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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Dmytruk K., Dmytruk O., Semkiv M., Vasylyshyn R., Fayura L., Sibirny A.

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

Institute of Cell Biology of the National Academy of Sciences of Ukraine,
14/16 Drahomanov St., Lviv, 79005, Ukraine
e-mail: dmytruk77@gmail.com

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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Dzanaeva L.[1], Dmytruk K.[1], Sibirny A.[1,2]

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

[1]Department of Molecular Genetics and Biotechnology, Institute of Cell Biology of the National Academy of Sciences of Ukraine, 14/16 Drahomanov St., Lviv 79005, Ukraine
[2]Institute of Biology and Biotechnology, University of Rzeszow,
4 Zelwerowicza St., Rzeszow 35-601, Poland
e-mail: liubadzanaieva@gmail.com

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