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OPTIMIZATION OF PALMITIC ACID PRODUCTION IN CHLOROIDIUM SP. UTEX 3007 AS A SUSTAINABLE SOURCE OF PALM OIL ALTERNATIVE

by

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Date & Venue

12:30 PM
Wednesday, 08 June 2022
https://eu.bbcollab.com/guest/0f5cd0bdc9d54d0d8150ba9968701075

Abstract

The increase in palm oil consumption and production has resulted in removing up to 90% of trees in some tropical forests. The green microalgae *Chloroidium* sp. UTEX 3007 can be an alternative sustainable source of palm oil due to its high content of palmitic acid. The objective of this study was to (i) generate a mutant *Chloroidium* population that is capable of producing and accumulating elevated levels of palmitic acid; and (ii) characterize the lipid contents in the obtained populations. The optimization of palmitic acid production in *Chloroidium* sp. UTEX 3007 was conducted via a workflow of ultraviolet (UV) mutagenesis and sorting of high-lipid producing mutants with Fluorescence-Activated Cell Sorting (FACS). The lipid composition for increased palmitic acid content in mutants was analyzed by ultra-high-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UHPLC-QToF-MS). Four iterative rounds of mutagenesis combined with FACS resulted in four mutant populations with increased lipid contents. Extracted lipids from the mutant populations by UHPLC-QToF-MS showed a significant increase in palmitic acid compared to the parental strain. in addition, the metabolomic analysis demonstrated higher palmitic acid and malonyl-CoA in the lipids of the fourth-mutant population. The latter is a precursor building block for palmitic acid as well as an inhibitor of fatty acid oxidation. Triacylglycerol (TAG) neutral lipids were higher in R4. Thus, the increase of malonyl-CoA may explain higher TAG and palmitic acid in R4. Most importantly, all other morphological characteristics and growth rates of the mutant populations were not significantly affected. The repetitive rounds of UV combined with FACS resulted in mutant populations with higher palmitic content than the wild-type strain. Individual cells isolated from these mutant populations may be further analyzed for their lipidomics and genomic alterations to identify promising candidates for palm oil alternative production.

Keywords: *Chloroidium* sp., Date oil, Fluorescence-activated cell sorting, Lipid, Palmitic acid, Ultraviolet mutagenesis.