiotic concentrations can paradoxically trigger hormesis and enhance virulence in *Staphylococcus aureus* (Müller, 2020; Prithviraj, 2021; Vargas-Cruz, 2022). Combining traditional antibiotics with cell-free metabolites from *Bacillus* probiotics offers a promising strategy to neutralize this stimulatory effect and combat bacterial resistance (Byun, 2023, Mazanko, 2026). This work aims to evaluate the efficacy of probiotic *Bacillus* metabolites in eliminating ceftriaxone-induced hormesis in *S. aureus*. You can review the complete research overview and proposal details.

Materials and Methods. The test strain *Staphylococcus aureus* 209 was used. Primary screening of the antagonistic potential of 7 *Bacillus* strains was performed via the deferred antagonism method on susceptibility agar (n = 3). Based on the results, the commercial probiotic "Biosporin" (LLC "PHC "Biopharma", Ukraine; *B. subtilis* UKM B-5007 and *B. licheniformis* B-5514) was selected. Its cell-free culture supernatant (CFCS) was obtained by cultivation in meat-peptone broth (MPB) for 48 h (37 °C), centrifugation, and filtration through 0.22 μm filters (Millipore).

The MIC of ceftriaxone and *S. aureus* growth kinetics were determined by serial microdilution in 96-well plates. The antibiotic range was 1.0–0.0075 μg/mL, the initial inoculum was $5 \cdot 10^5$ CFU/mL, and the final volume was 200 μL. Kinetics were monitored automatically using a microplate reader (OD_{560}, 48 h, 1-h interval, 37 °C). To evaluate the intrinsic effect of CFCS (dilutions 1:1–1:256) and the "ceftriaxone + CFCS" combination (final working dilution 1:1), double-strength MPB (2 x MPB) was used to maintain a standard

nutrient concentration. The control row contained equivalent volumes of 0.5 % NaCl. Viable pathogen cells after 24 h of co-cultivation were quantified by the Gould method, with results expressed as lg CFU/mL).

Results. The MIC of ceftriaxone against the *S. aureus* 209 test strain was determined to be 0.25 μg/mL. Under the influence of subinhibitory concentrations of the antibiotic (0.125–0.0075 μg/mL), a pronounced hormetic effect was observed. This phenomenon was manifested by a significant reduction in the lag phase: intensive exponential growth of the staphylococcal culture in the experimental wells initiated as early as 4–6 h of cultivation, compared to 24 h in the growth control. By 48 h, the optical density of the antibiotic-stressed cells (OD_{560} = 0.48–0.54) significantly exceeded that of the control (OD_{560} = 0.41), demonstrating profound metabolic stimulation of the bacterial population by low cephalosporin doses

In the first stage, a comparative assessment of the antagonistic activity of *Bacillus* strains was conducted. Among the commercial probiotic preparations, "Biosporin" (*B. subtilis* UKM B-5007 + *B. licheniformis* B-5514) demonstrated the highest efficacy against the *S. aureus* 209 test strain, with a growth inhibition zone of 19 ± 2.6 mm, whereas for "Subalin" and "Enterogermina" this parameter was only 14 ± 0.7 mm and 6 ± 3.2 mm, respectively. Given its high anti-staphylococcal potential, the cell-free culture supernatant (CFCS) of "Biosporin" was selected for further modeling of the antibiotic-induced hormesis elimination system.

When evaluating the intrinsic antimicrobial activity of various CFCS dilutions of "Biosporin", complete inhibition of *S. aureus* 209 growth (growth control – 8 lg CFU/mL was observed within the dilution range of 1:1 to 1:4. Starting from a 1:8 dilution, cell viability recovered 3 lg CFU/mL, gradually approaching the initial control values. The study of the combined effect confirmed the presence of antibiotic-induced hormesis: under subinhibitory concentrations of ceftriaxone (0.125–0.0075 μg/mL), the number of viable pathogen cells increased to 8.7–10 lg CFU/mL compared to the control. The maximum stimulatory effect was recorded at an antibiotic concentration of 0.015 μg/mL. The introduction of native "Biosporin" CFCS into the test system (final working dilution 1:1) resulted in the complete eradication of *S. aureus* 209 viability across the entire range of ceftriaxone concentrations. Thus, the complex of extracellular bacillary metabolites completely eliminated the hormetic growth surge of the staphylococcal culture induced by subinhibitory cephalosporin doses.

Conclusions. Subinhibitory concentrations of ceftriaxone induce a pronounced hormetic effect in *Staphylococcus aureus*, significantly accelerating its growth and increasing the final population titer compared to the control. The cell-free culture supernatant (CFCS) of the probiotic "Biosporin" exhibits high intrinsic antimicrobial activity against the staphylococcal culture, which is maintained even under significant dilution of the metabolites. The combined application of ceftriaxone and bacillary cell-free metabolites results in the complete elimination of antibiotic-induced hormesis and 100 % inhibition of pathogen viability, opening new avenues for optimizing antimicrobial therapy.

References

1. Byun H., Brockett M.R., Pu Q., Hrycko A.J., Beld J., Zhu J. (2023). An intestinal *Bacillus velezensis* isolate displays broad-spectrum antibacterial activity and prevents infection of both Gram-Positive and Gram-Negative pathogens *in vivo*. J Bacteriol, 205:e00133-23. https://doi.org/10.1128/jb.00133-23
2. Mazanko, M. S., Mazanko, E. V., Emelyantsev, S. A., Kozmenko, S. V., & Rudoy, D. V. (2026). Evaluation of the probiotic potential of *Bacillus* spp. strains isolated from river bottom sediments. Environments, 13(1), 13. https://doi.org/10.3390/environments13010013
3. Müller, A., Klöckner, A., & Schneider, T. (2020). Targeting the cell wall to win the battle against *Staphylococcus aureus*. International Journal of Medical Microbiology, 310(4), 151421. DOI: https://doi.org/10.1103/PhysRevE.102.032408

4. Prithviraj, B., Pillai, S. K., & Suresh, A. (2021). Hormetic dose responses induced by antibiotics in bacteria: A phantom menace in clinical settings. Microbial Pathogenesis, 158, 105021. https://doi.org/10.1016/j.scitotenv.2021.149255
5. Vargas-Cruz, N., Romero-Gómez, S., & Castro-Albarrán, J. (2022). Subinhibitory concentrations of antibiotics exacerbate staphylococcal infection by inducing bacterial virulence and modifying growth kinetics. Doi: 10.1128/spectrum.00640-22.

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ACTIVITY OF CURCUMIN METAL COMPLEXES AGAINST MATURE *PSEUDOMONAS AERUGINOSA* BIOFILMS

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Abstract. The aim of this study was to evaluate the antibiofilm activity of curcumin complexes with zinc, neodymium, ytterbium, and dysprosium against mature biofilms formed by *Pseudomonas aeruginosa* strains. The results demonstrated that the curcumin metal complexes had antibiofilm activity against mature biofilms formed by some *Pseudomonas aeruginosa* strains.
Keywords: *Pseudomonas aeruginosa,* curcumin, biofilm, antibiofilm activity, metal–curcumin complexes

Biofilm formation is an important stage in the development of many chronic infections, with the formation of the extracellular matrix being a key factor in this process. Together with the metabolic reprogramming of cells, the extracellular matrix provides protection to microorganisms (Osondu-Chuka et al., 2026). During biofilm maturation, cells form microcolonies and secrete an extracellular matrix, which may account for more than 90 % of the biofilm mass. Matrix components can bind antimicrobial compounds or promote their enzymatic degradation, thereby reducing the concentration of antimicrobial agents reaching bacterial cells. Thus, the matrix acts as a functionally active environment that maintains biofilm integrity and protects bacteria from environmental factors, particularly antimicrobial agents (Flemming et al., 2025). Given the high tolerance of mature biofilms to antimicrobial agents, the search for compounds capable of affecting their functioning is of particular relevance. Curcumin is one promising natural compound, and its metal-modified derivatives have been reported to exhibit antibacterial and antibiofilm activity against *P. aeruginosa* (Shariati et al., 2024; Mittal et al., 2024).

The antibiofilm activity of curcumin complexes with zinc, ytterbium, neodymium, and dysprosium was evaluated against strains of *P. aeruginosa* ATCC 15692, ATCC 10145, and ATCC 9027. Mature biofilms were formed for 48 h in polystyrene microplates, after which the studied compounds were added to the culture medium. Following an additional 24 h of incubation, biofilm biomass was determined spectrophotometrically using crystal violet staining (Haney et al., 2022).

None of the studied compounds exhibited antibiofilm activity against *P. aeruginosa* ATCC 15692 or ATCC 10145. In contrast, a reduction in biofilm biomass was observed for *P. aeruginosa* ATCC 9027. The most pronounced inhibitory effect was observed for the dysprosium complex, which reduced biofilm biomass by 32 % at 25 μM and 37 % at 50 μM

compared with the control. Zinc- and neodymium-containing complexes also exhibited activity at 5 μM, showing a higher inhibitory effect, whereas at 50 μM their activity was approximately twofold lower.

The increased sensitivity of the *P. aeruginosa* ATCC 9027 biofilm to the studied compounds may be primarily associated with strain-specific features of quorum-sensing regulation. A functional defect in the transcriptional regulator LasR, which plays a central role in the quorum-sensing regulatory hierarchy and the regulation of virulence, has been described in this strain (García-Reyes et al., 2020). Reduced LasR functionality may affect the expression of QS-dependent genes and the interplay between the Las, Rhl, and Pqs regulatory systems. Since quorum sensing contributes to the regulation of the biofilm phenotype, maturation, and spatial organization, atypical QS regulation in *P. aeruginosa* ATCC 9027 may contribute to differences in the physiological state of cells and the architecture of the resulting biofilm, which, in turn, may affect its susceptibility to the studied compounds (Chadha et al., 2022).

Another factor potentially contributing to the differential efficacy of the studied compounds against the biofilm of *P. aeruginosa* ATCC 9027 may be the specific composition of its extracellular matrix. The matrix of *P. aeruginosa* biofilms contains the polysaccharides Psl, Pel, and alginate, whose relative contributions and interactions may vary considerably among strains (Böhning et al., 2024). For *P. aeruginosa* ATCC 9027, the absence of the *mucA* gene, which encodes a negative regulator of alginate biosynthesis, has been described, providing a genetic basis for alterations in the polysaccharide profile of the matrix (Neubauer et al., 2021). Such differences may affect the spatial organization of the biofilm, the physicochemical properties of the matrix, and the accessibility of bacterial cells to external antimicrobial agents.

Therefore, the results obtained indicate the potential of curcumin metal complexes as promising antibiofilm agents capable of affecting mature *P. aeruginosa* biofilms. Further investigation of the matrix composition, spatial architecture, and physicochemical properties, combined with studies of the interactions between its components and metal complexes, may elucidate the basis of the identified differences, determine the factors underlying the varying susceptibility of individual strains, and provide a framework for developing more effective antibiofilm compounds.

References

1. Böhning, J., Tarafder, A. K., & Bharat, T. A. M. (2024). The role of filamentous matrix molecules in shaping the architecture and emergent properties of bacterial biofilms. *Biochemical Journal*, *481*(4), 245–263. https://doi.org/10.1042/BCJ20210301
2. Chadha, J., Harjai, K., & Chhibber, S. (2022). Revisiting the virulence hallmarks of *Pseudomonas aeruginosa*: A chronicle through the perspective of quorum sensing. *Environmental Microbiology*, *24*(6), 2630–2656. https://doi.org/10.1111/1462-2920.15784
3. Flemming, H.-C., van Hullebusch, E. D., Little, B. J., Neu, T. R., Nielsen, P. H., Seviour, T., Stoodley, P., Wingender, J., & Wuertz, S. (2025). Microbial extracellular polymeric substances in the environment, technology and medicine. *Nature Reviews Microbiology*, *23*(2), 87–105. https://doi.org/10.1038/s41579-024-01098-y
4. García-Reyes, S., Soto-Aceves, M. P., Cocotl-Yañez, M., González-Valdez, A., Servín-González, L., & Chávez, G. S. (2020). The outlier *Pseudomonas aeruginosa* strain ATCC 9027 harbors a defective LasR quorum-sensing transcriptional regulator. *FEMS Microbiology Letters*, *367*(16), fnaa122. https://doi.org/10.1093/femsle/fnaa122
5. Haney, E. F., TrIMVle, M. J., & Hancock, R. E. W. (2021). Microtiter plate assays to assess antibiofilm activity against bacteria. *Nature Protocols*, *16*(5), 2615–2632. https://doi.org/10.1038/s41596-021-00515-3
6. Mittal, A., Nagpal, M., Vashistha, V. K., Arora, R., & Issar, U. (2024). Recent advances in the antioxidant activity of metal-curcumin complexes: A combined computational and experimental review. *Free Radical Research*, *58*(1), 11–26. https://doi.org/10.1080/10715762.2023.2298857

7. Neubauer, D., Jaśkiewicz, M., Bauer, M., Olejniczak-Kęder, A., Sikorska, E., Sikora, K., & Kamysz, W. (2021). Biological and Physico-Chemical Characteristics of Arginine-Rich Peptide Gemini Surfactants with Lysine and Cystine Spacers. *International Journal of Molecular Sciences*, *22*(7), 3299. https://doi.org/10.3390/ijms22073299
8. Osondu-Chuka, G. O., Schandl, S., Scheberl, A., Guillaume, O., Ovsianikov, A., & Reimhult, E. (2026). Beyond the matrix: Rethinking antibiotic tolerance in CF biofilms using 3D models. *Npj Biofilms and Microbiomes*, *12*(1), 3. https://doi.org/10.1038/s41522-025-00869-6
9. Shariati, A., Noei, M., Askarinia, M., Khoshbayan, A., Farahani, A., & Chegini, Z. (2024). Inhibitory effect of natural compounds on quorum sensing system in *Pseudomonas aeruginosa*: A helpful promise for managing biofilm community. *Frontiers in Pharmacology*, *15*, 1350391. https://doi.org/10.3389/fphar.2024.1350391

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POTENTIAL PROBIOTIC PROPERTIES AND IN VIVO EFFECTS OF *SACCHAROMYCES CEREVISIAE* CL4

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Abstract. *Saccharomyces cerevisiae* CL4 was evaluated as a potential probiotic strain based on stress tolerance, safety-related properties, microbial interactions, and effects in rats. The strain grew at 37 °C and showed 71.0 % survival at pH 2.5 and 83.2 % survival under simulated gastric conditions. No hemolytic activity was detected. CL4 did not inhibit *Candida albicans*, but altered the phenotype of *Pseudomonas aeruginosa*, an effect also reproduced by cell-free culture fluid. In rats with metabolic syndrome, CL4 administration increased superoxide dismutase activity and affected other antioxidant defense parameters.
Keywords: *Saccharomyces cerevisiae*, probiotic yeast, antioxidant defense, tolerance assay, microbial interactions.

Probiotic yeasts are gaining increasing attention because of their ability to survive gastrointestinal conditions and potentially modulate host physiology. However, these properties are strongly strain-dependent, emphasizing the need for individual characterization of candidate strains (Ruchała, et al., 2026; Nenciarini, et al., 2024). Some studies using animal models also indicate the ability of probiotic yeasts to influence metabolic processes in the host organism, including carbohydrate and lipid metabolism and antioxidant defense (Egea, et al, 2023). The present study evaluated selected probiotic-associated properties of *Saccharomyces cerevisiae* CL4 and its effects on metabolic and antioxidant parameters in laboratory rats.

The obtained results indicate the tolerance of the studied strain to physiological temperatures of 28 and 37 °C, but not that of 42 °C, and to modeled conditions of the internal environment of the organism, the viability of cells being 71.0 % and 83.2 % after exposure to low pH and modeled gastric conditions respectively, compared with 45.7 % and 71.9 % for the reference strain *S. cerevisiae* BY4742.

The spot-on-lawn assay of the strain microbial interactions, no inhibition of *C. albicans* growth was observed under the tested conditions. In contrast, interaction with *P. aeruginosa* resulted in a distinct greenish-blue zone of altered bacterial phenotype. A similar effect was produced by cell-free culture fluid, suggesting the involvement of extracellular diffusible factor(s), although classical antibacterial activity was not demonstrated.

In rats with experimentally induced metabolic syndrome, administration of CL4 was associated with an 82.4 % increase in superoxide dismutase activity compared with the untreated metabolic-syndrome group, accompanied by changes in other components of the antioxidant defense system, including glutathione peroxidase activity and reduced glutathione levels.

Thus, *S. cerevisiae* CL4 combines several properties relevant to its probiotic potential, including tolerance to acidic and simulated gastric conditions and absence of detectable hemolytic activity. The observed modulation of the *P. aeruginosa* phenotype and changes in antioxidant defense parameters warrant further investigation of the mechanisms, safety, and probiotic efficacy of this strain.

References

1. Egea, M. B., Oliveira Filho, J. G., & Lemes, A. C. (2023). Investigating the Efficacy of *Saccharomyces boulardii* in Metabolic Syndrome Treatment: A Narrative Review of What Is Known So Far. *International journal of molecular sciences*, *24*(15), 12015. https://doi.org/10.3390/ijms241512015
2. Nenciarini, S., Amoriello, R., Bacci, G., Cerasuolo, B., Di Paola, M., Nardini, P., Papini, A., Ballerini, C., & Cavalieri, D. (2024). Yeast strains isolated from fermented beverage produce extracellular vesicles with anti-inflammatory effects. *Scientific reports*, *14*(1), 730. https://doi.org/10.1038/s41598-024-51370-7
3. Żywczak, G., Songdech, P., Vasylyshyn, R., & Ruchała, J. (2026). From Yeast Probiotics to Postbiotics: A Continuum Model and Yeast-Specific Functional Perspective. *Cells*, *15*(15), 1320. https://doi.org/10.3390/cells15151320

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MULTIDRUG RESISTANCE AND PANDRUG RESISTANCE GENES IN CLINICALLY IMPORTANT BACTERIAL VARIANTS

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Abstract. Mine-blast injuries pose a high risk of developing purulent complications caused by ESKAPE pathogens, which are characterized by multidrug resistance and pandrug resistance to antibiotics. Resistance genes are located on plasmids or within chromosomes and are capable of horizontal transfer within biofilm resistomes. Bacterial antibiotic resistance is combined with virulence factors. For the diagnosis of the wound healing process, it is advisable to employ rapid methods for detecting bacteria and resistance genes.
Keywords: antibiotic resistance genes, ESCAPE bacteria, purulent wounds.

Relevance of the topic: The rapid spread of multidrug-resistant (MDR) and pandrug-resistant (PDR) pathogens causing purulent-inflammatory infections in mine-blast injuries and burns necessitates an analysis of information regarding the genetics, mechanisms, and research methods of microorganisms as a foundation for diagnostic, therapeutic, and prophylactic procedures (Hrytsai, 2022).

Objective of the study: To analyze information on the genetic mechanisms of MDR and PDR, as well as modern methods of microbiological diagnosis, genodiagnostics, and antibiotic susceptibility testing.

Materials and methods: Information search within the PubMed, Google Scholar, Scopus, Web of Science, CARD, and NDARO search systems; selection and analysis of information; and formulating conclusions regarding the novelty, significance, and reliability of the publications.

Research results. These are predominantly bacteria of the ESKAPE group, which includes: *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp., including *Escherichia coli* (Singh, 2026). The clinical and microbiological features of these bacteria include widespread antibiotic resistance (AR), including MDR and PDR. The genetic basis of bacterial AR consists of chromosomal genes as well as plasmid genes that facilitate the horizontal gene transfer of antibiotic resistance genes (ARGs) (Nadeem, 2026). The ARGs of ESKAPE pathogens include those encoding: the synthesis of enzymes that degrade beta-lactams and carbapenems (genes *blaTEM*, *blaCTX-M*, *blaKPC*, *blaNDM-1*); the modification of antibiotic targets, such as genes modifying penicillin-binding proteins (PBP2a), which are targets for beta-lactams and carbapenems, for instance in *S. aureus*; while the *vanA* / *vanB* genes determine resistance to vancomycin. Antibiotic resistance to macrolides occurs due to the modification of the 23S rRNA fragment of the 50S ribosomal subunit (genes *erm(A)* and *erm(C)*). The *erm(B)* gene in *E. faecium* can be transferred by the *Tn1545* transposon, driving the horizontal transfer of AR. The target for tetracyclines is the 30S ribosomal subunit, which participates in elongation – the continuation of protein synthesis involving transfer RNAs (tRNAs). Resistance to tetracyclines is mediated by the *tet(M)* and *tet(O)* genes, which are localized on conjugative plasmids and can be transferred to *E. faecium*, *S. aureus*, and Gram-negative bacteria. The *tet(W)*, *tet(Q)*, and *tet(S)* genes mediate resistance to tetracyclines in ESKAPE pathogens causing wound infections. Furthermore, AR has also been reported against tigecycline, a latest-generation tetracycline belonging to last-resort antibiotics, which develops due to mutations in the chromosomal gene encoding 16S rRNA. The polymyxin antibiotic colistin is another vital last-resort antibiotic; however, MDR bacteria resistant to colistin have been increasingly identified. Under the influence of plasmid-borne and chromosomal genes, such as *pmrAB*, *phoPQ*, *parRS*, and *cprRS*, the LPS surface is modified with a positive charge, causing colistin molecules to be electrostatically repelled and fail to penetrate the cell. These mechanisms combine with other ARGs, leading to the development of MDR and PDR phenotypes, driven by the *phoP*/*phoQ* genes in *K. pneumoniae*, *mcr* and *lpxA*/*C*/*D* genes in *A. baumannii*, and *pmrAB*, *phoPQ*, *parRS*, and *cprRS* genes in *P. aeruginosa*.

A clinically crucial feature of ARGs is their capacity for horizontal transfer mediated by mobile genetic elements (MGEs), which drive the emergence of MDR and PDR phenotypes. These elements include R-plasmids, which can transfer a “resistance package” targeting multiple classes of antibiotics during conjugation; transposons – DNA fragments capable of migrating from chromosomes to plasmids and vice versa (for instance, *Tn4401* carries the *blaKPC* gene encoding carbapenem resistance); integrons, which are capable of capturing and transferring ARGs from the environment into the cell to form functional MDR complexes; and insertion sequences, which are genetic structures that activate ARGs within

the chromosome. Chromosomal AR develops as a result of point mutations and can combine with other AR mechanisms. The transmission of ARGs is highly effective within the resistomes of the intestinal tract or biofilms formed on wound surfaces and mucous membranes, as well as within plumbing fixtures, particularly hospital ancillary equipment throughout the evacuation stages.

The development of the infectious process involving ARGs is driven by the presence of a complex of virulence and toxigenicity factors in bacteria, the quorum sensing phenomenon, and the immunosuppressive effects of lipopolysaccharide endotoxin. Among the most dangerous pathogens of wound infections are *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and other bacteria of the ESKAPE group (Hrytsai, 2022). Crucial factors in the diagnosis and treatment of purulent complications in combat injuries include effective microbiological diagnosis utilizing automated microbiological systems, particularly matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), methods for determining the minimum inhibitory concentration of bacterial growth, and genodiagnostics via real-time PCR.

Conclusion. The rapid dissemination of MDR and PDR ESKAPE strains in combat injuries and burns is driven by the synergistic action of chromosomal mutations and horizontal transfer of resistance genes (specifically to colistin and tigecycline) via plasmids and transposons within biofilm resistomes. Effective treatment and prevention of purulent complications necessitate the integration of methods real-time PCR and MALDI-TOF MS as a foundation for personalized last-resort antibiotic therapy.

References

1. Hrytsai, M. P., Polyachenko, Yu. V., Tsokalo, V. M., Kolov, H. B., & Yevlantyeva, T. A. (2022). Features of the occurrence and development of infectious complications in patients with combat injuries of the musculoskeletal system (based on own experience). Visnyk Ortopedii, Travmatolohii ta Protezuvannia, (1), 53–68. (In Ukrainian).
2. Nadeem, M. S., Rasool, R., Afzal, M., Ullah, I., Murtaza, B. N., Daoub, R. M. A., Chaieb, K., Alzarea, S. I., Kazmi, I., & Ashraf, G. M. (2026). Genes and physiological strategies in bacterial antibiotic resistance. Frontiers in Microbiology, 17, Article 1811959. https://doi.org/10.3389/fmicb.2026.1811959
3. Singh, K. N., Agarwal, K., Singh, P., Rukadikar, A. R., Hada, V., Mohanty, A., Rana, S., Rahul, A., & Mishra, S. (2026). Prevalence and antibiotic susceptibility of ESKAPE pathogens in skin and soft tissue infections. Indian Journal of Dermatology, Venereology and Leprology, 92, 77–81. https://doi.org/10.25259/IJDVL_417_2025

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CURRENT SPECTRUM OF WOUND INFECTION PATHOGENS AND THEIR ANTIMICROBIAL RESISTANCE IN MILITARY PERSONNEL

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Abstract. The etiological structure of wound infections in military personnel was investigated, and the antimicrobial susceptibility profiles of the isolated microorganisms were determined. A broad spectrum of Gram-positive and Gram-negative pathogens was identified, with particular emphasis on

Pseudomonas aeruginosa, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and staphylococci. A high prevalence of multidrug-resistant and pandrug-resistant strains was observed, particularly among Gram-negative bacteria. Microorganisms isolated as components of microbial associations demonstrated less favorable antimicrobial susceptibility profiles than those recovered as monocultures. These findings highlight the challenges associated with selecting appropriate empirical antibacterial therapy for combat-related wounds. The results underscore the need for systematic local microbiological surveillance and continuous monitoring of antimicrobial resistance among wound infection pathogens.
Keywords: wound infections; combat wound microbiota; microbial associations; opportunistic microorganisms; antimicrobial resistance.

Introduction. In Ukraine, wound infections in military personnel represent a significant medical and social challenge. The development of infectious complications is associated with prolonged treatment, an increased risk of sepsis, disability, and mortality.

The situation is further aggravated by the rapid emergence and spread of antimicrobial resistance among potential wound infection pathogens. The use of antimicrobial agents without consideration of local resistance patterns, particularly inappropriate or purely empirical prescribing, exerts selective pressure and promotes the emergence and dissemination of multidrug-resistant strains. The accumulation of multiple resistance mechanisms may ultimately result in pandrug-resistant phenotypes, substantially limiting the therapeutic options available for effective antibacterial treatment.

The microbial spectrum of combat wounds is not static and may vary depending on the stage of medical evacuation, the conditions under which medical care is provided, and the characteristics of the healthcare environment. Therefore, characterization of the etiological spectrum of wound infections in military personnel and assessment of the antimicrobial susceptibility of isolated microorganisms are essential for developing evidence-based empirical treatment strategies, optimizing local antimicrobial stewardship policies, and preventing the further spread of resistant strains.

Thus, systematic investigation of the etiological structure of wound infections, combined with continuous surveillance of antimicrobial resistance among their causative agents, is an important prerequisite for improving the management of combat-related trauma and reducing infection-associated morbidity and mortality among military personnel.

Materials and Methods. Clinical specimens were collected from wound surfaces using sterile transport systems manufactured by FLmedical (Italy). Primary bacteriological examination was performed by inoculating the specimens onto appropriate culture media using the Gold sector-streaking method.

Selective and differential diagnostic media were used for the isolation and cultivation of specific groups of microorganisms. Sabouraud agar was used for the isolation of microscopic fungi. Blood agar, consisting of meat-peptone agar supplemented with 5 % blood, was used for the detection of representatives of the genera *Streptococcus* and *Neisseria*. Endo and Levine media (Farmaktiv, Ukraine) were used for the isolation of enterobacteria, while mannitol salt egg-yolk agar (Biolif-Italia) was used for the cultivation of *Staphylococcus* spp.

Species identification of the recovered isolates was based on a comprehensive assessment of their morphological, tinctorial, and biochemical characteristics using standardized test systems for microbiological identification.

Antimicrobial susceptibility testing of the isolated bacteria and microscopic fungi was performed using the disk diffusion method in accordance with the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Standardized antimicrobial susceptibility testing disks were used.

For inoculum preparation, 24-h microbial cultures were used. Colonies were suspended in sterile physiological saline to a density equivalent to a 0.5 McFarland standard, corresponding to

approximately $1.5 \cdot 10^8$ CFU/mL. The optical density of the microbial suspension was controlled using a densitometer. A 100-µL aliquot of the prepared suspension was evenly inoculated onto Mueller–Hinton agar for bacterial cultures or Sabouraud agar for microscopic fungi.

Following placement of the antimicrobial disks, bacterial cultures were incubated at 37 ± 2 °C for 24 h, whereas cultures of microscopic fungi were incubated at 35 ± 2 °C for up to 48 hours. Antimicrobial activity was assessed by measuring the diameters of growth inhibition zones around the disks in millimeters. The results were subsequently interpreted according to the applicable EUCAST criteria.

The susceptibility of the isolates to representatives of the cephalosporin, macrolide, fluoroquinolone, carbapenem, sulfonamide, and lincosamide classes was assessed. For microscopic fungi, susceptibility to antifungal agents was additionally determined.

Results. The study demonstrated substantial variability in antimicrobial susceptibility profiles depending on the taxonomic identity of the microorganisms and whether they were isolated as monocultures or as components of microbial associations. Overall, microorganisms recovered from microbial associations tended to exhibit higher levels of resistance than corresponding isolates recovered as monocultures.

Among Gram-positive cocci, *Staphylococcus aureus* isolates generally retained susceptibility to fluoroquinolones and reserve agents, including glycopeptides and oxazolidinones. At the same time, reduced susceptibility was observed to certain cephalosporins. *Staphylococcus epidermidis* exhibited a considerably less favorable susceptibility profile: most isolates demonstrated multidrug resistance, while strains recovered as components of microbial associations showed characteristics consistent with pandrug resistance.

Enterococcus faecalis isolates recovered as monocultures generally retained susceptibility to penicillins and reserve agents, although resistance to several other antibacterial classes was observed. When isolated as components of microbial associations, susceptibility to the principal β-lactam agents remained predominantly high, whereas carbapenems demonstrated limited activity.

A substantial proportion of pandrug-resistant strains was identified among *Streptococcus pyogenes* isolates. This finding indicates the emergence of unfavorable resistance patterns even among microorganisms traditionally regarded as highly susceptible to antibacterial therapy.

The most pronounced multidrug resistance was observed among Gram-negative bacteria. *Pseudomonas aeruginosa* and *Acinetobacter baumannii* isolates demonstrated high levels of resistance to the majority of the antibacterial agents tested, with susceptibility retained only to selected agents. Similar patterns were observed among members of the family Enterobacterales, particularly *Klebsiella pneumoniae* and *Enterobacter xiangfangensis*. The reduction in antimicrobial susceptibility was particularly pronounced when these microorganisms were isolated as components of microbial associations.

In contrast, *Escherichia coli* isolates demonstrated a relatively more favorable susceptibility profile toward selected antibacterial agents. Carbapenems and aminoglycosides retained comparatively higher activity. Nevertheless, *E. coli* isolates demonstrated resistance to cephalosporins and some fluoroquinolones.

Overall, the findings demonstrate a substantial prevalence of multidrug-resistant and pandrug-resistant microorganisms among wound infection pathogens in military personnel. The least favorable antimicrobial susceptibility profiles were observed among microorganisms isolated as components of microbial associations. This finding complicates the selection of appropriate empirical antibacterial therapy and emphasizes the importance of regular local microbiological surveillance.

Conclusions. The findings confirm the presence of a broad spectrum of microorganisms associated with wound infections in military personnel, with a substantial proportion of

multidrug-resistant and pandrug-resistant strains. The high prevalence of antimicrobial resistance among Gram-negative bacteria and microorganisms recovered from polymicrobial associations is of particular concern. The observed variability in antimicrobial susceptibility profiles highlights the limitations of an exclusively empirical approach to antibacterial therapy in the absence of local microbiological data.

Regular and systematic surveillance of the etiological structure of wound infections and the antimicrobial susceptibility of their causative agents is therefore essential. Further research should focus on monitoring temporal changes in resistance patterns and developing optimized approaches to antimicrobial treatment. Expansion of such investigations is an important prerequisite for establishing an effective antimicrobial stewardship strategy, improving the management of combat-related wounds, and limiting the further spread of antimicrobial resistance in military healthcare.

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ORAL MICROBIOME IN INFLAMMATORY PERIODONTAL DISEASES AND DEVELOPMENT OF PLANT-BASED CORRECTIVE ORAL CARE PRODUCTS

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Abstract. The study investigated the characteristics of the oral microbiota under various pathological conditions and evaluated the antimicrobial and antibiofilm activity of plant-derived essential oils. Complex aerobic–anaerobic microbial associations dominated by periodontal pathogens and opportunistic microorganisms were identified. Species-specific differences in the activity of essential oils against the studied microorganisms were established. The highest antimicrobial and antibiofilm activity was demonstrated by essential oils of *Thymus vulgaris* and *Origanum vulgare*. *Achillea millefolium* essential oil exhibited broad-spectrum antimicrobial activity against Gram-positive and Gram-negative microorganisms, as well as fungi of the genus *Candida*. The highest activity against anaerobic periodontal pathogens was observed for essential oils of *Pinus sylvestris*, *Eucalyptus globulus*, and *Anethum graveolens*. The findings support the potential use of essential oils as natural components of oral care products aimed at modulating the oral microbiota.
Keywords: oral microbiome, microbiome-associated markers, antimicrobial activity, essential oils.

The study of the oral microbiome is one of the promising areas of modern microbiology and dentistry, as it provides an opportunity not only to deepen our understanding of the mechanisms underlying various diseases of infectious and non-infectious origin but also to establish a scientific basis for the development of effective approaches to the correction of inflammatory diseases of the oral mucosa and periodontal tissues.

During 2016–2026, we investigated the composition and structure of the oral microbiota under various pathological conditions at the clinical departments of the Educational and Scientific Institute of Dentistry and Laboratory Medicine of Uzhhorod National University and at the University Dental Clinic. Microbiome-associated markers of the course of inflammatory periodontal diseases were identified using both conventional culture-based methods, including isolation and identification of anaerobic periodontal pathogens, and metagenomic analysis.

To identify promising natural antimicrobial agents, essential oils obtained from wild and cultivated plants were investigated, including *Eucalyptus globulus* Labill., *Lavandula angustifolia* Mill., *Origanum vulgare* L., *Mentha piperita* L., *Matricaria recutita* L., *Salvia officinalis* L., *Anethum graveolens* L., *Thymus serpyllum* L., *Pimpinella anisum* L., *Carum carvi* L., *Achillea millefolium* L., *Coriandrum sativum* L., and *Pinus sylvestris* L. The essential oils were produced by Calendula (Slovakia). Microbial susceptibility to the essential oils was determined using the agar well diffusion method, as well as by establishing the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).

It was established that inflammatory processes of various etiologies in the oral cavity are associated with the formation of complex aerobic–anaerobic microbial communities, with individual periodontal pathogens and opportunistic microorganisms predominating. These findings indicate a significant role of microbial imbalance in the maintenance and progression of inflammatory processes.

A comprehensive screening of isolated cultures for susceptibility to antimicrobial agents was performed, revealing species-specific antimicrobial effects depending on both the microorganism and the nature of the tested substance. Particular attention was paid to essential oils as natural multicomponent complexes exhibiting a broad spectrum of biological activities, including antimicrobial, antibiofilm, antioxidant, and deodorizing effects. These properties make essential oils promising active components of oral care products.

The assessment of antimicrobial and antibiofilm activity demonstrated that *Thymus vulgaris* L. and *Origanum vulgare* L. essential oils exhibited the highest activity among the tested oils. *Achillea millefolium* L. essential oil also demonstrated pronounced antimicrobial activity against a broad range of test cultures, including Gram-positive and Gram-negative microorganisms and microscopic fungi of the genus *Candida*.

The highest activity against anaerobic periodontal pathogens was observed for essential oils of *Pinus sylvestris* L., *Eucalyptus globulus* Labill., and *Anethum graveolens* L., indicating their potential for further investigation as natural components of products targeting the anaerobic component of dysbiotic oral microbial communities.

Based on the obtained results, formulations of oral care products were developed for the differentiated modulation of predominant members of oral microbial communities. The developed formulations contain plant-derived components with demonstrated antimicrobial activity, providing a basis for their further preclinical and clinical evaluation.

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TWO-QUARTET G-QUADRUPLEXES IN *MYCOBACTERIUM BOVIS RPOB* AND *RPOC* GENES ASSOCIATED WITH RIFAMPICIN RESISTANCE

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Abstract. Three putative G-quadruplexes (G4s) from two quartets were identified in the sense strand of the *rpoB* and *rpoC* genes of *Mycobacterium bovis*, which causes zoonotic tuberculosis, as well as two G4s in their antisense strands. Their 3D models were designed with atomic resolution. All

identified G4s are stabilized by two K+ ions, have a parallel topology, and are characterized by varying numbers of hydrogen bonds and stacking interaction modes. In addition to canonical (two-center) H-bonds between adjacent guanines, four G4s with bifurcated H-bonds were found.
Keywords: AlphaFold 3, G-quadruplex*, Mycobacterium bovis, rpoB, rpoC.*

G-quadruplexes (G4s) are located in guanine-rich sequences of nucleic acid molecules and have been identified in various species (Marsico et al., 2019). They represent planar G-quatets formed by the linking of guanine nucleotides by four Hoogsteen and four classical Watson-Crick hydrogen bonds. G4s are stabilized by stacking interactions between G-quatets, as well as by monovalent metal ions, primarily K+ (Kryachko et al., 2026). It has been established that in bacteria G4s are necessary for antigenic variability, regulating transcription and translation, acting as both activators and inhibitors (Cueny et al., 2022). Motifs with the potential to form G4s in the regulatory regions of a number of pathogenic bacteria have been identified by bioinformatics tools (Dey et al., 2021). Folding of stable G4 structures *in vitro* has been established for the promoter regions of four genes of different functional categories in *M. tuberculosis* (Perrone et al., 2017).

Increasing incidence of multidrug-resistant tuberculosis (MDR-TB), caused by simultaneously resistant mycobacterial strains to the first-line anti-TB drugs isoniazid and rifampicin is one of the public health problems. Rifampicin resistance in *M. tuberculosis* and *M. bovis*, the etiologic agent of zoonotic tuberculosis, is caused by mutations of 1 and 3 confidence grading in the *rpoB* gene (encodes the β-subunit of RNA polymerase) and by mutations of 5 confidence grading in the *rpoC* gene (encodes the β′-subunit of RNA polymerase) according to the WHO classification. The emergence of new genomic variants of *Mycobacterium tuberculosis* complex facilitates the search of putative targets for antibacterial therapy, including G4s.

In this study, DNA G-quadruplexes from two quartets in the sense strand of the *Mycobacterium bovis rpoB* and *rpoC* genes and their antisense strands were identified and their 3D models were designed. Several complexes of K+ ions with G4s in the *rpoB* and *rpoC* genes, mutations in which are linked to rifampicin resistance, were confirmed by AlphaFold 3 AI.

The nucleotide sequences of the *rpoB* and *rpoC* genes were obtained from the genome of the reference strain *M. bovis* AF2122/97 from the GenBank database (accession number LT708304).

Manipulation with gene fragments was performed by the MEGA12 software (version 12.0.9). The QGRS Mapper software was used to search for potential G4s. 3D models of oligonucleotides with G-rich motifs in complex with K+ ions were generated by AlphaFold 3 Artificial Intelligence. To assess the reliability of the model predictions, the pLDDT (predicted local distance difference test) parameter was used in AlphaFold 3. The value for a high level of model validity is above 90 on a scale from 0 to 100.

In the first stage, five putative G4s formed by two G-quartets with a loop of up to 5 nucleotides (nt) were found in the sense strand of the *M. bovis rpoB* gene, nine in the sense strand of the *rpoC* gene, three in the antisense strand of the *rpoB* gene, and five in the antisense strand of the *rpoC* gene with G-score from 32 to 36. In the second stage, for theoretically determined in the first stage G-rich motifs, three G4s in the sense strand of the *rpoB* gene and the *rpoC* gene, two G4s in the antisense strand of the *rpoB* gene and the *rpoC* gene were confirmed through the 3D models design. The generated 3D models are characterized by high reliability, as evidenced by the value of their pLDDT parameter for the models: 90 > pLDDT > 70 and pLDDT > 90.

To control the quality of the generated 3D models, we constructed a 3D model of a parallel three-quartet G4, whose structure was determined experimentally by NMR and atomic coordinates were deposited in the Protein Data Bank (PDB: 5I2V) (Sun et al., 2019). The

pLDDT parameter value for this control G4 (90 > pLDDT > 70) has the same value or lower than that predicted for the 3D models in the *rpoB* and *rpoC* genes. This indicates that all generated 3D models, including those with the parameter 90 > pLDDT > 70, are substantiated.

The generally accepted planar structure of the G-tetrad assumes the presence of a maximum of eight H-bonds through the linking of adjacent guanines by two H-bonds: one classical Watson-Crick (internal) bond and a Hoogsteen (external) bond. All identified G4s have a parallel topology and are characterized by varying numbers of H-bonds in the two quartets (from two to eight) and up to 4 stacking interactions between the corresponding guanines.

Along with the classical (two-center) H-bonds between adjacent guanines, four G4s with bifurcated H-bonds were found including one G4 in the *rpoB* gene, two G4s in the *rpoC* gene.

3D models of G-quadruplexes generated by the AlphaFold 3 AI with atomic resolution may indicate on putative targets for antimycobacterial drugs. Complex formation between determined G4 and a drug specific to the structure of certain G4 should result in its stabilization and subsequent inhibition of RNA polymerase, the subunits of which are encoded by *rpoB* and *rpoC* genes.

References

1. Cueny, R. R., McMillan, S. D., & Keck, J. L. (2022). G-quadruplexes in bacteria: insights into the regulatory roles and interacting proteins of non-canonical nucleic acid structures. *Critical Reviews in Biochemistry and Molecular Biology*, *57(5-6),* 539-561. Doi:10.1080/10409238. 2023.2181310.
2. Dey, U., Sarkar, S., Teronpi, V., Yella, V. R., & Kumar, A. (2021). G-quadruplex motifs are functionally conserved in cis-regulatory regions of pathogenic bacteria: An *in-silico* evaluation. *Biochimie, 184,* 40-51. Doi: 10.1016/j.biochi.2021.01.017.
3. Kryachko, E. S., MacDougall, P. J., & Neal, S. (2026). Unravelling the hydrogen bonding patterns in telomeric G-quadruplexes: from structure to function. *Molecular Physics, 124(3-4)*, e2390586. Doi:10.1080/00268976.2024.2390586.
4. Marsico, G., Chambers, V. S., Sahakyan, A. B., McCauley, P., Boutell, J. M., Di Antonio, M., & Balasubramanian, S. (2019). Whole genome experimental maps of DNA G-quadruplexes in multiple species. *Nucleic Acids Research, 47(8),* 3862-3874. Doi:10.1093/nar/gkz179.
5. Perrone, R., Lavezzo, E., Riello, E., Manganelli, R., Palu, G., Toppo, S., Provvedi, R., & Richter, S. N. (2017). Mapping and characterization of G-quadruplexes in Mycobacterium tuberculosis gene promoter regions. *Scientific Reports, 7(1)*, 5743. Doi:10.1038/s41598-017-05867-z.
6. Sun, Z.-Y., Wang, X.-N., Cheng, S.-Q., Su, X.-X., & Ou, T.-M. (2019). Developing novel G-quadruplex ligands: from interaction with nucleic acids to interfering with nucleic acid – protein interaction. *Molecules, 24,* 396. Doi:10.3390/molecules24030396.

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MICROBIAL DIVERSITY AND CATALASE PROFILE OF ORAL MICROORGANISMS IN CHILDREN WITH GASTRITIS AND GASTRODUODENITIS

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Abstract. The oral microbiome was compared between 33 children aged 5–18 years with gastritis or gastroduodenitis and multiple dental caries and 20 controls. Culture-based analysis revealed 16 taxa in controls and 20 identified taxa plus unidentified strains in the study group. Shannon diversity was higher in the study group (2.969 vs. 2.614), whereas Simpson dominance was lower (0.055 vs. 0.083). Bray-

Curtis dissimilarity reached 0.685, indicating marked restructuring of the microbial profiles. Catalase-positive phenotypes occurred among *Neisseria spp.*, *Rothia spp.*, *Staphylococcus spp.*, and *Candida albicans*, while isolated streptococci were catalase-negative. These findings demonstrate greater and more even microbial diversity in children with gastroduodenal pathology and multiple caries. The functional contribution of catalase-positive taxa to oxidative-stress tolerance in dental biofilms requires further investigation.
Keywords: oral microbiome, gastritis, gastroduodenitis, dental caries, catalase activity.

Dental plaque is a metabolically integrated microbial biofilm whose composition changes under ecological pressure (Marsh, 2006; Takahashi & Nyvad, 2011). In children, gastroduodenal disease may coincide with alterations in oral microbial homeostasis, while multiple caries is associated with acidogenic biofilm activity and impaired local protective mechanisms. Catalase decomposes hydrogen peroxide and may enhance microbial survival under oxidative stress; therefore, the distribution of catalase-positive and catalase-negative taxa can characterize the functional potential of the oral community.

The aim was to determine the qualitative and quantitative composition, diversity, and catalase profile of oral-fluid microorganisms in children aged 5–18 years with gastritis or gastroduodenitis and multiple caries in comparison with controls.

Materials and Methods. A clinical and microbiological study was conducted involving 33 children aged up to 18 years diagnosed with gastritis or gastroduodenitis and multiple dental caries. Dental examination was performed using generally accepted methods, including assessment of dental hard tissues and determination of caries intensity according to the child's age. Gastritis or gastroduodenitis was diagnosed based on clinical and instrumental examination findings.

The qualitative and quantitative composition of the microbiota was determined using a bacteriological method by inoculating the collected material onto appropriate culture media, followed by incubation. Microbial counts were expressed as colony-forming units per millilitre (CFU/mL).

Catalase activity of the isolated cultures was determined based on their ability to decompose hydrogen peroxide with the release of molecular oxygen. The appearance of gas bubbles after hydrogen peroxide solution was applied to the culture was considered a positive catalase reaction, whereas the absence of gas formation was considered a negative reaction. Based on the results, the ratio of catalase-positive to catalase-negative representatives of the oral microbiome was determined.

Detection frequency was expressed as an absolute count and percentage with its standard error. Community structure was assessed using the Shannon index, Simpson dominance and diversity indices, and Bray-Curtis dissimilarity after normalization of taxon detection frequencies.

Results and discussion. The microbial load ranged from 10^3 to 10^8 CFU/mL in both groups. Sixteen species were recovered from controls, whereas the study group yielded 20 identified species and a group of unidentified strains. In controls, the most frequent taxa were *Streptococcus mitis* (25.00 ± 9.68 %), *Streptococcus parasanguinis*, *Streptococcus mutans*, and *Veillonella parvula* (20.00 ± 8.94 % each). In children with gastroduodenal pathology, *Neisseria sicca* and unidentified strains were detected most often (21.21 ± 7.12 % each), followed by *Streptococcus mitis* and *Rothia mucilaginosa* (18.18 ± 6.71 % each) and *Lactobacillus fuchuensis* and *Candida albicans* (15.15± 6.24 % each). The study group also included *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumoniae*, *Acinetobacter johnsonii*, and *Klebsiella oxytoca*.

Only seven species were shared by both groups: *Streptococcus mitis*, *Streptococcus parasanguinis*, *Neisseria flavescens*, *Veillonella parvula*, *Lactobacillus plantarum*,

Actinomyces oris, and *Candida albicans*. The Shannon index was 2.614 in controls and 2.969 in the study group. Simpson dominance was 0.083 and 0.055, while Simpson diversity was 0.917 and 0.945, respectively. These values indicate a larger number of detected taxa, a more even frequency distribution, and weaker dominance in children with gastritis or gastroduodenitis. Bray-Curtis dissimilarity was 0.685; thus, the microbial profiles differed by 68.5 %, with a similarity of 31.5 %. These comparisons are descriptive because the statistical significance of between-group differences in diversity indices was not tested.

Catalase-positive phenotypes were recorded among *Neisseria spp.*, *Rothia spp.*, *Staphylococcus spp.*, and *Candida albicans*, whereas the isolated streptococci were catalase-negative. Catalase-positive members of a mixed biofilm may contribute to hydrogen peroxide decomposition and collective protection against oxidative stress, potentially supporting persistence of the microbial community. This functional interpretation should be confirmed in further studies.

In conclusion, children with gastritis or gastroduodenitis and multiple caries displayed a richer, more even, and compositionally distinct oral-fluid microbiome than controls. The presence of additional opportunistic taxa and catalase-positive microorganisms may reflect restructuring of oral microbial interactions. Larger studies controlling for age, oral hygiene, diet, dental status, and treatment are required to establish statistical and causal relationships.

References

1. Marsh, P. D. (2006). Dental plaque as a biofilm and a microbial community: Implications for health and disease. BMC Oral Health, 6(Suppl 1), S14. https://doi.org/10.1186/1472-6831-6-S1-S14
2. Takahashi, N., & Nyvad, B. (2011). The role of bacteria in the caries process: Ecological perspectives. Journal of Dental Research, 90(3), 294-303. https://doi.org/10.1177/ 0022034510379602

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THE ROLE OF *ARCANOBACTERIUM HAEMOLYTICUM* IN THE DEVELOPMENT OF A PURULENT-NECROTIC LESION: A CLINICAL AND MICROBIOLOGICAL CASE REPORT

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Abstract. *Arcanobacterium haemolyticum* is an under-recognised opportunistic pathogen that may contribute to skin and soft-tissue infections, particularly in patients with malignancy, tissue necrosis, impaired local blood supply, and immune dysfunction. This clinical and microbiological case report aimed to determine the significance of *A. haemolyticum* isolated from a purulent-necrotic lesion in a patient with recurrent basal cell carcinoma of the anterior chest wall. A male patient born in 1948 presented with an extensive malignant wound following repeated surgical interventions and radiotherapy, accompanied by sternal osteomyelitis, a pathological fracture, cachexia, and severe functional impairment. Bacteriological examination of an intraoperatively collected specimen from the deep area of the lesion yielded *A. haemolyticum* at a concentration of 10^5 CFU/mL, and species identification was confirmed by MALDI-TOF mass spectrometry. The clinically significant microbial load and pronounced local signs of infection suggest that the isolate was not merely a surface coloniser but a potential aetiological agent contributing to the persistence

of the inflammatory and necrotic process. This case highlights the importance of species-level identification and quantitative assessment of coryneform bacteria isolated from chronic malignant wounds to support individualised management of oncology and palliative care patients.
Keywords: *Arcanobacterium haemolyticum*; basal cell carcinoma; malignant wound; purulent-necrotic lesion; skin and soft-tissue infection; opportunistic infection; palliative care.

Background. Chronic ulcerative-necrotic lesions associated with malignant skin neoplasms create favourable conditions for colonisation and proliferation by opportunistic microorganisms, while impaired blood supply, tissue necrosis, a prolonged wound course, previous radiotherapy and surgical interventions, and the patient's poor general condition increase the risk of secondary bacterial infection.

One of the insufficiently investigated causative agents of skin and soft-tissue infections is *Arcanobacterium haemolyticum*. It is a Gram-positive, catalase-negative, facultatively anaerobic, coryneform bacterium predominantly associated with pharyngitis and skin and soft-tissue infections. Because of its slow growth, distinctive haemolytic properties, and morphological similarity to other coryneform bacteria, this microorganism may be misinterpreted as a component of the commensal microbiota. However, in patients with disrupted skin integrity, immune dysfunction, malignancy, cachexia, and limited mobility, *A. haemolyticum* may become clinically significant and contribute to the development of deep purulent-necrotic lesions.

Aim. To determine the clinical and microbiological significance of *Arcanobacterium haemolyticum* isolated from a purulent-necrotic lesion of the skin and soft tissues in a patient with recurrent basal cell carcinoma of the anterior chest wall.

Materials and Methods. Medical records and clinical and microbiological findings were analysed for a male patient born in 1948 with recurrent basal cell carcinoma of the anterior chest wall, previously treated surgically in 2004 and 2009 and with radiotherapy in 2022, who developed an extensive ulcerative-necrotic defect, putrefactive tissue necrosis, and sternal osteomyelitis with a pathological fracture. Clinical assessment included wound characteristics, signs of inflammation and necrosis, and functional status according to the Karnofsky, ECOG, and Barthel scales; treatment at the Fourth Hospital of the Second Medical Association of Lviv comprised repeated necrectomies and sequestrectomies, stabilisation of the sternal fragments, and correction of comorbidities. Intraoperative specimens collected aseptically from deep areas of the purulent-necrotic lesion were cultured to determine microbial load and isolate characteristics, followed by species-level identification using matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS).

Results. At the time of examination, the patient's general condition was severe and was determined by progression of the malignant neoplasm, the presence of an extensive ulcerative-necrotic defect, and marked functional impairment. A wound defect measuring approximately 15 × 15 cm and up to 1.5 cm in depth was identified on the anterior chest wall, and its bed was covered with fibrin and necrotic tissue. Irregular, infiltrated wound margins, purulent discharge, and a foul odour were observed, indicating secondary infection of the malignant wound. The patient's functional status was characterised by a substantial reduction in daily activity: the Karnofsky Performance Status score was approximately 30 %, the ECOG Performance Status score was 4, and the Barthel Index was below 25 points. The patient was highly dependent on assistance from others and required continuous care and palliative support.

Bacteriological examination of material obtained from the purulent-necrotic lesion yielded *Arcanobacterium haemolyticum* at a concentration of 10^5 CFU/mL, and the species-level identity of the isolate was confirmed by MALDI-TOF MS.

The detection of *A. haemolyticum* at a clinically significant concentration in a specimen obtained directly from the pathological lesion was accompanied by pronounced local signs of infection, including purulent discharge, necrosis, fibrinous deposits, and a foul odour.

These findings support the interpretation of the isolated microorganism not merely as a surface coloniser but as a potential aetiological agent contributing to the persistence of the inflammatory and necrotic process.

Considering the patient's clinical history, the pathogenic potential of the bacterium may have been facilitated by disruption of the skin barrier, the presence of necrotic tissue, impaired blood supply to the malignant wound, previous radiotherapy, repeated surgical interventions, progression of the malignancy, severe functional impairment, and weakened local and systemic antimicrobial defence mechanisms. The pathogenicity of *A. haemolyticum* is primarily associated with the production of phospholipase D, which damages cell membranes, increases tissue permeability, and facilitates bacterial dissemination. Haemolytic activity, neuraminidase production, adhesion capacity, and the ability to form persistent microbial communities within chronic wound tissues may also contribute to its pathogenicity.

The patient had a long-term history of malignancy and had previously received specific anticancer treatment. Progression of the underlying disease was accompanied by impaired nutritional status, cachexia, reduced functional activity, and prolonged immobilisation. Against this background, an extensive purulent-necrotic soft-tissue lesion developed.

Conclusions. The isolation of *A. haemolyticum* at a concentration of 10^5 CFU/mL from a deep area of the wound defect, together with the presence of purulent discharge, necrosis, and fibrinous deposits, supports its involvement in the persistence of the infectious process. Progression of basal cell carcinoma, repeated surgical interventions, previous radiotherapy, impaired local blood supply, and the patient's poor general condition created favourable circumstances for the expression of the microorganism's pathogenic potential. Coryneform bacteria isolated from chronic and malignant wounds should not automatically be regarded as contaminants without species-level identification and quantitative assessment of the microbial load. Microbiological examination of malignant wounds should constitute an integral component of the comprehensive management of oncology and palliative care patients and should inform the development of an individualised therapeutic strategy.

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IN VITRO EFFECT OF COLISTIN IN COMBINATION WITH MEROPENEM, RIFAMPICIN, TIGECYCLIN AND AMPICILLINE-SULBACTAME ON BIOFILM FORMATION IN EXTENSIVELY DRUG-RESISTANT *ACINETOBACTER BAUMANNII* ISOLATES

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Abstract. The effect of colistinin combinations with meropenem, rifampicin, tigecyclin and ampicilline-sulbactame on *Acinetobacter baumannii* was studied (17 clinical isolates with XDR phenotype, two of this colistin resistance). For colistin-susceptible isolates the combination of

colistin+meropenem exhibited the highest rate of synergism. For colistin-resistant isolates, the combination colistin + rifamicin was more effective. However, no combination was consistently synergistic against all strains tested. The most pronounced inhibitory effect on biofilm formation was confirmed for combinations colistin + rifamycin. The combination colistin + ampicilline-sulbactame was found to be active in preventing film formation and weakly active against already formed films. Combination colistin + tigecycline demonstrated limited syneggism and effect on biofilm formation.
Keywords: *Acinetobacter baumanii,* antimicrobials, colistin, synergism, biofilms ingibition.

The bacteria genus *Acinetobacter* is one of the most important pathogens of hospital-acquired infections in critically ill patients. *Acinetobacter* spp. are a serious problem due to their widespread distribution, unpretentiousness to living conditions, resistance to most antimicrobial drugs. Also important is the ability of this pathogen to form biofilms. A new approach to treat infections caused *by A. baumannii* is Combination therapy. Polymyxin-based combinations are considered the most promising (Karakonstantis et al, 2021, Mantzana et al, 2023, Oliva et al, 2019;).

A 17 *A. baumannii* clinical isolates with the XDR phenotype were included. The minimum inhibitory concentrations (MICs) of antimicrobial agents and their combinations were determined by the microdilution method in broth. The effectiveness of the combination of antimicrobial agents was assessed using a checkerboard test. The indicator of the type of antibiotic interaction *was* the index of fractional inhibitory concentrations (FICI). The intensity of biofilm formation inhibition was determined as a degree of inhibition of biofilms (I) using crystal violet assay.

No antimicrobials combinations were consistently synergistic against all strains tested. Most often, a synergistic effect (FICI ≤ 0,5) was observed for the combination colistin + meropenem – in 76.5 %. However, no synergism for this antimicrobial's combination was observed in colistin-resistant (with MIC colistin 16.0 mg/L) isolates. For the colistin + rifampicin combination, synergism was recorded in 64.7 %, including colistin-resistant isolates. However, such a combination of antibiotics is considered toxic during therapy. Furthermore, in this study, the MIC of colistin for both colistin-resistant isolates did not decrease to a clinically significant level (<4.0 mg/L) in combination with rifampicin.

Combination colistin+ampicilline-sulbactame demonstrated pronounced synergism in 47.1 % of cases, colistin + tigecycline – in 35.3 % of cases.

A. baumannii biofilm formation was most actively inhibited by the combination colistin+rifampicin: the biofilm inhibition indicator I = 73–85 % for colistin-susceptible isolates and I = 66–71 % – for colistin-resistance. Combination colistin + meropenem demonstrated I between 62 to 78 % for colistin-susceptible and between 54 to 66 % colistin-resistance isolates.

Combination colistin + ampicilline-sulbactame actively prevented the formation of biofilms (especially noticeable in colistin-sensitive isolates), however, it was weakly active against already formed films. Combination colistin + tigecycline demonstrated limited effect on biofilm formation.

Conclusion. The combinations of colistin + meropenem (for colistin-susceptible-isolates) and colistin + rifamicin (includet colistin-resistance isolates) exhibited the highest rate of synergism for *A. baumanii*;

The most pronounced inhibitory effect on *A. baumanii* biofilm formation among the tested combinations was confirmed for colistin + rifamycin, somewhat lower- for colistin + meropenem

The synergism degree and biofilm inhibition effect of antimicrobials combinations on depended on the initial level of colistin-sensitivity of the *A. baumanii* isolates.

References

1. Karakonstantis, S., Ioannou, P., Samonis, G., & Kofteridis, D. P. (2021). Systematic review of antimicrobial combination options for pandrug-resistant *Acinetobacter baumannii*. *Antibiotics, 10*(11), 1344. https://doi.org/10.3390/antibiotics10111344
2. Mantzana, P., Protonotariou, E., Kassomenaki, A., Meletis, G., Tychala, A., Keskilidou, E., Arhonti, M., Katsanou, C., Daviti, A., Vasilaki, O., Kagkalou, G., & Skoura, L. (2023). In vitro synergistic activity of antimicrobial combinations against carbapenem- and colistin-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae*. *Antibiotics, 12*(1), 93. https://doi.org/10.3390/antibiotics12010093
3. Oliva, A., Garzoli, S., De Angelis, M., Marzuillo, C., Vullo, V., Mastroianni, C. M., & Ragno, R. (2019). In-vitro evaluation of different antimicrobial combinations with and without colistin against carbapenem-resistant *Acinetobacter baumannii*. *Molecules, 24*(5), 886. https://doi.org/10.3390/molecules24050886

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METABOLIC PROFILE OF THE PROBIOTIC STRAINS *BACILLUS VELEZENSIS* AS THE POTENTIAL BIOAGENTS OF THE WOUND HEALING COMPOSITION

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Abstract. The metabolic profiles of the probiotic strains *Bacillus velezensis* IMV B-7142 and IMV B-7143 were investigated. Differences were identified in the metabolite spectra of lipid and nitrogen metabolism in the investigated strains, which complement each other, providing synergistic antimicrobial, antioxidant, and immunomodulatory potential of the probiotic composition. The efficacy of the developed composition was demonstrated in a model of infected wounds in Chinchilla rabbits.
Keywords: probiotics*, Bacillus velezensis,* metabolic profile, infected wounds, treatment.

Research and development of the latest probiotic preparations is currently a highly relevant field in microbiology, biotechnology and medicine. This opens up new prospects for the practical use of metabolites from probiotic bacterial strains to support the physiological homeostasis of the microbiome, and for the prevention and treatment of pathological conditions (Fu et al., 2023).

At present, aerobic spore-forming bacteria of the genus *Bacillus* are of considerable interest as a basis for the development of antimicrobial biopreparations. This is particularly relevant in view of the rapid spread of antibiotic-resistant pathogenic microorganisms (Safronova et al., 2024). *Bacillus* strains are components of probiotic preparations for the treatment of various pathological conditions in humans and animals, including infections of the skin and adjacent tissues, owing to their ability to synthesize a complex of biologically active metabolites (antibiotic substances, enzymes, antioxidants, amino acids, polysaccharides, vitamins and others) (Lu et al., 2022).

The aim of this study was to investigate the metabolic profiles of the probiotic strains *Bacillus velezensis* IMV B-7142 and IMV B-7143 and to determine the potential for using a composition of these strains in the prevention and treatment of infected wounds.

Metabolomic profiling of ethyl acetate extracts of cell-free supernatants from the *B. velezensis* IMV B-7142 and IMV B-7143 strains was carried out using gas chromatography-mass spectrometry (GC-MS). It was found that their profiles contained similar classes of compounds with anti-inflammatory and antiseptic activity (organic acids, fatty acids, nitrogenous bases), but differed significantly in the relative content of major components. Thus, the *B. velezensis* IMV B-7142 strain was characterized by a significantly higher level of synthesis of lipid metabolism metabolites – derivatives of glycerol and fatty acids. In particular, the content of glycerol monopalmitate and glycerol monostearate in its metabolome exceeded 20 per cent for each, whereas in the *B. velezensis* IMV B-7143 strain these figures were less than 1 per cent.

The exometabolome of the *B. velezensis* IMV B-7142 strain also revealed significantly higher levels of nitrogen metabolism compounds: the urea content was 17.35 %, whereas in the *B. velezensis* IMV B-7143 strain it was 0.79 %; at the same time, the uracil content was five times higher than that in the *B. velezensis* IMV B-7143 strain.

Urea, as a natural moisturizing factor, can maintain an optimal moist environment within the wound, which is critical for the rapid migration of keratinocytes and wound closure. It can also help to soften and exfoliate necrotic tissue and dry scabs without damaging living cells. Urea has the ability to stimulate the production by keratinocytes of their own protective antimicrobial peptides, which reduces the risk of wound infection. Uracil (and its clinical analogue, methyluracil) is a potent reparative agent. Uracil derivatives are capable of enhancing the phagocytic activity of macrophages, thereby accelerating the biological cleansing of the wound, and modulating inflammation, shifting it into the recovery phase.

Phenylalanine (2.13 %) has been identified from the amino acid pool in the metabolic profile of the *B. velezensis* IMV B-7142 strain; it is absent from the major metabolites of the *B. velezensis* IMV B-7143 strain. Phenylalanine is an important substrate for the synthesis of tissue proteins and acts as a mediator that activates regeneration and collagen formation processes.

At the same time, the *B. velezensis* IMV B-7143 strain had a more complex profile with a large number of small peaks (over 160), including various specific components – derivatives of organic acids, particularly 2-furancarboxylic acid (0.32 per cent), known to have detoxifying, antimicrobial and biocontrol properties.

Thus, the *B. velezensis* IMV B-7142 strain demonstrated a specialized metabolism directed towards the synthesis of monoglycerides, while the *B. velezensis* IMV B-7143 strain had a more balanced and diverse lipid composition containing unsaturated compounds and sterol precursors, in particular, squalene, which is responsible for antimicrobial, immunemodulatory and antioxidant effects.

Laboratory experiments have confirmed the protective activity of *B. velezensis* IMV B-7142 and IMV B-7143 against infectious agents. Under *in situ* and *in vivo* conditions, the composition based on these strains has been shown to play an antagonistic and adaptogenic role in enhancing local immunity and resistance to pathogens. The efficacy of the formulation was demonstrated in model experiments on Chinchilla rabbits during the treatment of purulent wounds infected with *Staphylococcus aureus* B-4001 ATCC 6538A and *Klebsiella pneumoniae-R* at a dose of 100 µl ($1 \cdot 10^8$ cells/mL), where its use significantly accelerated wound healing.

Consequently, the probiotic *B. velezensis* IMV B-7142 and IMV B-7143 strains are promising bioagents for the development and clinical implementation of new safe preparations for the treatment of wounds complicated by infection.

References

1. Fu, Y., Dou, Q., Smalla, K., Wang, Y., Johnson, T. A., Brandt, K. K., Mei, Z., Liao, M., Hashsham, S. A., Schäffer, A., Smidt, H., Zhang, T., Li, H., Stedtfeld, R., Sheng, H., Chai, B., Virta, M., Jiang, X., Wang, F., Zhu, Y.-G., & Tiedje., J. M. (2023). Gut microbiota research nexus: One Health relationship between human, animal, and environmental resistomes. *mLife,* 2, 350–364. https://doi.org/10.1002/mlf2.12101
2. Lu, S., Na, K., Li, Y., Zhang, L., Fang, Y., & Guo, X. (2022). Bacillus-derived probiotics: metabolites and mechanisms involved in bacteria-host interactions. *Crit Rev Food Sci Nutr, 6*, 1–14. https://doi.org/10.1080/10408398. 2022.2118659
3. Safronova L. A., Pylypiuk Y. V., Skorochod I. O., Polova Z. M. (2024). Probiotics and their potential for the prevention and treatment of infections. *Microbiological journal.* 6, 74–91. https://doi.org/10.15407/microbiolj86.06.074

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MONITORING OF BACTERIAL DISEASE OUTBREAKS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) TO IMPROVE PREVENTIVE STRATEGIES FOR THE NATIONAL AQUACULTURE

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Abstract. During monitoring studies of trout farms 3 strains of *A. salmonicida* (A.salmUA1/2025RTV, A.salmUA1/2026WFV and A.salmUA2/2026BTCh), 2 strains of *F. psychrophila* (F.psyUA1/2025RTV and F.psyUA1/2026RTCh) and one strain of *Y. ruckeri* bacteria (Y.ruckUA1/2026RTZ) were isolated from rainbow trout, whitefish and brook trout. Using PCR, fragments of species-specific genes and the 16S rRNA gene of the isolated bacteria were amplified for further genetic characterization. Development of inactivated autogenous vaccines for oral administration based on isolated bacterial strains will help to decrease antibiotic use at the local level and prevent economic losses of trout farms in Ukraine.
Keywords: bacterial diseases, aquaculture, PCR, 16SrDNA, vaccine, rainbow trout.

Today, aquaculture is the most dynamic and fastest growing sector of the animal agriculture and is crucial for food security in many countries. Like other livestock sectors, aquaculture requires adequate veterinary support and control of infectious diseases (Buchatsky et al., 2020). Vaccination of fish with locally developed autogenous vaccines can provide a local and cost-effective means of reducing mortality in aquaculture facilities, as well as contributing to the rational use of antibiotics. A comprehensive scientific and methodological approach to assessing the possibilities of introducing autogenous vaccination in national aquaculture will allow using a number of key advantages of immunobiotechnological tools in terms of efficiency and flexibility of animal welfare, transboundary biosecurity, and the economy of local farms (Rud et al., 2022).

Pathogenic bacteria from the genera *Aeromonas*, *Flavobacterium* and *Yersinia* cause morbidity and high mortality in salmon farming, which encourages producers to constantly use antimicrobial drugs for therapeutic and prophylactic purposes. The lack of immunoprophylactic agents for aquaculture on the market and the constant growth of production volumes require comprehensive solutions to improve the epizootic situation in fish farms. Monitoring studies of trout farms and assessment of the epizootic situation are very important for the biosecurity of the industry and make it possible to respond in a timely manner to the outbreaks (Vinh et al., 2026). However, for the prevention and control of infectious diseases in aquaculture, it is important to introduce immunoprophylactic agents that can solve the global problem of antimicrobial resistance at the local level. Therefore, the goals of our research were to monitor the main bacterial diseases of rainbow trout in national aquaculture and select strains of pathogenic microorganisms for the development of inactivated autogenous vaccines.

In our monitoring studies, we focused our efforts on the identification of three main pathogenic bacteria: namely the causative agent of bacterial cold water diseases (BCWD) and rainbow trout fry syndrome (RTFS) bacteria *Flavobacterium psychrophila*; the causative agent of furunculosis *Aeromonas salmonicida*; and the causative agent of enteric red mouth disease or "yersiniosis" – *Yersinia ruckeri*. For this, we used selective media ECA, FLPA, BHIA, TSA+CBB and SWA. Gram stain and PCR methods were used to identify the strains. We also applied the diffusion disk method to determine susceptibility to the main antibiotics allowed in aquaculture.

Identification of *A. salmonicida, F. psychrophila* and *Y. ruckeri* bacteria was carried out in rainbow trout (*Oncorhyncyus mykiss*) and American char (*Salvelinus fontinalis*) with characteristic external clinical signs of diseases. To isolate *A. salmonicida* strains, trout or char with boils (furuncles) on the side or in the tail part of the body were selected. *F. psychrophila* bacteria were isolated from rainbow trout fry from ulcers formed in the form of a "saddle" behind the dorsal fin and from internal organs. In the case of *Y. ruckeri* bacteria, characteristic external and internal hemorrhages, exophthalmos and redness of the oral cavity in rainbow trout were recorded. For molecular diagnostics of bacteria, the target genes 16S rRNA, VapA and gyrB for *A. salmonicida*, the ompA gene for *Y. ruckeri* and the 16S rRNA gene for *F. psychrophila* were used.

As a result of our research conducted in 2025–2026, 3 strains of *A. salmonicida* bacteria (A.salmUA1/2025RTV, A.salmUA1/2026WFV and A.salmUA2/2026BTCh) were isolated from rainbow trout, whitefish (*Coregonus sp*.) and brook trout, respectively, two strains of *F. psychrophila* bacteria (F.psyUA1/2025RTV and F.psyUA1/2026RTCh) were isolated from rainbow trout and one strain of *Y. ruckeri* bacteria (Y.ruckUA1/2026RTZ) was isolated from rainbow trout. Using PCR, fragments of species-specific genes and the 16S rRNA gene of the isolated bacteria were amplified for further genetic characterization.

The isolated bacteria *A. salmonicida, F. psychrophila* and *Y. ruckeri* were characterized by a significant level of antibiotic resistance, which indicates an incorrect approach to the treatment of infectious diseases by national producers, who often do not turn to specialized laboratories, but select a treatment method empirically. Most often, the studied bacteria were resistant to oxytetracycline, trimethoprim and sulfamethoxazole. Since the rational use of antibiotics is a global public health issue, in which attention is focused on the supply of food products within the framework of the "One Health" concept, the creation of inactivated autogenous vaccines for oral administration based on isolated bacterial strains will help to solve the global problem of antimicrobial resistance at the local level and prevent economic losses of trout farms in Ukraine.

References

1. Buchatskyi L.P., Rud Yu.P., Matvienko N.M. Viral infections of sturgeons and salmon. Kyiv: DIA, 2020. 240 p. ISBN 978–617–7785–10–0.
2. Rud Yu.P., Zaloilo O.V., Zaloilo I.A., Buchatskyi L.P. Antiviral vaccines for fish (review) / Ribogospod. nauka Ukr. 2022. No. 3(61). P. 71—104. https://doi.org/10.15407/fsu2022.03.071
3. Vinh, N. T., Dong, H. T., Turner, J. K., Senapin, S., & Turner, W. A. (2026). Back to basics: locally produced vaccines offer a practical alternative to antibiotics for prevention of streptococcosis in farmed tilapia (*Oreochromis* spp.). Frontiers in Veterinary Science, 13. https://doi.org/10.3389/fvets.2026.1740757

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DIAGNOSTIC CAPABILITIES OF MALDI-TOF MS IN THE IDENTIFICATION OF RARE BACTERIAL ISOLATES

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Abstract. MALDI-TOF mass spectrometry has transformed clinical microbiology by enabling rapid, highly accurate identification of microorganisms based on unique protein spectral profiles, overcoming the limitations of conventional biochemical panels when applied to atypical strains. To characterize rare bacterial isolates identified by MALDI-TOF MS and assess their clinical significance. Methods: Clinical specimens (blood, sputum, wound discharge, urine, respiratory material) collected in 2025/2026 at the Center for Microbiological Research, Danylo Halytsky Lviv National Medical University, were analyzed using a Bruker MALDI-TOF mass spectrometer with reference database comparison. Results: Six rare pathogens were identified – *Elizabethkingia meningoseptica*, *Chryseobacterium indologenes*, *Ochrobactrum anthropi*, *Myroides odoratimimus*, *Sphingomonas paucimobilis*, and *Kocuria kristinae* – many of which are difficult to distinguish from more common species by conventional culture-based methods, risking misclassification and diagnostic delay.
Keywords: MALDI-TOF, rare pathogens, microorganism identification, clinical microbiology.

Introduction. The introduction of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) into clinical microbiology practice has opened fundamentally new possibilities for rapid and highly accurate identification of microorganisms. Unlike conventional biochemical panels, which often prove uninformative or misleading when applied to strains of pathogens with atypical properties, MALDI-TOF enables identification of microorganisms based on a unique protein spectral profile considerably faster than conventional methods.

Aim. To characterize rare isolates recovered from clinical material and identified by MALDI-TOF mass spectrometry, and to assess their clinical significance.

Materials and methods. The study was conducted at the Center for Microbiological Research of Danylo Halytsky Lviv National Medical University using a MALDI-TOF mass spectrometer (Bruker, Germany). Strain identification was performed by comparing the acquired spectral profiles with a reference database. The material comprised clinical specimens (sputum, blood, wound discharge, urine, and respiratory material) collected during 2025/2026.

Results. Among the atypical isolates examined, the study identified a number of rare pathogens, including:

- *Elizabethkingia meningoseptica* – a Gram-negative non-fermenting rod associated with nosocomial infections (meningitis in neonates, bacteremia in immunocompromised patients), characterized by intrinsic resistance to multiple antibiotic classes (isolated from a patient's blood).
- *Chryseobacterium indologenes* – an opportunistic non-fermenting microorganism causing infections in intensive care unit patients associated with catheters and mechanical ventilation (isolated from a patient's sputum).
- *Ochrobactrum anthropi* – a rare opportunistic pathogen of catheter-associated bacteremia, difficult to differentiate by conventional methods owing to phenotypic similarity to *Brucella* species (isolated from a patient's sputum).
- *Myroides odoratimimus* – a non-fermenting gram-negative rod with intrinsic multidrug resistance, causing wound and urinary tract infections predominantly in debilitated individuals (isolated from a wound in a military serviceman).
- *Sphingomonas paucimobilis* – a rare pathogen detected in catheter-associated infections and in immunosuppressed patients (isolated from a patient's sputum).
- *Kocuria kristinae* – gram-positive cocci of the family *Micrococcaceae*, with isolated cases described as a cause of bacteremia and endocarditis (isolated from a patient's urine).

Identification of the above microorganisms by conventional culture-based and biochemical methods is frequently complicated by their phenotypic similarity to more common species, which leads to misclassification or a considerable increase in the time required to establish the etiology of the pathological process. The use of MALDI-TOF enabled accurate and maximally rapid verification of all isolates listed.

Conclusions. MALDI-TOF mass spectrometry is a highly effective tool for the rapid identification of rare and atypical pathogens of infectious diseases that are verified with considerable difficulty by conventional methods. Timely and accurate identification of such microorganisms is of fundamental importance for selecting appropriate antimicrobial therapy, epidemiological surveillance, and preventing the spread of healthcare-associated infections of various profiles within clinical settings.

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EFFECT OF THE BACTERIOPHAGE PREPARATION PYOFAG ON PHAGOCYTIC ACTIVITY AND POLARIZATION-ASSOCIATED MARKER EXPRESSION IN RAW264.7 MACROPHAGES

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Abstract. This study evaluated the effects of the polyvalent bacteriophage preparation Pyofag on phagocytic activity and polarization-associated marker expression in RAW264.7 macrophages. Pyofag did not significantly alter the proportion of phagocytic cells, although bead-associated mean fluorescence intensity (MFI) showed a non-significant upward trend. The CD80-positive fraction remained

unchanged, while CD80 MFI tended to increase; CD206 MFI increased 1.6-fold versus the vehicle control ($p = 0.0003$). These findings indicate preserved phagocytic function and a mixed polarization-associated response dominated by enhanced CD206 expression.
Keywords: bacteriophages, Pyofag, RAW264.7, phagocytosis, macrophage polarization.

Background. Antimicrobial resistance is a major global public health threat, driving interest in bacteriophage therapy as a promising alternative or adjunct to conventional antimicrobial treatment (Cieślik et al., 2026). In vivo, phages interact with innate immune cells, and macrophages can contribute to therapeutic phage uptake and clearance, potentially affecting phage persistence (Zborowsky et al., 2025). Macrophage activation is highly plastic and spans a spectrum from predominantly pro-inflammatory and antimicrobial to anti-inflammatory and tissue-reparative states, commonly referred to as M1-like and M2-like phenotypes. Phagocytic function is also important during bacterial infection, as macrophages clear bacteria, dead cells, and tissue debris, contributing to immune defense and tissue homeostasis (Cieślik et al., 2026). Bacteriophages can exert complex, context-dependent effects on macrophages: gut-derived virulent phages induced initial M1-like activation and enhanced phagocytosis followed by tolerance to LPS, whereas M13 phage-coated surfaces promoted an anti-inflammatory M2-like phenotype and enhanced efferocytosis (Hou et al., 2025; Safari et al., 2022). These contrasting effects indicate that macrophage responses to bacteriophages are context-dependent and may extend beyond a simple pro-inflammatory/anti-inflammatory dichotomy. The aim of this study was to evaluate the effect of the polyvalent bacteriophage preparation Pyofag on phagocytic activity and CD80/CD206 expression in RAW264.7 macrophages in vitro.

Materials and methods. RAW264.7 murine macrophages ($5 \cdot 10^5$ cells/sample) were exposed to Pyofag, a polyvalent bacteriophage mixture, at an approximate cell:phage ratio of 1:2 for 30 min at 37 °C, 5 % CO_2. LPS (1 μg/mL) was used as a pro-inflammatory control, while the corresponding phage-free formulation solution served as the vehicle control. After exposure, cells were pelleted, reseeded in complete medium based on high-glucose DMEM, and cultured for 24 h under the same conditions. Phagocytic activity was assessed by incubating cells with 1-μm fluorescent microspheres at a cell:bead ratio of 1:100 for 1 h, followed by washing and flow cytometric analysis. RAW264.7 cells were gated based on FSC/SSC characteristics with subsequent singlet discrimination. Phagocytic activity was quantified as the percentage of bead-positive cells and bead-associated mean fluorescence intensity (MFI), used as an indicator of relative particle uptake. CD80 and CD206 expression was analyzed in separate samples using FITC-conjugated anti-mouse CD80 and PE-conjugated anti-mouse CD206 antibodies. Cells were detached and stained according to the manufacturers' instructions for 30 min at 4 °C, washed, and analyzed by flow cytometry. Marker expression was evaluated as the percentage of positive cells and MFI. Statistical analysis was performed in GraphPad Prism 9.0.0 ($n = 3$ biological replicates; $p \leq 0.05$).

Results. Pyofag did not significantly affect the proportion of phagocytic RAW264.7 cells, which remained high across all groups. Bead-associated MFI tended to increase after Pyofag exposure ($1.26 \cdot 10^6$ vs $0.95 \cdot 10^6$ in the vehicle control) and was comparable to that observed after LPS stimulation ($1.24 \cdot 10^6$), although the difference was not statistically significant. This tendency is consistent with previous observations of enhanced particle phagocytosis following bacteriophage exposure in macrophage models (Hou et al., 2025). LPS significantly increased the proportion of CD80-positive cells compared with the vehicle control ($p = 0.0211$), whereas Pyofag did not significantly alter the CD80-positive fraction; however, CD80 MFI showed a non-significant upward trend. The proportion of CD206-positive cells remained unchanged; however, CD206 MFI increased 1.6-fold after Pyofag exposure compared with the vehicle control ($p = 0.0003$) and was also significantly higher

than after LPS stimulation ($p = 0.0357$). The significant increase in CD206 MFI, together with the non-significant upward trend in CD80 MFI, suggests that the response to Pyofag may involve a mixed pattern of polarization-associated marker expression rather than a unidirectional M1- or M2-like shift. Given its polyvalent composition, the response to Pyofag should be considered a composite effect of multiple phages.

Conclusions. The pattern of polarized activation of RAW264.7 macrophages following exposure to Pyofag does not conform to the classical M1/M2 dichotomous paradigm. A moderate increase in phagocytic activity and a pronounced upregulation of CD206, indicative of an anti-inflammatory metabolic shift, accompanied by a trend toward increased CD80 expression, suggest a complex and multidirectional modulation of macrophage functional state rather than a unidirectional polarization. These findings support the view that Pyofag may induce a mixed or intermediate macrophage activation phenotype. A more precise characterization of this response will require a broader functional assessment, including cytokine production, reactive oxygen species generation, and arginine metabolism.

References

1. Cieślik, M., Gębicka, M., Wójcicki, M., Górski, A., & Jończyk-Matysiak, E. (2026). When bacteriophages encounter macrophages during their journey through the human body. *Frontiers in Cellular and Infection Microbiology, 16*, 1857968. Doi:10.3389/fcIMV.2026.1857968.
2. Hou, X., Ding, H., Wang, H., Wang, J., Liu, H., Hu, H., Billington, C., Wang, R., & Zhang, L. (2025). Gut bacteriophages induce specific-reprogramming of macrophages to generate a protective innate immunity response to lipopolysaccharide exposure. *Gut Microbes, 17*(1), 2524540. Doi:10.1080/19490976.2025.2524540.
3. Safari, Z., Sadeghizadeh, M., Asgaritarghi, G., Bardania, H., Sadeghizadeh, D., & Soudi, S. (2022). M13 phage coated surface elicits an anti-inflammatory response in BALB/c and C57BL/6 peritoneal macrophages. *International Immunopharmacology, 107*, 108654. Doi:10.1016/j.intimp.2022.108654.
4. Zborowsky, S., Seurat, J., Balacheff, Q., Ecomard, S., Mulet, C., Minh, C. N. N., Titécat, M., Evrard, E., Rodriguez-Gonzalez, R. A., Marchi, J., Weitz, J. S., & Debarbieux, L. (2025). Macrophage-induced reduction of bacteriophage density limits the efficacy of in vivo pulmonary phage therapy. *Nature Communications, 16*(1), 5725. Doi:10.1038/s41467-025-61268-1.

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SELENIUM NANOPARTICLES SYNTHESIZED USING *LACTOBACILLUS PLANTARUM* IMV B-7679 AND SYNERGISTIC EFFECT OF SELENIUM NANOPARTICLES-ANTIBIOTIC COMBINATIONS

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Abstract. Selenium nanoparticles (Nano-Se) were obtained by using *Lactobacillus plantarum* IMV B-7679. In vitro studies have shown that Nano-Se possess antimicrobial properties and exhibited significant synergistic effects when Nano-Se were combined with traditional antibiotics. The results of this study confirmed that nanoselen could be a potential carrier system for cefotaxime targeting

Gram-negative and Gram-positive microorganisms. The prepared nanoparticles are considered stable because they are of biogenic origin and show uniformity in size as well as shape.
Keywords: green synthesis, selenium nanoparticles, probiotic strains, *L. plantarum* IMV B-7679, antibiotics, Selenium nanoparticles-antibiotic combinations, antimicrobial activity, Cefotaxime, disk diffusion test.

The synergistic action of antibiotics and biogenic nanoselen is one of the most promising directions in modern nanobiotechnology and pharmacology, aimed at overcoming multidrug resistance of bacteria (Ridha et al., 2024).

The purpose of our work was to obtain Nano-Se produced by *L. plantarum* IMV B-7679, to study their antimicrobial properties and synergistic action with antibiotics *in vitro* to reduce their dose during combined use and increase the sensitivity of bacteria to drugs.

The ability of *L. plantarum* IMV B-7679 to quickly reduce sodium selenite with the formation of Nano-Se has been established (Tymoshok et al., 2023).

Thus, during the cultivation of the culture, after the introduction of sodium selenite at 5 ppm by Se, after 48–72 hours, changes in the color of the suspension of *L. plantarum* IMV B-7679 were noted, and according to the data of transmissible electron microscopy, spherical Nano-Se granules measuring 150–180 nm were noted. Such selenium nanoparticles, exceeding the size of 100 nm, were called lactomicrosel. *L. plantarum* IMV B-7679, rich in bioactive metabolites including those acting as a natural antibiotic, has been proposed as a suitable candidate for the synthesis of biogenic Nano-Se.

It should be noted that Nano-Se play a vital role in overcoming antibiotic resistance (Truong et al., 2021). The antibacterial activity of Nano-Se, which was obtained with the strain IMV B-7679, was confirmed using a disk diffusion test, according to CLSI (2015). The resulting Nano-Se in an amount of 100 mg were suspended in 0.15 M NaCl to obtain a concentration of 100 mg/mL. The resulting solution of Nano-Se suspension was applied with a volume of 100 µL to discs of filter paper (ø 6 mm) and allowed to dry for use in the test. Using disco-diffusion analysis, experimental Nano-Se nanoparticles demonstrated significant antimicrobial activity against *Staphylococcus aureus* and *Eshcherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans*.

Antibacterial activity studies were conducted with both cases only Nano-Se and in Selenium nanoparticles-antibiotic combinations. It was established that *S. aureus* UKM B-918 (ATCC 6538) was sensitive to selenium nanoparticles, so the growth inhibition zone was –16 ± 0.2 mm. However, *S. aureus* UKM B-4001 (ATCC 6538 P) was found to be non-sensitive because the inhibition zone was 8 ± 0.3 mm. According to the work Jahdaly Al et al. (2021) the Inhibition zone <10 mm is considered non-sensitive.

The selenium nanoparticles-antibiotic combinations for activity against *S. aureus* and *E. coli*, *P. aeruginosa*, *C. albicans* were studied. Antibacterial activity of lincomycin, kanamycin, cefotaxime against *S. aureus* strains (MSSA), *C. albicans* increases in the presence of Nano-Se. Antibacterial activity of gentamicin, cefotaxime increases in the presence of Nano-Se against *P. aeruginosa*. While Cefotaxime and Monomycin increase activity in the presence of Nano-Se against *E. coli*. The combination of Nano-Se with generation I aminoglycosides and generation III cephalosporins, a bactericidal effect that is associated with impaired cell wall protein and/or mucopeptide formation, proved promising. The ability of Nano-Se to enhance the antibacterial activity of cefotaxime, an inhibitor of cell wall synthesis, opens up new possibilities for influencing the macromolecular targets of gram-positive and gram-negative pathogens. The synergistic action of antibiotics inhibiting the synthesis of cell wall proteins and/or mucopeptides and biogenic Nano-Se *in vitro* at the nanolevel, as opposed to free antibiotics, may be an improved broad-spectrum strategy to prevent resistance mechanisms in pathogens.

References

1. Jahdaly Al, B. A., Al-Radadi, N. S., Eldin, G. M., Almahri, A., Ahmed, M. K., Shoueir, K., & Janowska, I. (2021). Selenium nanoparticles synthesized using an eco-friendly method: dye decolorization from aqueous solutions, cell viability, antioxidant, and antibacterial effectiveness. *Journal of materials research and technology*, 11, 85–97.
2. Performance standards for antimicrobial susceptibility testing: 25th informational supplement (M100-S23) Wayne PA: Clinical and Laboratory Standard Institute (CLSI); 2015.
3. Ridha, D. M., Al-Awady, M. J., Abd Al-Zwaid, A. J., Balakit, A. A., Al-Dahmoshi, H. O., Alotaibi, M. H., & El-Hiti, G. A. (2024). Antibacterial and antibiofilm activities of selenium nanoparticles-antibiotic conjugates against anti-multidrug-resistant bacteria. *International Journal of Pharmaceutics*, *658,* 124214.
4. Truong, L. B., Medina-Cruz, D., Mostafavi, E., & Rabiee, N. (2021). Selenium Nanomaterials to Combat Antimicrobial Resistance. *Molecules*, 26(12), 3611. https://doi.org/ 10.3390/ molecules 26123611
5. Tymoshok, N. O., Demchenko, O. A., Bityutskyy, V. S., Tsekhmistrenko, S. I., Kharchuk, M. S., & Tsekhmistrenko, O. S. (2023). Bionanotechnology of selenite ions recovery into nanoselenium by probiotic strains of lactobacteria and tolerance of lactobacteria to sodium selenite. *Mikrobiolohichnyi Zhurnal*, 85(4), 9–20.

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ANTIMICROBIAL ACTION AND PHYTOCHEMICAL PROFILES OF SELECTED PLANT EXTRACTS AGAINST *BACILLUS SUBTILIS*

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Abstract. This study evaluates the phytochemical profiles and antimicrobial efficacy of seven plant species (*Vaccinium corymbosum*, *Ampelopsis brevipedunculata*, *Hemerocallis fulva*, *H. citrina*, *Glechoma hederacea*, *Galinsoga parviflora*, and *Solidago canadensis*) against *Bacillus subtilis*. Antibacterial activity strongly depended on solvent choice, organ specificity, phenological phase, and extraction method. Hydroethanolic extracts of *S. canadensis* and *H. fulva* flowers produced inhibition zones (up to 20 mm), while reflux extraction optimized *A. brevipedunculata* flavonoid yield to 28.76 mg/g.
Keywords: *Bacillus subtilis*, plant extracts, flavonoids, antimicrobial activity, biotechnological potential.

Widespread antibiotic use in medicine and agriculture has accelerated antimicrobial resistance, driving the search for novel, eco-friendly plant-derived bioactive compounds. The Gram-positive, spore-forming bacterium *Bacillus subtilis* serves as a classic model for evaluating plant metabolites due to its labile cell membranes during the vegetative phase and spore-forming capability under stress (Su et al., 2020). Although natural antibacterial targets remain insufficiently explored, plant flavonoids act via mechanisms distinct from conventional antibiotics, offering potential to bypass resistance (Pandey & Kumar, 2013). Disruption of cell membrane integrity directly triggers metabolic dysfunction and cell death (Hartmann et al., 2010), though flavonoid–membrane lipophilic interactions remain a subject of debate (Sanver et al., 2016). Key flavonoid antibacterial mechanisms include enzyme

inhibition (Havsteen, 2002) and DNA gyrase blockade–either through direct DNA binding or ATP-binding site inhibition (Plaper et al., 2003). Quercetin specifically alters membrane permeability, causes physical envelope damage, suppresses protein and nucleic acid synthesis, reduces virulence factor expression, disrupts mitochondrial function, and blocks biofilm formation (Nguyen & Bhattacharya, 2022). Quercetin-loaded nanoparticles induce cell wall destruction in resistant *Escherichia coli* and *B. subtilis* (Yang et al., 2017). Furthermore, quercetin complexes with Mn^{2+}, Hg^{2+}, Co^{2+}, and Cd^{2+} exert bactericidal activity against *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *E. coli*, and *Klebsiella pneumoniae*, whereas free quercetin at identical concentrations remains inactive (Bravo & Anacona, 2001). Beyond susceptibility, *B. subtilis* can metabolically transform toxic phenols; for instance, *B. subtilis* ATCC 23857 biotransforms quercetin into apigenin-7-O-pentoside and 9 other derivatives with enhanced antitumor activity (El Gendy et al., 2025), underscoring the broader biotechnological potential of this bacterium (van Dijl & Hecker, 2013).

Aim of the study: to analyse and systematise research findings on the effects of aqueous and aqueous-ethanolic extracts of plants from the Lviv region on *Bacillus subtilis*.

Materials and methods. Research objects: *B. subtilis* (VKM B408) and plant raw materials, including *V. corymbosum*, *A. brevipedunculata*, *H. fulva*, *H. citrina*, *G. hederacea*, *G. parviflora*, and *S. canadensis*. Maceration and reflux methods were used to obtain aqueous and aqueous-ethanol extracts. Distilled water and aqueous ethanol solutions of various concentrations (20 %, 60 %, 70 %, and 96 %) were employed as extractants. The total content of polyphenols and flavonoids was determined spectrophotometrically (Yavorska et al., 2023). Antimicrobial activity was determined by the agar diffusion method (using 6 mm diameter wells) on tryptic soy agar. Inoculum standardization was adjusted to 0.5 on the McFarland scale. Ciprofloxacin (0.3 %) and pure extractants served as controls. Statistical analysis was carried out using the Jamovi 2.3 software and MS Excel.

Results. Aqueous and hydroethanolic extracts of seven plant species revealed pronounced selective sensitivity in *Bacillus subtilis*, governed by extractant polarity, extraction method, and raw material phytochemical profile. High-concentration hydroethanolic solvents (60–80 %) maximized polyphenol and flavonoid yields, directly enhancing antibacterial efficacy. In particular, *H. fulva* flower extracts and *S. canadensis* extracts formed growth inhibition zones of up to 20 mm, which directly correlated with high levels of flavonoids (254.30 ± 0.93 μg/g) and total phenols (8.55–9.92 mg/g). A clear organ specificity of biologically active substance accumulation (the activity of *H. fulva* flowers versus that of *H. citrina* leaves) and a dependence on the phenophase (peak activity of *V. corymbosum* in the post-fruit ripening phase) were revealed. In addition, the use of reflux extraction increased the yield of *A. brevipedunculata* flavonoids to 28.76 mg/g (compared to 22.33 mg/g in the case of maceration), resulting in the inhibition of *B. subtilis* up to 16 mm. At the same time, aqueous extracts of *G. hederacea* and *A. brevipedunculata* also inhibited *B. subtilis* (SIR: up to 20 mm and 16 mm, respectively).

Conclusions and future directions. The plant extracts studied exhibit a pronounced selective antimicrobial effect against *B. subtilis*, which directly correlates with the polyphenol and flavonoid content, and depends upon extractant polarity, organ specificity, phenophase and extraction method. Further work will involve investigating the effect of sub-inhibitory concentrations of these extracts on sporulation and biofilm formation.

References

1. Bravo, A., & Anacona, J. R. (2001). Metal complexes of the flavonoid quercetin: Antibacterial properties. *Transition Metal Chemistry*, 26(1), 20–23. https://doi.org/10.1023/A:1007128325639

2. El Gendy, S. N., Elmotayam, A. K., Samir, R., Ezzat, M. I., Abo-Elfadl, M. T., & El Sayed, A. M. (2025). Biotransformation of quercetin by *Bacillus subtilis* and anticancer activity evaluation: In vitro and in silico. *AMB Express*, 15(1), Article 58. https://doi.org/10.1186/s13568-025-01860-2
3. Hartmann, M., Berditsch, M., Hawecker, J., Ardakani, M. F., Gerthsen, D., & Ulrich, A. S. (2010). Damage of the bacterial cell envelope by antimicrobial peptides gramicidin S and PGLa as revealed by transmission and scanning electron microscopy. *Antimicrobial Agents and Chemotherapy*, 54(8), 3132–3142. https://doi.org/10.1128/AAC.00124-10
4. Havsteen, B. H. (2002). The biochemistry and medical significance of the flavonoids. *Pharmacology & Therapeutics*, 96(2–3), 67–202. https://doi.org/10.1016/s0163-7258(02)00298-x
5. Nguyen, T. L. A., & Bhattacharya, D. (2022). Antimicrobial activity of quercetin: An approach to its mechanistic principle. *Molecules*, 27(8), Article 2494. https://doi.org/10.3390/molecules 27082494
6. Pandey, A. K., & Kumar, S. (2013). Perspective on plant products as antimicrobials agents: A review. *Pharmacologia*, *4*(7), 469–480. https://doi.org/10.5567/pharmacologia.2013.469.480
7. Plaper, A., Golob, M., Hafner, I., Oblak, M., Solmajer, T., & Jerala, R. (2003). Characterization of quercetin binding site on DNA gyrase. *Biochemical and Biophysical Research Communications*, *306*(2), 530–536. https://doi.org/10.1016/s0006-291x(03)01006-4
8. Sanver, D., Murray, B. S., Sadeghpour, A., Rappolt, M., & Nelson, A. L. (2016). Experimental modeling of flavonoid-biomembrane interactions. *Langmuir*, 32(50), 13234–13243. https://doi.org/10.1021/acs.langmuir.6b02219
9. Su, Y., Liu, C., Fang, H., & Zhang, D. (2020). *Bacillus subtilis*: A universal cell factory for industry, agriculture, biomaterials and medicine. *Microbial Cell Factories*, 19(1), Article 173. https://doi.org/10.1186/s12934-020-01436-8
10. van Dijl, J. M., & Hecker, M. (2013). *Bacillus subtilis*: From soil bacterium to super-secreting cell factory. *Microbial Cell Factories*, 12, Article 3. https://doi.org/10.1186/1475-2859-12-3
11. Yang, X., Zhang, W., Zhao, Z., Li, N., Mou, Z., Sun, D., Cai, Y., Wang, W., & Lin, Y. (2017). Quercetin loading CdSe/ZnS nanoparticles as efficient antibacterial and anticancer materials. *Journal of Inorganic Biochemistry*, 167, 36–48. https://doi.org/10.1016/j.jinorgbio.2016.11.023
12. Yavorska, H. V., Vorobets, N. M., Yavorska, N. Y., & Fafula, R. V. (2023). Screening of anticandidal activity of *Vaccinium corymbosum* shoots' extracts and content of polyphenolic compounds during seasonal variation. *Studia Biologica*, 17(1), 3–18. https://doi.org/ 10.30970/ sbi.1701.699

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Zhylikhovska T., Hryshyna O., Tymoshok N., Lazarenko L., Spivak M.

EFFECTIVENESS OF *LACTOBACILLI* AND CERIUM DIOXIDE NANOPARTICLES IN THE TREATMENT OF INFECTED WOUNDS

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Abstract. The dynamics of reparative processes in wound tissue and the microbial status of the wounds were evaluated. Structural changes in the skin at different stages of healing were examined using histological methods. The antibacterial activity of CeO_2 nanoparticles, *L. plantarum* IMV B-8195, and the biocomposite against *S. aureus* was determined. The potential of these agents as promising components for the prevention and treatment of infectious skin lesions was investiga.
Keywords: cerium dioxide (CeO_2), *Lactobacillus*, wound, skin, *Staphylococcus aureus*.

The search for new effective agents for the local therapy of infected wounds remains one of the pressing problems of modern medicine, particularly under the conditions of the full-scale armed conflict in Ukraine. This is due primarily to the increased risk of complications in the wound process and the growing antibiotic resistance of pathogenic microorganisms. Probiotic bacterial strains, nanomaterials, and their biocomposites, which possess proven antioxidant, antimicrobial, and reparative properties, may find wide application in regenerative medicine and in the treatment of infectious-inflammatory diseases.

The aim of the study was to determine the efficacy of cerium dioxide (CeO_2) nanoparticles, the probiotic strain *Lactiplantibacillus plantarum* IMV B-8195, and a biocomposite obtained by cultivating this strain with the nanomaterial, on the healing of experimental infected skin wounds on the backs of BALB/c mice induced by *Staphylococcus aureus* B 918 (ATCC 6538).

It was established that treatment with CeO_2 nanoparticles, *L. plantarum* IMV B-8195, and the biocomposite (each individually) accelerated the healing rate of infected wounds compared with untreated wounds (control). Thus, on day 7 of the experiment, under the influence of CeO_2 nanoparticles, *L. plantarum* IMV B-8195, and the biocomposite, the area of the infected wounds decreased to 8 ± 2 mm², 13 ± 2 mm², and 10 ± 2 mm², respectively ($p < 0.05$), compared with 16 ± 2 mm² ($p < 0.05$) in untreated wounds (control). The results of histological examination confirmed the cessation of necrotic processes, preservation of epidermal structure, and active formation of granulation tissue starting from day 3 after treatment of infected wounds with CeO_2 nanoparticles, *L. plantarum* IMV B-8195, and the biocomposite.

CeO_2 nanoparticles, *L. plantarum* IMV B-8195, and the biocomposite showed high antibacterial efficacy against *S. aureus*. It should be noted that CeO_2 nanoparticles provided a rapid antimicrobial effect at the early stages of the infectious process – on days 3–7 of the experiment. *L. plantarum* IMV B-8195 and the biocomposite suppressed pathogen development and promoted restoration of normal microbial status over days 7–10 and 3–10, respectively.

The results obtained indicate that CeO_2 nanoparticles, the probiotic strain *L. plantarum* IMV B-8195, and the biocomposite are promising agents for the local therapy of infected wounds, stimulating physiological healing processes. CeO_2 and the biocomposite provide a rapid antibacterial effect during the acute phase of inflammation, while *L. plantarum* IMV B-8195 supports prolonged suppression of the pathogen. This allows these approaches to be considered as a promising alternative or complement to traditional antibiotic therapy.

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