Title:	Establishing the role of the conserved regulatory domain of arabidopsis thaliana MTHFR & Quantifying the oxidative stress responses in articulospora tetracladia
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Abstract:	This thesis is divided into two parts. The first part aims to establish the role of the conserved regulatory domain of AtMTHFR. Methylenetetrahydrofolate reductase (MTHFR) catalyzes the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate and is known to be allosterically inhibited by S-adenosylMethionine (SAM) at its regulatory domain, which is conserved across species. Previous studies from the lab found a highly conserved seven amino acid stretch in the regulatory domain. Mutations in this region lead to SAM insensitivity with mutants exhibiting a growth defect on methionine media. Despite this conservation, Arabidopsis thaliana MTHFR (AtMTHFR) is SAM insensitive. Also, it contains single point asparagine to histidine or glutamine mutations at the 355th position which lies in the seven amino-acid stretch. So, a histidine to asparagine and glutamine mutation was created in AtMTHFR and yeast MTHFR (MET13) at the 355th position. Both the mutants complement growth in met13Δ but not in met13Δmet15Δ. Major portions of the mutant proteins appeared in the insoluble portion and could not be purified. The purification of these proteins is needed for an in vitro enzymatic assay to be performed which will give us better insight into the regulatory domain of AtMTHFR1. The second part of this thesis pertains to the quantification of stress responses in Articulospora tetracladia, a species belonging to aquatic hyphomycetes. These freshwater fungi are usually found in cool and unpolluted streams; however, many species have been found in streams polluted with heavy-metals. The presence of these metals has been correlated to increased ROS production and programmed cell death. Carotenoids have been known to provide chemical reactivity against free radicals and act as a stress-protectant. The presence of adaptive mechanisms like accumulation of carotenoids in providing tolerance to such species was hypothesized. The presence of higher ROS content was observed in strains isolated from arsenic-rich streams in comparison to others. Upon stress induction by nitrates, strains from low-arsenic streams showed a higher increase in the production of ROS in comparison to the strains from Ar-rich waters. The stress responses induced in these strains were also quantified using HPLC for carotenoid detection. However, the low amount of carotenoids in samples could not be detected with HPLC. The samples need to be concentrated for the detection and quantification of fungal carotenoids. This would provide a better insight into the adaptive mechanisms adopted by aquatic hyphomycetes to combat oxidative stresses.
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