Biosynthesis of long chain aliphatic compounds from Nannochloropsis spp.

Director of Studies: Sergio Balzano

Department: Marine Biotechnology

Project Summary

Nannochloropsis spp. contain biosynthetically related primary/secondary bifunctional aliphatic compounds consisting of 26-34 carbons, the long chain hydroxy fatty acids (LCHFAs) and long chain alkyl diols (LCDs). Both compound classes might encounter some biotechnological applications for the development of fuel lubricants and polymers. *Nannochloropsis* spp. are already considered suitable biofuel candidates because of their high growth rate and lipid content, the use of LCHFAs along with ordinary C_{14-18} fatty acids as substrates for methanol transesterification might provide a better quality biodiesel fuel. Furthermore, since petrochemical diols are currently used by the plastic industry for the synthesis of aliphatic carbonates, polyesters, and polyurethanes, and diols from terrestrial plants are being tested as suitable alternatives, LCDs from *Nannochloropsis* might also be considered as starters for polymer synthetic processes. Furthermore, LCDs are typically present in the outer cell wall of *Nannochloropsis* spp. as part of an ether-bond polymer known as algaenan. The biosynthetic pathways of LCDs and LCHFAs have been recently predicted by combining culturing experiments, stable isotope incubation, genome and transcriptome analyses, although the mechanisms for polymerization into algaenans are still unclear. LCDs are thought to derive from the reduction of the carboxylic group of LCHFAs which, in turn, are likely to be formed after 6-7 elongation cycles of C_{16-18} fatty acids, with the secondary alcohol group resulting from an incomplete elongation occurring at the first step. Genome analyses revealed indeed that *Nannochloropsis* spp. code a polyketide synthase (PKS) that can catalyse incomplete fatty acid elongations leading to the formation of 3-OH-fatty acids as well as fatty acid elongases (FAE) that could elongate 3-OH-fatty acids to longer products such as LCHFAs. Transcriptome analyses confirmed that the genes coding both PKS and FAE are up-regulated under culturing conditions promoting the accumulation of LCDs within *Nannochloropsis* cells.

The project will evaluate the production rate of both LCHFAs and LCDs in *Nannochloropsis* cells, aiming to enhance their production through environmental or genetic modulation. The enzymatic activities of both FAE and PKS will be confirmed by heterologous expression of genes coding FAE and PKS in bacteria (*Escherichia coli)* and/or yeasts (*Saccharomyces cerevisiae*)as well as genetic knock-down and overexpression of such genes within *Nannochloropsis* spp.. The relationship between enzyme structure and product stereochemistry (chain length and point of functionalization) will be investigated. The project would finally aim in comparing the production rate of both LCHFAs and LCDs in order to delineate the most suitable synthesis method for these biotechnologically relevant products.