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CENTER FOR ADVANCED BIOENERGY AND BIOPRODUCTS INNOVATION

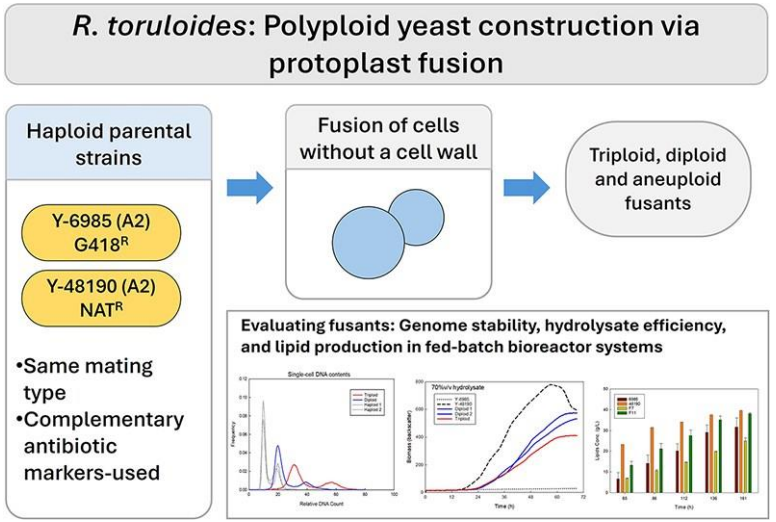
Protoplast Fusion as a Strategy to Increase Ploidy in *Rhodotorula toruloides* for Strain Development

Background/Objective

Rhodotorula toruloides has growing commercial interest because of its hardiness and exceptional lipid production capacity. Because it is a basidiomycete yeast with a complex life cycle, many classical breeding methods used with ascomycetes are unavailable for strain improvement, and previous studies of breeding methods are outdated and incomplete. Our goal was to develop a reliable protoplast fusion protocol, then measure how increased ploidy affects lipid production and genomic stability, to serve as another tool for strain-development.

Approach

Two diploids and one triploid of *R. toruloides* were constructed by fusing protoplasts from two parents with the same mating type, Y-6985 (A2) and Y-48190 (A2), which had been transformed with complementary antibiotic markers. Their stability was tested on non-selective medium after several growth cycles under antibiotics, and flow cytometry and standard cell cycle analysis confirmed their DNA contents. Mitochondrial restriction fragment length polymorphism (RFLP) analysis showed fusants inherited mitochondria from a single parent. The phenotypic properties of the parents and fusants were compared in glucose fed-batch bioreactor studies and cellulosic sugar batch cultures.



Constructing better *Rhodotorula* yeast strains for industrial use by fusing together two parental strains.

Results

Lipid titers in fed-batch cultures produced 25–40 g/L, with Y-6985 and the diploid and triploid performing the best and worst, respectively. The fusants demonstrated intermediate growth hardiness on switchgrass hydrolysate pretreated with dilute-acid and were outperformed by Y-48190. Unlike one haploid parent, the fusants grew in 70% v/v hydrolysate, though not as fast as the other parent.

Significance/Impacts

This modernized protoplast fusion method is a useful tool that expands strain development in *R. toruloides*.

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