New discovery can improve industrial yeast strains

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Baker's yeast, Saccharomyces cerevisiae, is used industrially to produce a great variety of biochemicals. These biochemicals can be produced from waste material from the agricultural or forest industry (second-

generation biomass). During the mechanical and enzymatic degradation of biomass acetic acid is released. Acetic acid inhibits the growth and the biochemical production rate of yeast. Now, researchers at Chalmers have used high-resolution CRISPRi library screening to provide a new understanding of the stress response of yeast, and they found new target genes for the bioengineering of efficient industrial yeast.

"We are presenting a massive dataset that offers an extraordinary resolution of the functional contribution of essential genes in baker's yeast under acetic acid stress. This was never attempted before," says Vaskar Mukherjee, researcher at the Division of Industrial Biotechnology at Chalmers, first author of the study.

Yvonne Nygård is Associate Professor at Chalmers and last author of the study. She says that "in the strain library we screened, the expression of all essential genes was altered, something which was very difficult to do before the discovery of the CRISPR-Cas9-technology," she adds.

Reduced expression of essential genes using CRISPRi

CRISPR interference (CRISPRi) is a powerful tool to study cellular physiology under different growth conditions. With this derivative of the Nobel prize winning CRISPR-Cas9-technology genes are not inserted or deleted, but the regulation of the target gene can be altered. Using CRISPRi technology, the researchers can reduce the expression of the essential genes (i.e., genes that on deletion kills the organism), and thus, reduce the level of the protein encoded by the target gene.

"For most of the essential genes, this keeps the organism viable, and we also get to see the functional contribution of that gene at different expression levels under different nutrient or environmental conditions, in this case under acetic acid stress," says Vaskar Mukherjee.

Proteosomal genes involved in acidic acid tolerance

In the study a CRISPRi library consisting of more than 9,000 yeast strains was used and over 98 per cent of all essential and respiratory growth-essential genes were targeted. The results showed that fine-tuning of the expression of proteasomal genes lead to increased tolerance to acetic acid. The proteosome is protein complexes which degrade redundant or damaged proteins by spending ATP, i.e. an organic compound that provides energy to drive many processes in living cells and particular essential in large amount in yeast cells to cope with acetic acid stress.

The authors proposed that adaptation of proteasomal degradation of oxidized proteins saves ATP and thereby increases acetic acid tolerance. The results are of wide interest, suggesting these genes can be targeted for bioengineering of improved industrial cells.

"Our results allowed us to build rational mechanistic models that expand our current understanding of molecular biology of yeast under acetic acid stress. I am sure our footsteps will be followed by many researchers to screen essential genes under many other different conditions. I believe our dataset will be used by academia or industries to identify novel genetic candidates to bioengineer robust acetic acid tolerant yeast strains," says Vaskar Mukherjee."

More research on yeast and second-generation biomass

Currently, the Chalmers' researchers are working on three different projects where they use similar technologies, among them a project where CRISPRi technology is used to identify novel bioengineering genetic candidates to improve co-utilization of glucose and xylose during

biochemical fermentation using second-generation biomass.

Wild S. cerevisiae cannot metabolize xylose and a xylose utilizing engineered strain of S. cerevisiae prefers glucose over xylose as the primary carbon source. As a result, consumption of xylose is often incomplete in industrial second-generation biochemical fermentation and remains as one of the major bottlenecks for the commercial production of second-generation biochemicals.

The research was published in *mSystems*.

More information: Vaskar Mukherjee et al, A CRISPR Interference Screen of Essential Genes Reveals that Proteasome Regulation Dictates Acetic Acid Tolerance in Saccharomyces cerevisiae, *mSystems* (2021). DOI: 10.1128/msystems.00418-21

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