

Evidence of horizontal gene transfer of carotenogenesis in the heterotrophic stramenopile, *Aurantiochytrium limacinum*

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Carotenogenesis, the biosynthetic pathway by which carotenoids are produced, is found across all three domains of life and among all photosynthetic organisms. Within the Stramenopilian branch of the eukaryotes, thraustochytrids are the only heterotrophic group known to produce carotenoids. Thraustochytrids are ubiquitous marine heterokonts important in supporting oceanic food webs through *de novo* omega-3 polyunsaturated fatty acid production. Interestingly, unlike its closest carotenoid-producing relatives (e.g. photosynthetic diatoms and brown algae), the thraustochytrid, *Aurantiochytrium limacinum*, has a single fused gene (Aurli150841) encoding three carotenogenesis-specific proteins (phytoene synthase, phytoene desaturase, and lycopene cyclase). This striking difference in gene structure and metabolic mode raises the question regarding the evolutionary origins of thraustochytrid carotenogenesis: did thraustochytrids inherit carotenogenesis from a common ancestor with photosynthetic stramenopiles or by horizontal gene transfer (HGT)? We used phylogenetic analyses of each domain to address this question. The squalene/phytoene synthase domain (SQS_PSY): PF00494 identified in EMBL-EBI's Protein Family Database (PFAM) was used in conjunction with HMMER (hmmsearch) to extract domains from a comprehensive proteome database based on UniProt, MMETSP, JGI, BROAD, and other sources. Resulting protein sequences were classified into phytoene synthase (OG5_131363, 52%), squalene synthase (OG5_129470, 26%) or other orthologous groups by OrthoMCL. For each group MUSCLE version 3.6 was used to make an alignment and FastTree version 2.1.9 SSE3 was used in constructing phylogenetic and constrained phylogenetic trees. Our phylogenetic analyses demonstrate that *A. limacinum*'s phytoene synthase domain is most closely related to that of Actinobacteria. The hypothesis of carotenogenesis acquisition resulting from horizontal inheritance was statistically tested using the Approximately Unbiased (AU) test as well as several others. These results support the rejection of the null hypothesis (vertical inheritance of carotenogenesis), and therefore uphold the likelihood of the introduction of the carotenogenesis to thraustochytrids via horizontal gene transfer.