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Prokopiv T., Stasyuk N., Gonchar M.

NANOZYME CAN SUBSTITUTE A NATURAL *OGATAEA POLYMORPHA* CATALASE ENZYME *IN VIVO*

Institute of Cell Biology of the National Academy of Sciences of Ukraine,
14/16 Drahomanova St., Lviv, 79005, Ukraine
e-mail: tetyanaprokopiv@gmail.com

Abstract. Nanozymes are promising artificial enzyme substitutes, but their ability to compensate for natural enzyme deficiency in living cells remains insufficiently explored. In this study, catalase-like platinum (nPt) and platinum–gold (nPtAu) nanoparticles were tested in the methylotrophic yeast *Ogataea polymorpha*. Both nanozymes exhibited catalase-like activity *in vitro*. The catalase-deficient *O. polymorpha* C-105 strain was able to grow on methanol-containing media only in the presence of nanozymes. Nanozyme treatment also reduced intracellular hydrogen peroxide levels. Transmission electron microscopy confirmed the presence of nanoparticles inside the yeast cells. These findings demonstrate that synthetic catalase-like nanozymes can functionally replace deficient natural enzymes *in vivo*.
Keywords: nanozyme; platinum nanoparticles; catalase-like activity; *Ogataea polymorpha*; hydrogen peroxide.

Reactive oxygen species (ROS) are continuously generated during cellular metabolism and play important roles in physiological redox signaling. However, excessive ROS accumulation results in oxidative stress and may damage cellular macromolecules, including DNA and proteins (Murphy et al., 2022). Catalase is an important component of the antioxidant defense system because it decomposes hydrogen peroxide into water and molecular oxygen. In methylotrophic yeasts, catalase is particularly important for growth on methanol as the sole carbon and energy source (Gonchar et al., 1990).

Nanozymes are nanomaterials exhibiting enzyme-like catalytic properties and have attracted considerable attention as potential alternatives to natural enzymes because of their high stability and tunable catalytic properties (Wu et al., 2019; Yang et al., 2021). Among them, catalase-like nanozymes are capable of decomposing hydrogen peroxide and therefore have potential applications in the regulation of intracellular oxidative stress (Xu et al., 2022). Platinum-group-element-based nanozymes are of particular interest because of their high catalytic activity, stability, and potential biomedical applications (He et al., 2021). Nevertheless, whether synthetic nanozymes can functionally replace deficient natural enzymes directly within living cells remains insufficiently investigated.

The aim of this study was to determine whether catalase-like platinum-containing nanozymes can compensate for the deficiency of natural catalase in catalase-deficient *O. polymorpha* cells. Platinum nanoparticles (nPt) were synthesized using a chemical reduction method. The resulting nPt nanoparticles were subsequently used as seeds for the

preparation of platinum–gold core–shell nanoparticles [nPt(core)Au(shell), nPtAu]. The synthesized nPt nanoparticles had sizes of 68.1 and 91.3 nm, whereas the corresponding nPtAu nanoparticles were 531.2 and 615.1 nm in size. Both nPt and nPtAu exhibited catalase-like activity in vitro, confirming their ability to decompose hydrogen peroxide (Prokopiv et al., 2023).

The catalase-deficient *O. polymorpha* C-105 strain was used to investigate whether the catalytic activity of the nanoparticles could compensate for the absence of natural catalase *in vivo*. This strain carries a defect in the *CATX* gene and is unable to grow normally under conditions requiring efficient hydrogen peroxide detoxification. Catalase-deficient mutants of methylotrophic yeasts have previously been shown to exhibit impaired regulation of alcohol oxidase synthesis and altered metabolism during growth on methanol (Gonchar et al., 1990). The synthesized nanozymes were introduced into *O. polymorpha* C-105 cells, and their effects on cell growth and intracellular hydrogen peroxide levels were investigated. In the presence of either nPt or nPtAu, the catalase-deficient yeast cells were able to grow on methanol as well as on a mixture of glucose and methanol. In contrast, no growth was observed in the absence of the nanozymes. Importantly, restoration of cell growth was accompanied by a decrease in intracellular hydrogen peroxide levels (Prokopiv et al., 2023).

The ability of nanoparticles to enter living cells is an important factor determining their biological effects. Cellular uptake of nanoparticles depends on their physicochemical properties as well as their interactions with the cell surface and intracellular structures (Behzadi et al., 2017). In the present study, transmission electron microscopy confirmed the presence of nPt and nPtAu nanoparticles within *O. polymorpha* cells, demonstrating that the nanozymes can reach the intracellular environment where hydrogen peroxide is generated. The obtained results demonstrate that synthetic catalase-like nanozymes can functionally compensate for the deficiency of a natural antioxidant enzyme in living microbial cells. The restoration of growth under methanol-containing conditions, together with the simultaneous decrease in intracellular H_2O_2 levels, indicates that the nanoparticles can at least partially substitute for the missing catalase function. These findings provide experimental evidence that nanozyme catalytic activity can be expressed functionally within living cells rather than being restricted to cell-free systems.

In conclusion, platinum and platinum–gold nanozymes were able to compensate for deficient catalase activity in *O. polymorpha* C-105 cells. The nanozyme-mediated decrease in intracellular hydrogen peroxide levels was associated with the restoration of yeast growth under methanol-containing cultivation conditions. These findings highlight the potential of catalase-like nanozymes as intracellular artificial enzyme substitutes and provide a basis for their further application in microbial biotechnology, the screening of novel nanozymes, and the development of cell-based biosensing systems.

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Semaniuk U.[1,2], Lac D.[3], Bozdag G.[3], Ratcliff W.[3], Saarikangas J.[2]

METABOLIC REMODELING DURING THE EVOLUTIONARY TRANSITION TO MACROSCOPIC MULTICELLULARITY

[1]Vasyl Stefanyk Carpathian National University,
57 Shevchenko St., Ivano-Frankivsk, 76018, Ukraine
[2]Helsinki Institute of Life Science, HiLIFE, and Faculty of Biological and Environmental Science, University of Helsinki, Helsinki, Finland
[3]School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA
e-mail: uliana.semaniuk@cnu.edu.ua

Abstract. The evolution of macroscopic body size in multicellular organisms is a key but poorly understood step in the history of life. Using the yeast long-term evolution experiment, in which selection for larger size drove clonal clusters of the budding yeast *Saccharomyces cerevisiae* to become macroscopic over more than 1,000 daily rounds of selection, we combined steady-state metabolomics, ^{13}C metabolic flux analysis, and transcriptomics to characterize the accompanying metabolic changes. We found a system-wide metabolic reallocation from growth toward maintenance, marked by depletion of amino acids, lipids, nucleotides, and central-carbon metabolites, reduced growth rate, and a sharp rise in the protective sugar trehalose. These results indicate that metabolic remodeling, and trehalose accumulation in particular, might be a major driver of the evolutionary transition to macroscopic multicellularity in this system.
Keywords: multicellularity; snowflake yeast; metabolic remodeling; trehalose; proteostasis

The evolution of multicellularity was a major step in the rise of complex life on Earth, yet our understanding of this transition is still incomplete. Progress has been hindered by the ancient origins of extant multicellular lineages, making it difficult to reconstruct the changes that accompanied the emergence of multicellularity. In particular, it remains largely unknown whether metabolic changes facilitated early multicellular evolution.

To test whether metabolism reorganizes during the transition to multicellularity, we used yeast evolved in the Multicellularity Long-Term Evolution Experiment (MuLTEE), an experimental evolution system that lets us examine this transition as it unfolds. In the MuLTEE, replicate populations of *Saccharomyces cerevisiae* founded from a common ancestor undergo daily selection for larger size through rapid settling, driving the evolution