Many relevant options to improve efficacy and kinetics of sucrose metabolism in Saccharomyces cerevisiae and, thereby, the economics of sucrose-based processes remain to be investigated. An essential first step is to identify all native sucrose-hydrolysing enzymes and sucrose transporters in this yeast, including those that can be activated by suppressor mutations in sucrose-negative strains. A strain in which all known sucrose-transporter genes (MAL11, MAL21, MAL31, MPH2, MPH3) were deleted did not grow on sucrose after 2 months of incubation. In contrast, a strain with deletions in genes encoding sucrose-hydrolysing enzymes (SUC2, MAL12, MAL22, MAL32) still grew on sucrose. Its specific growth rate increased from 0.08 to 0.25 h⁻¹ after sequential batch cultivation. This increase was accompanied by a 3-fold increase of in vitro sucrose-hydrolysis and isomaltase activities, as well as by a 3- to 5-fold upregulation of the isomaltase-encoding genes IMA1 and IMA5. One-step Cas9-mediated deletion of all isomaltase-encoding genes (IMA1-5) completely abolished sucrose hydrolysis. Even after 2 months of incubation, the resulting strain did not grow on sucrose. This sucrose-negative strain can be used as a platform to test metabolic engineering strategies and for fundamental studies into sucrose hydrolysis or transport.

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