Engineering of carboxylation in *Saccharomyces cerevisiae*: PEPCK and malic enzyme

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Pyruvate carboxylase is the sole anaplerotic enzyme in glucose-grown cultures of wild-type *Saccharomyces cerevisiae*. Pyruvate carboxylase-negative (Pyc^-) *S. cerevisiae* strains cannot grow on glucose unless media are supplemented with C_4 compounds, such as aspartic acid. In several succinate-producing prokaryotes, phosphoenolpyruvate carboxykinase (PEPCK) fulfills this anaplerotic role. However, the *S. cerevisiae* PEPCK encoded by *PCK1* is repressed by glucose and is considered to have a purely decarboxylating and gluconeogenic function. This study investigates whether and under which conditions PEPCK can replace the anaplerotic function of pyruvate carboxylase in *S. cerevisiae*. Pyc^- *S. cerevisiae* strains constitutively overexpressing the PEPCK either from *S. cerevisiae* or from *Actinobacillus succinogenes* did not grow on glucose as the sole carbon source. The inability of pyruvate-carboxylase-negative (Pyc^-) *S. cerevisiae* strains to grow on glucose suggested that malic enzyme (Mae1p) cannot act as a pyruvate-carboxylating, anaplerotic enzyme. In this study, relocation of malic enzyme to the cytosol and creation of thermodynamically favorable conditions for pyruvate carboxylation by metabolic engineering, process design and adaptive evolution, is investigated to enable malic enzyme to act as sole anaplerotic enzyme in *S. cerevisiae*. Carboxylation though PEPCK or malic enzyme produces one ATP per carboxylation event, whereas the original route through pyruvate kinase and pyruvate carboxylase is ATP neutral. This increased ATP yield may prove crucial for engineering in *S. cerevisiae* of efficient and low-cost anaerobic production of C_4 dicarboxylic acids and other compounds that require carboxylation.