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- / Master of Science (/jspui/handle/123456789/2)
- / MS-10 (/jspui/handle/123456789/447)

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Title: Identifying genes to increase flux in the isoprenoid pathway in S. cerevisiae

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Keywords: Isoprenoids

Biology

Organic Compounds

Enzymes DNA E. coli

Issue Date: 29-Jul-2015

Publisher: IISER M

Abstract:

Isoprenoids (also called terpenoids) are a large and diverse class of naturally occurring organic compounds derived from five carbon isoprene units. Previous research in the lab has led to cloning and expression of the carotenoid producing enzymes of the red yeast in S. cerevisiae to yield a red pigmented strain. Current efforts are to identify genes and mutants which increase the flux in the isoprenoid pathway. In the present study, we have used two approaches towards this goal. The first approach is to find out the SNPs in natural isolates of S. cerevisiae that can lead to increase in flux by finding out SNPs that lead to increased pigmentation. The second approach is to develop a color based assay by exclusive biosynthesis of lycopene as a marker for identification of genes that increase flux in the isoprenoid pathway in S. cerevisiae. In the first approach, SNPs of different natural isolates of S. cerevisiae were examined for their impact on carotenoid pigmentation and a few candidate genes were examined. In the second approach, since mixtures of carotenoids interfere in a genetic screen (based on previous work in the lab), we developed a screen wherein only lycopene was biosynthesized by heterologous expression of A. thaliana phytoene synthase (AtPS) gene along with R. toruloides geranylgeranyl diphosphate synthase (RtGGPPS) and phytoene dehydrogenase (RtCRTI) gene. We show in this strain, a significant increase in the pigmentation level with over expression of tHMG1 (a known flux increasing gene) which validates genetic screen.

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