Exploring plasma membrane transporters to improve organic acid production in yeast – Characterization and engineering

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Organic acids are industrially relevant building-block chemicals obtainable from renewable feedstocks by utilization of microbial cell factories. With a wide variety of applications, including bioplastics synthesis, microbially produced organic acids have the potential to replace petroleum-derived commodity chemicals that are obtained through unsustainable production processes. Yeasts commonly represent the organisms of choice for production of organic acids, namely due to their tolerance of low pH environments, since such production conditions allow for direct formation of the desired protonated form of the acid and thus cut downstream processing costs. Efficient product export over the plasma membrane in such conditions is particularly demanding, therefore expression of membrane transporters with adequate substrate specificity and transport mechanism is often the determining factor at acquiring competitive product titres. Here, we are characterizing and engineering plasma membrane transporters with the final aim to improve production of dicarboxylic acids, namely succinic acid, in yeast. This includes transporters that have already been described as efficient dicarboxylate transporters, as well as promising transporters from the AceTr family. First, we perform functional characterization by studying transporter kinetics, energetics and specificity, as well as site-directed mutagenesis, to acquire insight into functional-structural relationship of transporters. This insight further uncovers engineering targets that can lead to improved transporter activity as well as altered substrate specificity. Finally, the performance of these transporters can be assessed via their expression in *S. cerevisiae* that is engineered for succinic acid production.