ENGINEERING XYLOSE METABOLISM IN THRAUSTOCHYTRID T18

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Thraustochytrids spp are oleaginous marine protists with significant potential for biofuel production at industrial levels; however, the cost of feedstocks has been a major challenge in making this process economical. On a quest for cheaper and renewable feedstocks, we investigated the ability of Thraustochytrid strain T18 to grow in the presence of xylose and demonstrated its ability to produce xylitol. However, genome sequencing and in vivo enzymatic assays revealed the presence of a xylose isomerase which indicates there are two xylose metabolism pathways in Thraustochytrid T18: a xylose reductase/xylitol dehydrogenase pathway as well as an isomerase pathway. Characterization of the two native pathways suggested that xylitol production is a bottleneck to T18 xylose metabolism. Through various strain improvement strategies, including over-expression of the endogenous xylose isomerase and heterologous xylulose kinases, we enhanced xylose usage while reducing xylitol production by >50% and 80%, respectively, compared to wild-type. Highest levels of xylose metabolism were obtained through selection of strains possessing multiple copies of the transgenes. The xylose usage of the best xylose metabolizing isolate was further validated through fermentation. These newly engineered strains pave the way to using T18 for biofuel production using hemicellulosic feedstocks.