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Feshchuk O.[1,2], Vasylyshyn R.[2], Ruchała J.[3], Nagalievska M.[1], Sibirny A.[2,3]

NOVEL POSITIVE SELECTION APPROACH FOR THE YEAST *OGATAEA POLYMORPHA*

[1]Ivan Franko National University of Lviv, 4 Hrushevsky St, Lviv, 79005, Ukraine
[2]Institute of Cell Biology of the National Academy of Sciences of Ukraine, 14/16 Dragomanov St, Lviv, 79005, Ukraine
[3]Institute of Biotechnology, University of Rzeszow, 1 Pigonia St., Rzeszów 35-310, Poland
e-mail: oleksandrf6801@gmail.com

Abstract. A novel positive selection approach for *Ogataea polymorpha* based on *CAT8* overexpression and metformin-mediated growth inhibition was investigated. Overexpression of *CAT8* conferred a growth advantage under selective conditions with glycerol as the carbon source, demonstrating the potential of this system for selection of recombinant *O. polymorpha* strains.
Keywords: *Ogataea polymorpha*, *CAT8*, metformin, positive selection, metabolic engineering.

The thermotolerant methylotrophic yeast *Ogataea polymorpha* is widely used as a model organism for studying methanol metabolism, peroxisome biology and thermotolerance and represents a promising platform for heterologous protein production and high-temperature fermentation. Further development of genetic engineering approaches for this yeast requires additional selection systems that could complement conventional antibiotic-resistance markers.

In our previous studies, metformin was identified as a potential selective agent for the development of a metabolic selection system in *O. polymorpha*. Growth of the parental NCYC495 strain on glycerol-containing medium was strongly inhibited by metformin, with complete growth inhibition observed at 50 mg/mL. Since utilization of glycerol requires an active gluconeogenic pathway, overexpression of the transcription factor gene *CAT8*, involved in the regulation of gluconeogenesis and utilization of non-fermentable carbon sources, was investigated as a basis for positive selection.

Transformation of *O. polymorpha* with a construct carrying a *CAT8* overexpression cassette restored growth under selective conditions on YNB medium containing glycerol and metformin. The presence of the target DNA sequence in the obtained transformants was confirmed by PCR. These results demonstrate that *CAT8* overexpression provides a selective growth advantage under conditions in which growth of the parental strain is inhibited by metformin.

The developed approach provides a basis for a positive metabolic selection system in *O. polymorpha* and may complement conventional selection based on antibiotic-resistance markers. Further optimization of the selective conditions and validation of the system with different genetic constructs could facilitate its application in metabolic engineering of this thermotolerant yeast.

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