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engineering, and adaptive laboratory evolution. This study explored the possibility of using syngas-derived acetate or ethanol as the new carbon source for biochemical production, which would inspire the research for cost-effective biorefinery.

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IB-101

A counter-selection system exploiting phenotypic heterogeneity for improved production of glutathione from yeast

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Gene expression noise (variation in gene expression among individual cells of a genetically-uniform cell population) can result in heterogenous metabolite production by industrial microorganisms, with cultures containing both low- and high-producing cells. The presence of low-producing individuals may be a factor limiting the potential for high yields. This study tested the hypothesis that low-producing variants in yeast cell populations can be continuously counter-selected, to increase net production of glutathione (GSH) as an exemplar product. A counterselection system was engineered in Saccharomyces cerevisiae based on the known feedback-inhibition of gamma-glutamylcysteine synthetase (GSH1) gene expression, which is rate limiting for GSH synthesis: the GSH1 ORF and the counter-selectable marker GAP1 were expressed under control of the TEF1 and GSH-regulated GSH1 promoters, respectively. An 18% increase in the mean cellular GSH level was achieved in cultures of the engineered strain supplemented with D-histidine to counter-select cells with high GAP1 expression (i.e. low GSH-producing cells). The phenotype was non-heritable and did not arise from a generic response to D-histidine, unlike that with certain other test-constructs prepared with alternative markers, corroborating that the system improves GSH production by targeting low-producing cells. The results support the potential for exploiting metabolite-promoter interactions to enrich high-producing cells in phenotypically heterogeneous populations in order to improve metabolite production by yeast.

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IB-102

In vitro protein synthesis versus *E. coli* expression - a comparison using the acetate transporter SatP

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In-vitro protein synthesis uses components essential for transcription and translation to produce protein, without relying on living organisms. Compared to conventional E. coli expression, which includes cell cultivation, cell lysis, protein purification etc., in-vitro methods are less sophisticated while saving time and costs. Transferring protein synthesis from E. coli expression to an in-vitro synthesis method significantly speeds up overall protein production. In addition, in-vitro protein synthesis can benefit membrane protein expression since expressing in E. coli may cause membrane destabilization and introduce toxic effects, although differing from a native membrane environment may impact the folding and functionality of in-vitro expressed membrane proteins.

To explore the possibility of in-vitro synthesis on membrane transporters, SatP was produced from two different sources - in-vitro protein synthesis and E. coli expression, then biophysical characterization was performed on both. SatP was expressed and purified from E. coli using detergent DDM. For in-vitro synthesis, SatP was expressed in an E. coli extract-based cell-free expression system, assisted by liposomes. No specific phospholipid preference for membrane insertion was found in SatP generated from either method. However, when acetate transport activity was measured by single-channel recording, a lower transport activity was observed from SatP expressed in-vitro.

In conclusion, in-vitro synthesis is a fast protein synthesis approach compared to E. coli expression. However, in the case of SatP, in-vitro synthesis compromised protein quality and resulted in lower protein activity. Further studies characterizing the compromised activity from in-vitro synthesized SatP might be helpful to the development of in-vitro protein synthesis.

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IB-103

Cellular responses of *Cupriavidus necator* H16, a bioplastic producer, to organic acids: a comparative analysis

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Cupriavidus necator H16 serves as a model organism for polyhydroxyalkanoate (PHA) polyester production. PHA, a biodegradable, biocompostible, and biocompatible thermoplastic material, can be synthesized by C. necator with remarkable efficiency, accounting for up to 90% of its dry cell weight. The organism demonstrates versatility in utilizing various carbon sources, including carbohydrates, glycerol, organic acids, and C1 compounds like CO_2. To enhance the economic viability of bioplastics synthesized by microorganisms, the exploration of low-cost and renewable carbon feedstocks such as lignocellulosic hydrolysate becomes crucial. However, these feedstocks often contain microbial inhibitors, among which aliphatic and aromatic organic acids are notable. In this study, we conducted a comparative growth analysis of C. necator across four organic acids –lactic acid, butyric acid, benzoic acid, and 3-hydroxypropionic acid –across a spectrum of concentrations. This examination was juxtaposed with cultivation in the absence of organic acids. Subsequently, label-free quantitative proteomics was employed to discern up- and down-regulated genes, along with pathway enrichment analysis. The overarching objective of this study encompassed three important aspects: Firstly, to ascertain if the investigated organic acids are substrates for C. necator. Secondly, to unravel the shared and acid-specific cellular responses elicited under organic acid stress conditions. Thirdly, to formulate a strategic framework for strain engineering aimed at enhancing the resilience of C. necator to organic acids, thus facilitating broader applications within industrial biotechnology. The nitrogen regulatory protein GlnK exhibited significant up-regulation across all tested organic acids, underscoring its crucial physiological function in orchestrating the cellular response to organic acid stress.

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