Production and biosynthesis of secondary metabolites from *Nannochloropsis oceanica* (Eustigmatophyceae)

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Abstract

The exploitation of sustainable compounds from microalgae is attracting the interest of biotechnological research for their potential applications in the field of renewable energies, biomaterials, and pharmaceuticals. Within this context, *Nannochloropsis* spp. (Eustigmatophyceae, Heterokontophyta) are considered as suitable candidates for biotechnological purposes because of their fast growth and their high lipid content. In addition to triacylglycerols (TAGs) and polyunsaturated fatty acids (PUFAs), that can be biosynthesised by most eukaryotic phytoplankton, *Nannochloropsis* spp. produce $C_{30\text{-}32}$ hydroxy fatty acids (HFAs) that might reveal biotechnologically interesting similarly to shorter the plant-derived ricinoleic and lesquerolic acids. Ricinoleic and lesquerolic acids have been investigated for their biofuel potential resulting as potential alternatives to petroleum-based fuels, and revealing more efficient as lubricants compared to methyl esters from commercially available vegetable oils. Yet, hydroxy fatty acids are highly suitable for the chemical modifications required during the industrial synthesis of polyester and polyurethane.

Nannochloropsis spp. produce a series of long chain hydroxy fatty acids (LCHFAs) with the hydroxy group at position n-18, the most abundant being the 13-OH-$C_{30:0}$ and the 15-OH-$C_{32:0}$ fatty acids. Furthermore, *Nannochloropsis* spp. do also contain two classes of compounds similar to LCHFAs in terms of length and position of functionalization: the long chain diols (LCDs) and long chain alkenols (LCAs). The latter are thought to be strongly bound between each other and with other lipids forming the algaenans, polymers that are acid and base-resistant aliphatic polymers present in Eustigmatophyceae.

Although fatty acid derivatives from microalgae still encounter few applications compared to aliphatic lipids from plants (e.g., ricinoleic acid, waxes), the high genetic and chemical diversity of microalgae makes their metabolites promising for the production of environmentally sustainable fuels, biomaterials, as well as pharmaceuticals, nutraceuticals, and cosmeceutical products.

My PhD project aimed at obtaining increased knowledge of these functionalized aliphatic compounds in *Nannochloropsis oceanica* to expand the range of lipids exploitable from this microalga for biotechnological purposes. Firstly, we investigated the impact of light intensity on LCHFA, LCD, and LCA production in *N. oceanica*. Experiments under different light intensities (25, 150, and 400 µE m^{-2} s^{-1}) and prolonged darkness were conducted. In the second part of the project, we focused on the elucidation of the role of polyketide synthase (PKS) enzyme in the biosynthetic pathway of LCHFAs, LCDs, and LCAs by generating *N. oceanica* mutant lines with non-functional PKS using the Crispr-Cas9 technology. Cell growth, morphology, and lipid content of mutant strains were evaluated and compared to the wild type *N. oceanica* strain. In the third part, we investigated the interest of *N. oceanica* wild type as well as the abovementioned mutants for toxic metal bioremediation. Indeed, given the key role of cell walls, and thus potentially of LCDs and LCAs, we assessed the tolerance of *N. oceanica* wild type as well as mutant strains to a mixture of toxic metals and their efficiency in removing them from the medium.

The findings of this project have shed more light on LCHFA, LCD and LCA presence, modulation, and role in *N. oceanica*. This will support the potential of *Nannochloropsis* spp. as living cell factories of compounds exploitable for biotechnological and industrial purposes.