Determining Mating Type and Ploidy in *Rhodotorula toruloides* and its Effect on Growth on Sugars from Lignocellulosic Biomass

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Background/Objective

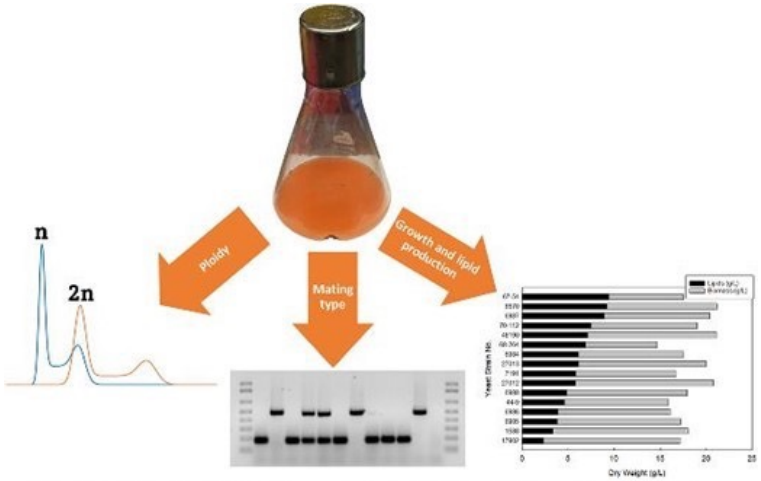
Rhodotorula toruloides has favorable physiology for industrial biotechnology processes, such as an ability to store large amounts of lipids as intracellular lipid bodies and to microbially convert lignocellulosic-based sugars into fuels and chemicals. An important area of continuing research is identifying strains that are robust for growth and product yield on unrefined hydrolysate sugars, with a focus on inhibitor tolerance. Evidence suggests diploids can be more rugged than haploid strains for growth in hydrolysate. Here we seek to clarify how ploidy affects growth and lipid production in *R. toruloides*.

Approach

Nineteen strains were characterized for mating type (A1, A2, A1/A2), ploidy (haploid, diploid/aneuploid, triploid), robustness for growth, and lipid accumulation on inhibitory switchgrass hydrolysate (SGH). Mating type was determined using a novel PCR-based assay and validated using a classical microscopic test, ploidy was determined by flow cytometry, and growth and production performance were measured by growth experiments on different hydrolysate concentrations.

Results

A diverse set of mating types and ploidy in *R. toruloides* strains were identified. Three strains were heterozygous for mating type (A1/A2), and the novel PCR assay method provided faster and more accurate identification than microscopy. Strains exhibited different growth patterns and lipid production abilities on SGH, and four strains had significantly higher lipid yields, one of which was not previously described in the literature. Further experiments revealed that certain A1/A2 strains outperformed their parent strains, showing enhanced robustness towards inhibitors present in SGH.



***R. toruloides* yeast strains were characterized for ploidy, mating type, and growth and lipid production on switchgrass hydrolysate.**

Significance/Impacts

More accurate identification and characterization of mating types and ploidy variation in *R. toruloides* strains, aided by the new assay to determine mating type and paired with a better understanding of robustness and growth of under stressful conditions such as hydrolysate sugars, informs better engineering decisions for yeast strains for biofuel and chemical production.

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