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Title: Evaluation of the promoters of the mevalonate biosynthetic pathway genes of the red yeast

Rhodosporidium toruloides

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Abstract:

The mevalonate biosynthetic pathway (MVA) or the Isoprenoid pathway is essential in mammals, plants, and yeast to synthesise sterols and isoprenoids. There are also many valuable terpenoids that are produced from these pathways in different plant organisms. Rhodosporidium toruloides is emerging as a potential host organism for heterologously producing these terpenoids and isoprenoids. However, few tools are available to modify this yeast genetically. Also, there is limited knowledge regarding promoters and their regulation in R. toruloides in the MVA pathway. The goal was to evaluate R. toruloides promoters in R. toruloides and S. cerevisiae and to find any possible bifunctional promoter that may work in both yeasts. As part of this project, in silico work was followed by experiments towards this goal. In silico work was carried out to compare the promoters by phylogenetic footprinting to identify conserved motifs. Several promoters of the mevalonate pathway of R. toruloides were cloned, and evaluated in S. cerevisiae, followed by a directed evolution approach to make functional Rt HMG1 promoter in S. cerevisiae. We also evaluated selected promoters for their function in the native host, R. toruloides. Promoter deletions were also created of the RtHMG1 promoter and preliminary evaluation of a few of them was carried out. The GFP reporter was used to evaluate promoter strength of MVA pathway genes. The modified R. toruloides vector which was also developed in this project, would be helpful as a genetic engineering tool in R. toruloides based research.

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