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Decompartmentalization of the Yeast Mitochondrial Metabolism to Improve Chemical Production in *Issatchenkia orientalis*

Background/Objective

Metabolic pathways are typically localized in the cytosol in yeast-based metabolic engineering. However, the production of highly reduced chemicals from glucose is often limited by an inadequate provision of reducing power in the cytosol, due to the highly compartmentalized nature of cofactor metabolism. Here, we performed cofactor engineering through the decompartmentalization of mitochondrial metabolism, redistributing mitochondrial functions to the cytosol to enhance succinic acid (SA) production in *I. orientalis*.

Approach

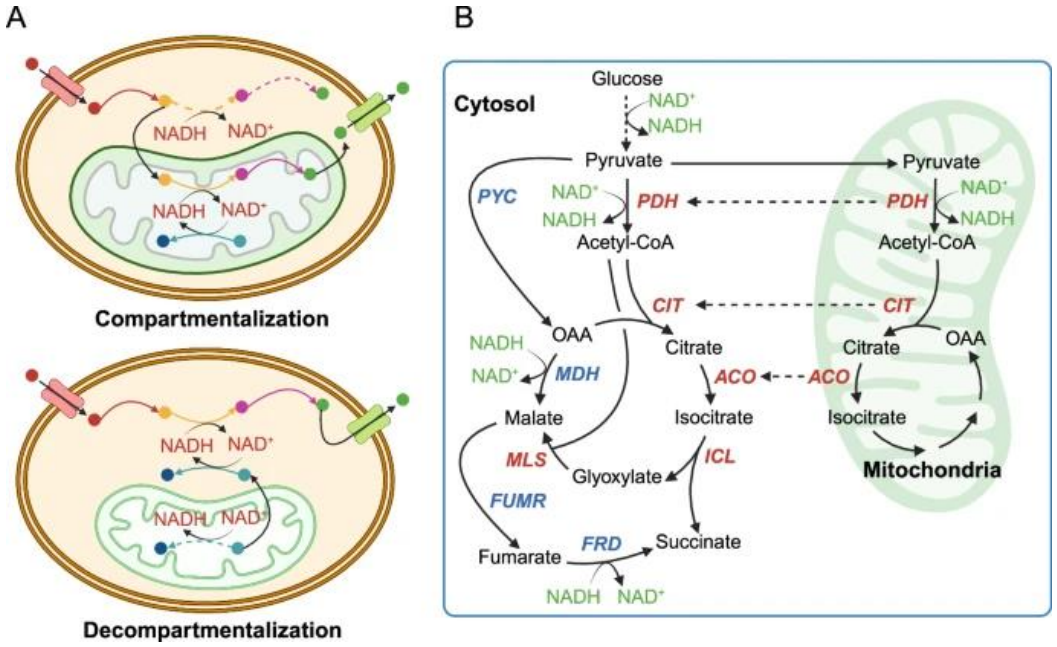
We localize the reducing equivalents of mitochondrial NADH to the cytosol through cytosolic expression of its pyruvate dehydrogenase (PDH) complex and couple a reductive tricarboxylic acid pathway with a glyoxylate shunt, partially bypassing an NADH-dependent malate dehydrogenase to conserve NADH.

Results

Cytosolic SA production reached a titer of 104 g/L and a yield of 0.85 g/g glucose, surpassing the yield of 0.66 g/g constrained by cytosolic NADH availability. Additionally, cytosolic PDH expression enhanced acetyl-CoA-derived citramalic acid and triacetic acid lactone production by 1.22- and 4.35-fold, respectively.

Significance/Impacts

This work establishes *I. orientalis* as a versatile platform to produce markedly reduced and acetyl-CoA-derived chemicals.



A) An illustration of compartmentalization vs. decompartmentalization. Dots indicate metabolites; arrows indicate transport or enzymatic reactions. B) A schematic diagram for enhanced SA production by cofactor engineering.

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