

Methylotrophic yeast *Komagataella phaffii* as Neoleukin producer

Anastasia S. Makeeva, Maria A. Shubert,
Ousama R. Al Shanaa, Andrei M. Rumyantsev
Saint Petersburg State University, Saint Petersburg, Russia

Methylotrophic yeast *Komagataella phaffii* (also known as *Pichia pastoris*) is widely applied in biotechnology for recombinant protein production. *K. phaffii* particularly proved to be a successful host system for the synthesis of immunomodulators such as interferons [1].

In this study, we engineered *K. phaffii* strains capable of producing the immunomodulatory protein Neoleukin (Neo-2/15). Neo-2/15 is an interleukin-2 mimetic, designed by *in silico* methods [2]. In preclinical studies on murine cancer models, Neo-2/15 showed superior therapeutic effect to interleukin-2 with reduced toxicity.

In this work, we show that *K. phaffii* can successfully synthesize and secrete Neo-2/15. We have obtained a number of *K. phaffii* strains, including Mut^s and Mut^r, with different Neo-2/15 expression cassettes integrated into the genome, carrying up to five copies of Neo-2/15 gene. In fact, the higher number of Neo-2/15 gene copies in *K. phaffii* genome allowed a higher protein yield.

In this study, we further developed a split marker approach [3] for yeast transformation using two DNA fragments, comprising of the expression cassette and the overlapping fragments of the marker gene. This allowed us to generate Mut^s strains with two copies using pPICZαB vector, which is not originally intended for Mut^s strain generation.

As a result, we demonstrated that *K. phaffii* is a perspective producer of Neo-2/15, providing wide opportunities to increase the production of this therapeutic protein.

REFERENCES

1. Tsygankov MA, Zobnina AE, Padkina MV. Synthesis of recombinant gamma interferons resistant to proteolysis in the yeast *Pichia pastoris*. *Appl Biochem Microbiol*. 2014;50(4):429–436. (In Russ.) DOI: 10.7868/s055510991404028x
2. Silva D-A, Yu S, Ulge UY, et al. De novo design of potent and selective mimics of IL-2 and IL-15. *Nature*. 2019;565(7738):186–191. DOI: 10.1038/s41586-018-0830-7
3. Heiss S, Maurer M, Hahn R, et al. Identification and deletion of the major secreted protein of *Pichia pastoris*. *Appl Microbiol Biotechnol*. 2013;97(3):1241–1249. DOI: 10.1007/s00253-012-4260-4

AUTHORS' INFO

Anastasia S. Makeeva, Research Engineer, Department of Microbiology. Saint Petersburg State University, Saint Petersburg, Russia. SPIN: 1412-8449; e-mail: anastasimakeeva@mail.ru

Maria A. Shubert, Research Assistant, Department of Genetics and Biotechnology. Saint Petersburg State University, Saint Petersburg, Russia. E-mail: marishubert01@gmail.com

Ousama Al Shanaa, Junior Researcher, Department of Genetics and Biotechnology. Saint Petersburg State University, Saint Petersburg, Russia. SPIN: 7453-9258; e-mail: st072427@student.spbu.ru

Andrei M. Rumyantsev, PhD, Senior Researcher, Department of Genetics and Biotechnology. Saint Petersburg State University, Saint Petersburg, Russia. SPIN: 9335-1184; e-mail: rumyantsev-am@mail.ru