Generation of improved *Chlorella sorokiniana* UV mutants with enhanced lipid accumulation capacity

Papapanagiotou G.[1], Samara C.[1], Arora N.[2,3], Lo E.[2], Philippidis G.P.[2], Chatzidoukas C.[1]

[1] *Department of Chemical Engineering, School of Engineering, Aristotle University of Thessaloniki, Greece*
[2] *Patel College of Global Sustainability, University of South Florida, Tampa, FL, USA*
[3] *Department of Cell, Microbiology and Molecular Biology, University of South Florida, Tampa, FL, USA*

Introduction

Microalgae are a prominent natural source of macronutrients. However, due to their low production capacity, strain improvement is the key to increasing biosynthesis of targeted biomolecules. Random mutagenesis causes genetic alterations that can potentially lead to the enhanced accumulation of desired intracellular components such as lipids [1]. Such gene modifications can be evoked by chemical or physical mutagens, like ultraviolet (UV) radiation [2].

Aim & Objectives

The aim of this research was the generation of UV-induced *C. sorokiniana* mutants with enhanced lipid productivity in the context of the development of valuable strains for the food industry. The main objectives of this research were the evaluation of the superiority of the UV-induced mutants of *C. sorokiniana* against the wild type (WT) cells, as well as the stability of the mutants over time.

Material & Methods

The mutagenesis protocol comprises culture exposure to UV-C light for sufficient time to attain cell fatality higher than 50%, followed by a relief period of 24-h in the dark. Attainment of the desired cell fatality was monitored by O.D. (750 nm) and dry cell weight (DCW) measurements of the mutated strains and comparison against WT (same treatment without UV exposure). Upon determination of the appropriate time of UV treatment, the protocol was applied on fresh *C. sorokiniana* cells.

After the 24-h dark period, the cells were spread onto BBM (Bold's Basal Medium) agar plates and incubated until algal colonies appeared. The biggest and greenest mutant colonies were inoculated in 5 mL of fresh BBM in 25-mL Erlenmeyer flasks and cultivated for 5 days at 25°C under diel cycle of 16/8 hours light/dark. The success of the triggered mutagenesis, in terms of the intracellular lipid content, was assessed by staining the cells with Nile red [3] and comparing against the wild type. The mutants exhibiting more intensive fluorescence than the wild type were selected for subsequent lipid quantification with the sulfo–phospho–vanillin method [4].

The most productive mutants were cultivated in a shaking incubator for 10 cycles of 12 days each to ensure that their growth and lipid accumulation rate remained stable.

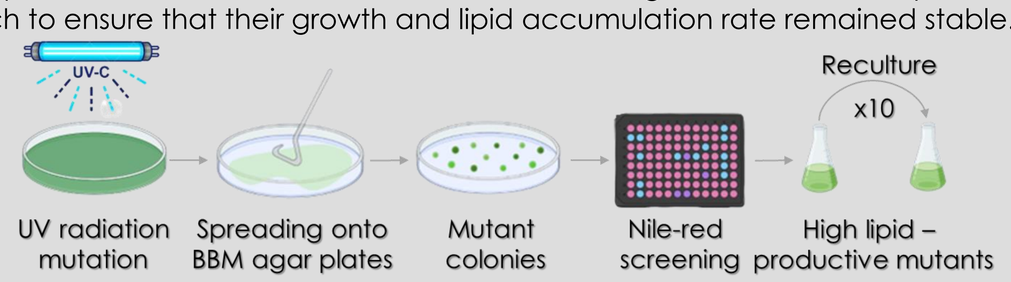
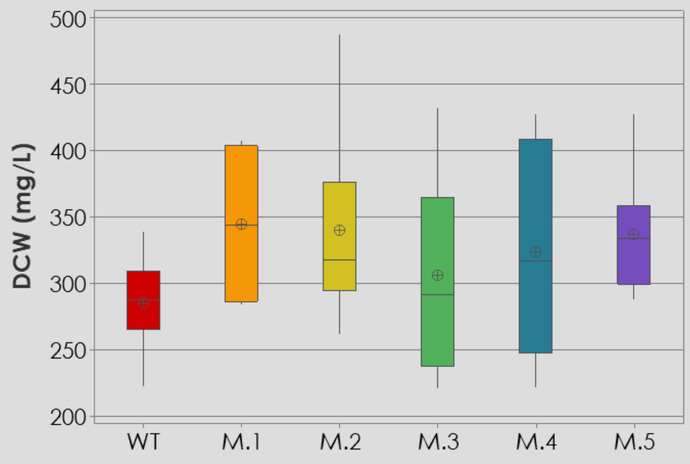


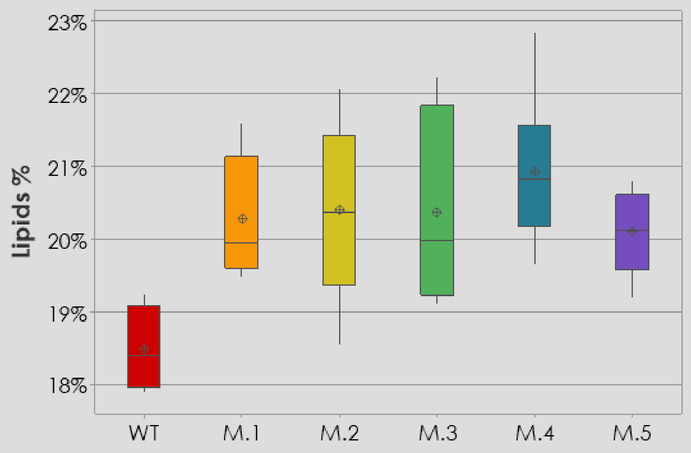
Fig.1 | Main mutagenesis processes

The results of biomass productivity, lipid content and productivity of 10 cultivation cycles performed are presented as box-plots. MiniTab software was used for data representation; statistical analysis was performed via one- way analysis of variance (ANOVA, $p < 0.05$) followed by Dunnett's multiple comparison.

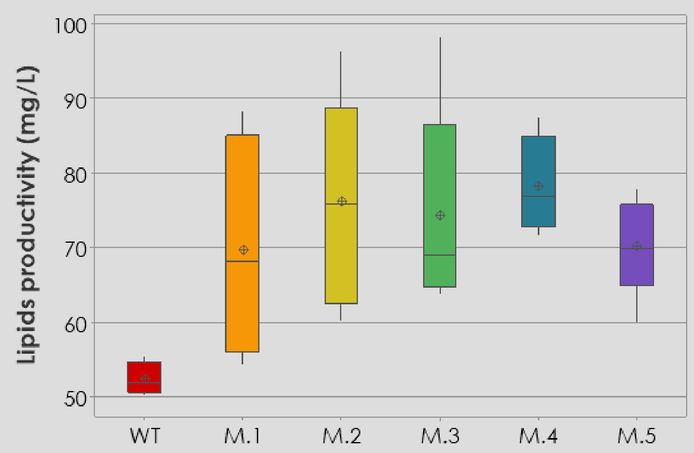
Results



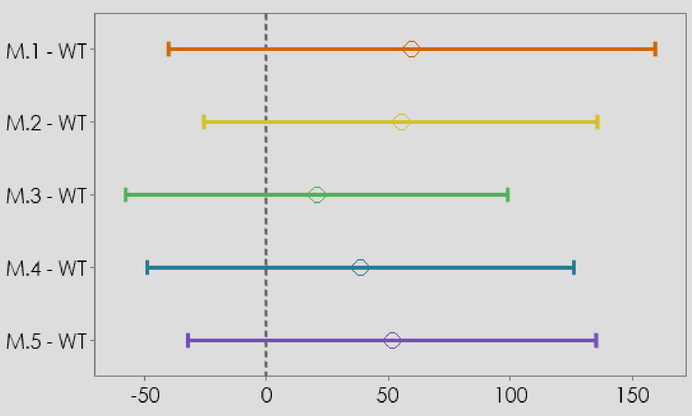
Graph 1 | Box-plot of DCW (mg L^{-1}) productivity of W.T and UV-induced mutants based on the results of 10 cultivation cycles



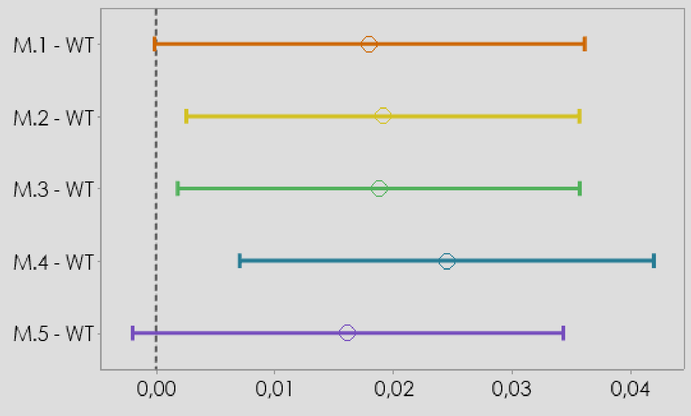
Graph 2 | Box-plot of lipids % content of W.T and UV-induced mutants based on the results of 10 cultivation cycles



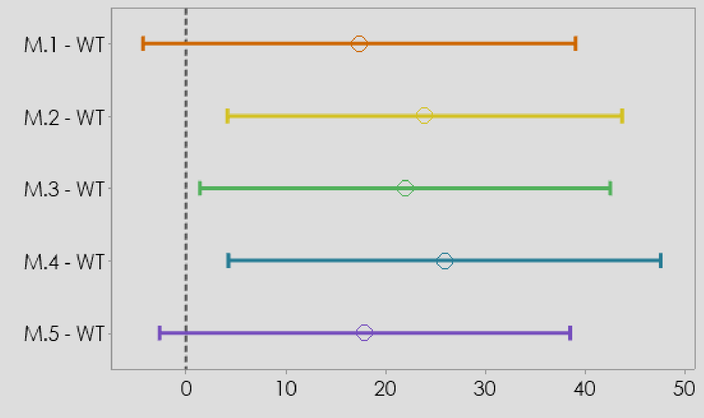
Graph 3 | Box-plot of lipids productivity of W.T and UV-induced mutants based on the results of 10 cultivation cycles



Graph 4 | Dunnett's Multiple Comparison in response to biomass productivity



Graph 5 | Dunnett's Multiple Comparison in response to lipid content



Graph 6 | Dunnett's Multiple Comparison in response to lipid productivity

Discussion

At the level of biomass productivity, no statistically significant differentiation was observed between the studied strains. However, in the case of lipids, both in terms of their cellular content and total productivity, a statistically significant predominance of 3 UV- induced mutants over WT cell was observed. Among the three mutants the M.4 was classified first and then followed the M.2 and then the M.3, while for the mutants M.1 and M.5 no statistically significant superiority was recorded based on the results obtained from the 10 cultivation cycles.

Conclusion

This research enabled the generation of three (3) stable *C. sorokiniana* UV-induced mutants with improved lipid content and productivity. Further effort is needed to achieve even greater enhancement of intracellular lipid accumulation, as well as the evaluation of differentiation between WT and UV-induced mutants in terms of fatty acid profile.

References

[1] E. Lo, N. Arora, and G. P. Philippidis, 'Physiological insights into enhanced lipid accumulation and temperature tolerance by *Tetraselmis suecica* ultraviolet mutants', *Science of The Total Environment*, vol. 839, p. 156361, Sep. 2022.

[2] R. Sivaramakrishnan and A. Incharoensakdi, 'Enhancement of lipid production in *Scenedesmus* sp. by UV mutagenesis and hydrogen peroxide treatment', *Bioresour Technol*, vol. 235, pp. 366–370, Jul. 2017.

[3] W. Chen, C. Zhang, L. Song, M. Sommerfeld, and Q. Hu, 'A high throughput Nile red method for quantitative measurement of neutral lipids in microalgae', *J Microbiol Methods*, vol. 77, no. 1, pp. 41–47, Apr. 2009.

[4] S. K. Mishra, W.I. Suh, W. Farooq, M. Moon, A. Shrivastav, M.S. Park, J.W. Yang 'Rapid quantification of microalgal lipids in aqueous medium by a simple colorimetric method', *Bioresource Technology*, vol. 155, pp. 330–333, Mar. 2014.

Acknowledgements

This research was supported by (1) European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation under the call RESEARCH-CREATE-INNOVATE (project T2EDK-02279 "Human nutrition, animal and fish feeding on microalgae derived products through sustainable photosynthetic autotrophic cultures"); and (2) the Greek Diaspora Fellowship Program funded by the Stavros Niarchos Foundation and administered by the Institute of International Education (New York, NY, USA) in collaboration with the Fulbright Foundation in Greece.

ALGAEUROPE2022 · ARISTOTLE UNIVERSITY OF THESSALONIKI · UNIVERSITY OF SOUTH FLORIDA · Sun Feeding

European Union, European Regional Development Fund · HELLENIC REPUBLIC, MINISTRY OF DEVELOPMENT AND INVESTMENTS, SPECIAL SECRETARIAT FOR ERDF & CF PROGRAMMES, MANAGING AUTHORITY OF 'COMPETITIVENESS' PROGRAMME · EPAnEK 2014-2020 OPERATIONAL PROGRAMME COMPETITIVENESS ENTREPRENEURSHIP INNOVATION · ΕΣΠΑ 2014-2020, ανάπτυξη - εργασία - αλληλεγγύη, Partnership Agreement 2014 - 2020

Co-financed by Greece and the European Union

Greek Diaspora Fellowship Program