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.

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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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