Oxidative Pentose Phosphate Pathway is the Major Cytosolic NADPH Source in *Rhodosporidium toruloides*

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Project Goals: Understand the metabolic pathways in *R.toruloides*.

Understanding the native metabolism of microbes is important for facilitating metabolic engineering efforts. *Rhodosporidium toruloides* is a promising yeast for fatty-acids production, but relatively understudied. Nitrogen limitation elevates *R. toruloides*' already high native production of fatty acids. Fatty acid production causes a high demand for cytosolic NADPH. Here we investigated the source of cytosolic NADPH in *R. toruloides*. Specifically, we employed a ^{2}H-glucose strategy to trace directly the source of the redox-active hydride of NADPH, an approach that had not previously been applied to any yeast. This strategy requires complementary deuterated water tracing experiments to determine the extent of ^{1}H-^{2}H exchange in NADPH, which otherwise leads to underestimation of pathway contributions. By this approach, we find that the oxidative pentose phosphate pathway (oxPPP) contributes most of *R. toruloides*' cytosolic NADPH. The oxidative pentose phosphate pathway contribution is equivalent in both batch growth and N-limitation. 1,2-^{13}C-glucose tracing data indicate that the ratio of oxidative pentose phosphate pathway flux to glycolysis is also similar in these two conditions. These data suggest a shift during nitrogen limitation in NADPH utilization from reductive nutrient assimilation to fat synthesis. These observations lay foundation for future efforts to enhance fatty acid production in *Rhodosporidium* species via metabolic engineering.

References

1. Zhang, Z., Chen, L. *et al.* Chemical basis for deuterium labeling of fat and NADPH. *JACS,* **139**, 14368-14371 (2017).