

Effect of biotin starvation on gene expression in industrially significant yeast *Komagataella phaffii*

Anastasia S. Makeeva, Anton V. Sidorin, Valeriia V. Ishtuganova,
Marina V. Padkina, Andrei M. Romyantsev

Saint Petersburg State University, Saint Petersburg, Russia



Komagataella phaffii (*Pichia pastoris*) is known to be an excellent producer of recombinant proteins for industrial and research purposes. Protein synthesis improvement in this yeast includes selection of optimal cultivation parameters [1, 2]. Therefore, much attention is paid to the influence of media components on physiology of this yeast [3–5].

One of the essential media components is biotin. In yeast cells it plays a crucial role as a cofactor of enzymes, providing carboxylation reactions in lipo-, gluconeogenesis, and nitrogen metabolism. *K. phaffii* is biotin auxotrophic organism unable to synthesize this vitamin *de novo*. Thus, it necessarily requires adding of biotin in the media.

In this study, we analyzed the effect of biotin starvation on gene expression in *K. phaffii* cells during its growth on methanol- and glycerin-containing media. These carbon sources are the most commonly used in standard protocols for recombinant protein biosynthesis in *K. phaffii*.

It was shown, that biotin starvation cell response significantly depends on carbon source. In glycerol-containing media biotin deficiency enhanced transcription of genes involved in biotin and thiamine metabolism, glyoxylate cycle, synthesis of acetyl-CoA in cytoplasm and its carnitine-mediated transport into mitochondria. Genes involved in biosynthesis of lipids and glucose were repressed in media with glycerol. In methanol-containing media the biotin deficiency effect was more pronounced and led to repression of numerous genes involved in protein and amino acids synthesis and activation of cell response to oxidative stress.

The obtained results are thought to be important for optimizing the culture conditions in the *K. phaffii* expression systems.

Keywords: *Komagataella phaffii*; gene expression; biotin.

REFERENCES

1. Joseph JA, Akkermans S, Cornillie E, Deberlanger J, and Van Impe JFM. Optimal culture medium selection and supplementation for recombinant thaumatin II production by *Komagataella phaffii*. *Food Bioprod. Process.* 2023;139(12):190–203. DOI: 10.1016/j.fbp.2023.04.001
2. Ergün BG, Berrios J, Binay B, Fickers P. Recombinant protein production in *Pichia pastoris*: from transcriptionally redesigned strains to bioprocess optimization and metabolic modelling. *FEMS Yeast Res.* 2021;21(7):foab057. DOI: 10.1093/femsyr/foab057
3. Ianshina T, Sidorin A, Petrova K, et al. Effect of methionine on gene expression in *Komagataella phaffii* cells. *Microorganisms.* 2023;11(4):877. DOI: 10.3390/microorganisms11040877

4. Rumiantsev AM, Padkina MV, Sambuk EV. Effect of nitrogen source on gene expression of first steps of methanol utilization pathway in *Pichia pastoris*. *Genetika*. 2013;49(4):454–460. (In Russ.) DOI: 10.7868/S0016675813040115
5. Rumjantsev AM, Bondareva OV, Padkina MV, and Sambuk EV. Effect of nitrogen source and inorganic phosphate concentration on methanol utilization and *PEX* genes expression in *Pichia pastoris*. *Scientific World Journal*. 2014;2014:743615. DOI: 10.1155/2014/743615

AUTHORS' INFO

Anastasia S. Makeeva, Research Engineer, Department of genetics and biotechnology; Saint Petersburg State University, Saint Petersburg, Russia; ORCID: 0000-0002-7181-0495; eLibrary SPIN: 1412-8449; e-mail: anastasimakeeva@mail.ru

Anton V. Sidorin, Master of Science, PhD Student, Department of Genetics and Biotechnology; Saint Petersburg State University, Saint Petersburg, Russia; eLibrary SPIN: 1056-3113; e-mail: spacerocketpilot@gmail.com

Valeriia V. Ishtuganova, Student, Department of Genetics and Biotechnology; Saint Petersburg State University, Saint Petersburg, Russia; e-mail: st086860@student.spbu.ru

Marina V. Padkina, Dr. Sci (Biol.), Professor of the Department of Genetics and Biotechnology; Saint Petersburg State University, Saint Petersburg, Russia; ORCID: 0000-0002-4051-4837; eLibrary SPIN: 7709-0449; e-mail: m.padkina@spbu.ru

Andrei M. Rumyantsev, Cand. Sci. (Biol.), Senior Researcher of the Department of Genetics and Biotechnology; Saint Petersburg State University, Saint Petersburg, Russia; ORCID: 0000-0002-1744-3890; eLibrary SPIN: 9335-1184; e-mail: rumyantsev-am@mail.ru