*https://doi.org/10.30970/apmicro.2026.06*

**Fedorovych D.[1], Dmytruk K.[1], Sibirny A.[1,2]**

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

*[1]Institute of Cell Biology of the National Academy of Sciences 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: fedorovych.d@gmail.com*

**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.

The work was partially performed with the support of the National Research Foundation of Ukraine, project 2023.03/0035.