Title:	Attempts to identify folate transporters in saccharomyces cerevisiae
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Abstract:	Folate is an important micronutrient for all the organisms. Many prokaryotes and eukaryotes can synthesize folates de novo, but humans require a dietary intake of folates. Folate uptake in human cells is mediated by transporters on the plasma membrane namely SLC46A1 and SLC19A1, but there are no known homologs in yeast. Although yeast can synthesize folates, it can also take folates exogenously, suggesting the presence of folate transporters. Yet, folate transporters have not been identified in yeast. So, the study aims to identify folate transporters in yeast. As there were no detectable homologues of SLC19A1 and SLC46A1 in yeast, a strategy was designed making use of synthetic lethality of yeast fol2 mutant with putative transporter deletion mutants. The FOL2 gene of Saccharomyces cerevisiae encodes the enzyme GTP cyclohydrolase. Hence fol2Δ was to be created in a library of putative folate transporter deletion strains ordered from EUROSCARF. The fol2Δcassette was improved by increasing the length of the homologous region for better in vivo recombination in yeast. Yeast spotting assay was performed to examine if any of the double deletion strains exhibited growth defectsin suboptimal concentrations of folinic acid ranging from nil, suboptimal and optimal folinic acid concentrations. With fol2 deletion background, the strains with ORFs YER039cΔ, YER060w-aΔ, YJL163cΔ, YOR306cΔ, YOR071CΔ, YBR132CΔ, YBR220cΔ, YCL049cΔ, YNR062cΔ, YML038cΔ, YGL186cΔ, YDR406wΔ, YPL274wΔ, YGR260wΔ, YOL162wΔ, YIR028WΔ showed distinct slow growth compared to BY4741 with fol2Δ.One possible explanation for the result is that there might be more than one folate transporter in yeast. Insilico analysis of the promoters of the major genes of folate biosynthesis pathway, namely FOL1, FOL2, FOL3, MTD1, DHFR, MET7 was performed for four Saccharomyces species i.e. Saccharomyces cerevisiae, Saccharomyces paradoxus, Saccharomyces mikatae, Saccharomyces bayanus to identify some conserved boxes. Though some conserved regions were identified no region was conserved across the genes highlighting that the genes are regulated via different modes if at all any regulation exists. Further narrowing down to FOL2 gene promoter to check it is regulated in the presence of folinic acid. 800bp upstream of FOL2gene was cloned under lacZ reporter gene. Currently, I am testing it for positive clones; the cloning is ongoing, and assay will be performed after that.
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