

Generating microbes with useful properties is quicker, easier if multiple genes are modified at the same time

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Genetically engineered microorganisms with improved properties are of vital interest in the advancement of modern medicine, as well as the agriculture and food industry. Biotechnology enables modification of specific genes in an organism to produce desirable properties—for example, the ability to withstand extreme environmental conditions or to catalyze a chemical reaction—but modifying complex traits can be time-consuming and expensive due to the large number of genes involved.

Hua Zhao and co-workers at the A*STAR Institute of Chemical and Engineering Sciences have now developed a technique called error-prone whole genome amplification (WGA) that enables modification of numerous genes at the same time. To illustrate the potential of the new technique, the researchers applied it to create <u>yeast cells</u> capable of surviving high levels of ethanol.

Metabolism of ethanol in yeast is a complex trait that requires the action of 40 to 60 genes. The researchers isolated DNA from Saccharomyces cerevisiae—one of the most useful forms of yeast widely used in baking and brewing since ancient times—and copied it using the powerful polymerase chain reaction (PCR) technique that amplifies DNA sequences. The key to error-prone WGA is the introduction of random DNA copying errors through imperfect reaction conditions during PCR. The researchers established the mutagenic reaction conditions by adding gene-damaging manganese chloride to the reaction mixture in order to



produce DNA with plenty of mutations.

Zhao and co-workers introduced copies of mutated DNA back into S. cerevisiae—a process known as transformation. Normal yeast cells are capable of surviving on a medium containing 7% ethanol. The transformed cells were grown on a medium initially comprising 8.5% ethanol.

The researchers harvested DNA from cells that survived on the highethanol medium, and then repeated the error-prone PCR and transformation cycle twice. By the third cycle, cells that were able to survive on a medium containing 9% ethanol had been isolated. The method is an example of directed evolution, which uses the power of natural selection to speed up the process of adapting to changes in environmental conditions in order to develop microorganisms with properties that are biotechnologically useful.

Error-prone WGA is unique in that its direct manipulation of DNA in vitro is slower and more complex than in vivo methods. "The new method enables rapid evolution of complex phenotypes of microorganisms", says Zhao, whose team has already begun to characterize the proteins and genes in the ethanol-tolerant yeast cells using proteomic and whole genome studies. In future, error-prone WGA may also be extended to other <u>microorganisms</u>.

More information: Luhe, A. L., Tan, L., Wu, J. & Zhao, H. Increase of ethanol tolerance of Saccharomyces cerevisiae by error-prone whole genome amplification. Biotechnology Letters 33, 1007–1011

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