Title: Investigation of xylose metabolism in *Rhodosporidium toruloides* using a modular cloning kit (RT-EZ)

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Project Goals: Understanding xylose metabolism in *Rhodosporidium toruloides*, and enhancing xylose utilization efficiency using a modular cloning kit

Abstract Text:

Rhodosporidium toruloides is an oleaginous yeast strain, which has the ability to utilize diverse kinds of carbon sources including glucose, xylose, fructose, xylitol, arabitol, galactitol and etc. [1]. However, its preference is highly biased on glucose, showing slower consumption rate and growth rate (50% ~) under different carbon source such as xylose. In addition, significant amount of arabitol is generated as a byproduct when xylose is provided as a sole carbon source, which is re-consumed after depletion of xylose in the media. Hence, this study aimed to improve the xylose utilization efficiency of *R. toruloides*, which would allow better growth of the strain in hydrolysates containing xylose as well. First of all, in order to reduce the troubles often caused by high GC-contents (62.01%) of *R. toruloides* during vector construction, we developed a toolkit (RT-EZ) composed of genetic modules that allows hierarchical assembly based on Golden Gate cloning prior to actual cloning [2]. The toolkit contains uni-/bi-directional promoters, antibiotic markers, terminators, 2A linkers and etc. Using the toolkit, heterologous *Xyl1*, *Xyl2*, and *Xyl3* genes from *Pichia stipitis*, encoding xylose reductase (*p*XR), xylitol dehydrogenase (*p*XDH), and xylulose kinase (*p*XK), respectively, were expressed in *R. toruloides*. Although expression of *p*XR or *p*XDH did not show much difference compared to the parental strain, expression of *p*XK resulted in improved growth rate with doubled maximum growth rate. Surprisingly, the accumulation of arabitol, which reached up to 15 g/L in parental strain, was completely removed from the *p*XK expressing strain as well, implying improved overall sugar consumption rate. The low expression level of endogenous XK in *R. toruloides* with xylose [3] suggests malfunction, mis-annotation or lack of the XK activity, explaining the distinct effect of *p*XK expression. We believe that our findings will help understanding the xylose metabolism in *R. toruloides*, and also the developed toolkit to boost the research on this high-potential oleaginous yeast strain.

References/Publications

1. *Yaegashi* et al, *Biotechnol. Biofuels*, 2017, 10:241
2. *Weber* et al, *Plos one*, 2011, 18:6(2)
3. *Jagtap* et al, *Appl Microbiol Biotechnol*. 2021, 105(19)

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