

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 B₂ 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 understanding of cellular defense mechanisms.

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