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Citric acid is one of the most important organic acids produced by fermentation with the filamentous fungus *Aspergillus niger* using glucose as substrate. This organic acid is extensively used in food, cosmetic and pharmaceutical industries with a production capacity of nearly 2 million tons per year (Steiger et al., 2017). Citric acid is produced in the tricarboxylic acid cycle (TCA) from oxaloacetate and acetyl-CoA. In glycolysis, 1 mol glucose is oxidized and converted into 2 mol of pyruvate. 1 mol of pyruvate is carboxylated to oxaloacetate, the other is decarboxylated to acetyl-CoA, resulting in the net reaction:

$$\text{Glucose} + 3\ NAD^+ + H_2O \rightleftharpoons \text{Citrate} + 3\ NADH$$

While this pathway leads to a theoretically balanced carbon yield it is not redox balanced and re-oxidizing the NADH leads to high oxygen consumption and heat release (Karaffa & Kubicek, 2003). Mixed-substrate conversion allows to incorporate CO_2, a cheap carbon source, into products (e.g. organic acids) with higher oxidation states than the co-substrate (e.g. glucose). This is a promising strategy to fix CO_2 in an industrial process and increase the total carbon yield of the process without requiring oxygen as an electron acceptor, hence reducing the need for extensive cooling (Steiger et al., 2017).
The aim of this research is to increase the carbon efficiency of citric acid production by developing a synthetic pathway that avoids decarboxylation, hence leading to a net CO_2 assimilation during the mixed-substrate production of citric acid. The pathway, expressing the respective genes under control of methanol regulated promoters, is being incorporated into the yeast *Komagataella phaffii* (*Pichia pastoris*) to create an orthogonal test system. Preliminary results of this research have shown that the citric acid transporter genes of *Yarrowia lipolytica* and *Aspergillus niger* were successfully expressed in *K. phaffii* (Fig.1). After evaluation, the best pathway variants will be transferred into *A. niger*.

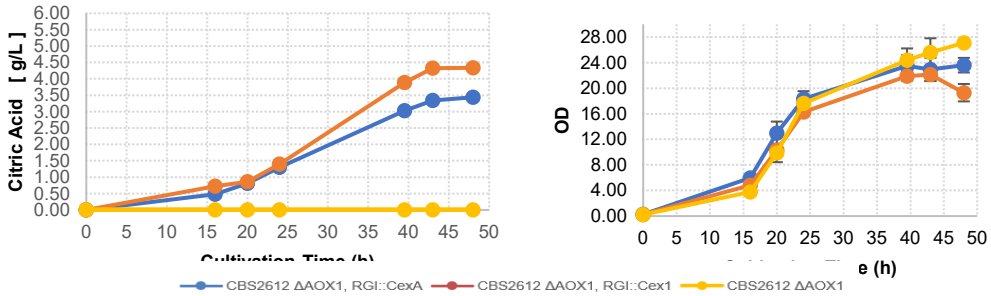

Figure 1: Growth and extracellular concentration of citric acid in Pichia pastoris strains overexpressing the citrate exporter Cex1 from Yarrowia lipolytica or CexA from Aspergillus niger.

References:
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