A GROWTH SELECTION SYSTEM FOR THE DIRECTED EVOLUTION OF SUCROSE SYNTHASES

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UDP-glucose is a common sugar donor in nature for the biosynthesis of diverse glycosides[1,2] with commercial potential in the food, health and cosmetic industries among others. Chemical methods for UDP-Glc synthesis have been developed due to its high cost and difficulty in extraction from natural sources. However, the up-scaling of UDP-Glc synthesis by chemical catalysis is hindered by lengthy reaction times and multistep procedures[3]. Biotechnological approaches have focused on sucrose synthases (SuSys). These enzymes catalyze the conversion of sucrose and UDP into UDP-Glc and fructose, allowing the recycling of the expensive sugar donor in biocatalytic glycosylations with catalytic amounts of UDP and inexpensive sucrose as the ultimate glucose donor[4]. However, the limited stability[4] of SuSy limits its practical applications in industrial-scale biocatalysis.

SuSy engineering is limited by the lack of a high-throughput screening method. Incremental improvements to half life have been achieved[5]. Here, we report a growth selection system based on SuSy activity to address the efficient screening of large mutant libraries. The bacterial platform is based on an *E. coli* strain evolved for sucrose utilization[6], which was further engineered to avoid sucrose hydrolysis through the invertase CscA. When growing on minimal media with sucrose as the only carbon source, this strain relies on SuSy activity to produce fructose and sustain growth. Future applications of this platform will be focused on improving both actvitity and stability.

References:

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