Development and characterization of lipid-based nanovectors as green delivering systems for agro-technological applications

Felicia Menicucci (1), Marco Michelozzi (2), Aida Raio (2), Mario Tredici (3), Mattia Belli (4), Gabriele Cencetti (2), Marina Carta (2), Alessio Papini (4), Ilaria Clemente (1), Cristina Gonnelli (4), Sandra Ristori (1)

(1) Department of Chemistry, University of Florence; (2) National Research Council of Florence; (3) DISPAA, University of Florence; (4) Department of Biology, University of Florence.

1 LIPID EXTRACTION FROM NANNOCHLOROPSIS SP. AND DEVELOPMENT OF LIPID-NANOVECTORS

2 GC-MS ANALISYS: CHARACTERIZATION OF THE MICROALGA'S LIPID COMPOSITION

3 GC-MS ANALISYS: DETECTION OF THYMOL CONTENT

4 STRUCTURAL CHARACTERIZATION OF NANOVECTORS BY DLS AND SAXS

5 AGRO-TECHNOLOGICAL APPLICATION -> BACTERIAL GROWTH INHIBITION TEST

The aim of this work was the development of engineered lipid-nanovectors (NVs) employing lipids extracted from the marine microalga *Nannochloropsis sp.* This autothrophic microrganism is naturally rich in lipids (39-68%)[1], representing a precious source for the manufacturing of our biocompatible nanovectors. The obtained nanocarriers were loaded with thymol, a natural antibacterial and fungicidal monoterpene[2], and their efficacy was tested against the bacterium *Xanthomonas campestris* pv. *vesicatoria* (Xcv) that commonly affects tomato plants[3].

LIPID EXTRACTION FROM NANNOCHLOROPSIS SP. AND DEVELOPMENT OF NANOVECTORS

I LIPID EXTRACTION by FOLCH METHOD

II ADDITION of THYMOL

III EVAPORATION

IV REHYDRATION with MILLIQ WATER

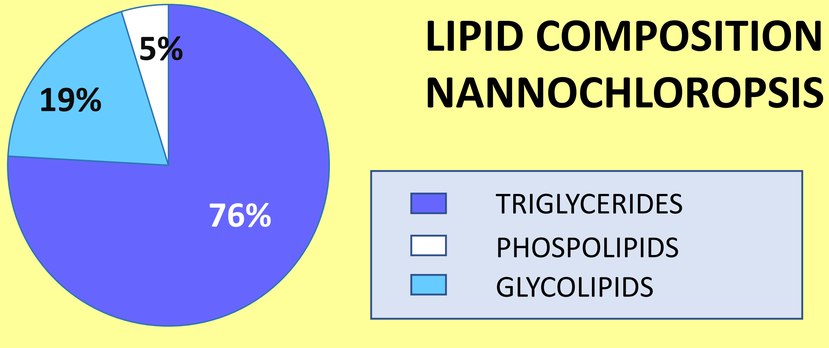
V FREEZING-THAWING

VI SONICATION

VII UV-LIGHT STERILIZATION

GC-MS ANALISYS

LIPID COMPOSITION OF NANNOCHLOROPSIS SP.

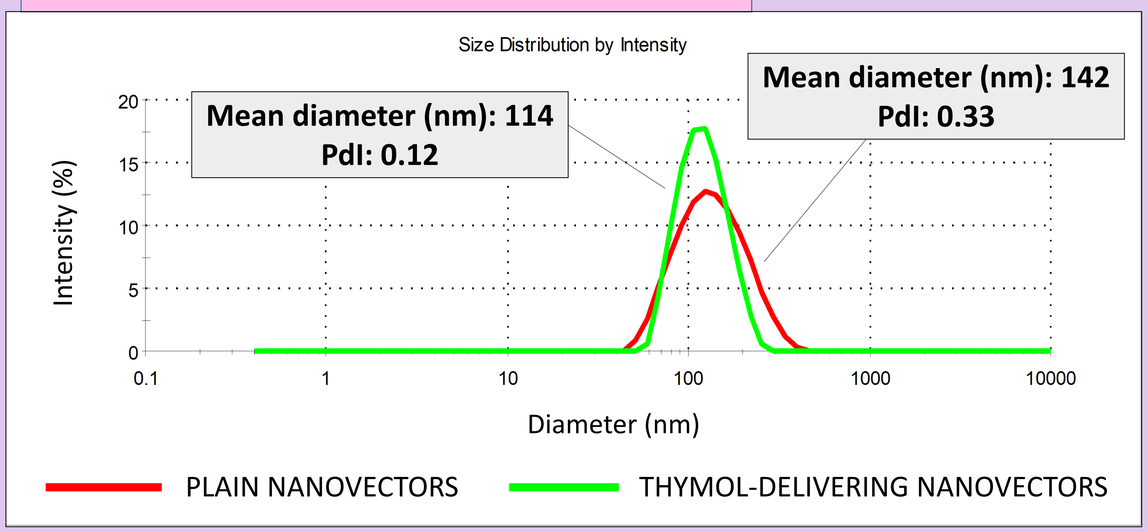


QUANTIFICATION OF THYMOL LOADED IN NANOVECTORS

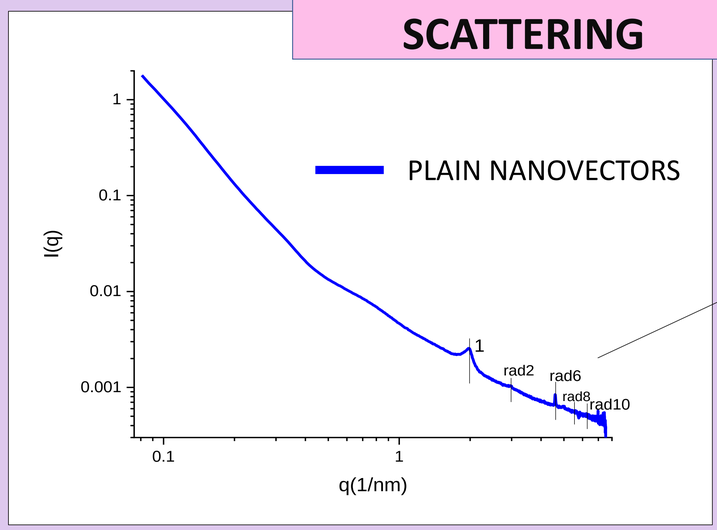
Nannochloropsis-derived NVs	
Thymol content (ug/ml)	Loading efficiency (%)
906.0	92.5

STRUCTURAL CHARACTERIZATION

DYNAMIC LIGHT SCATTERING

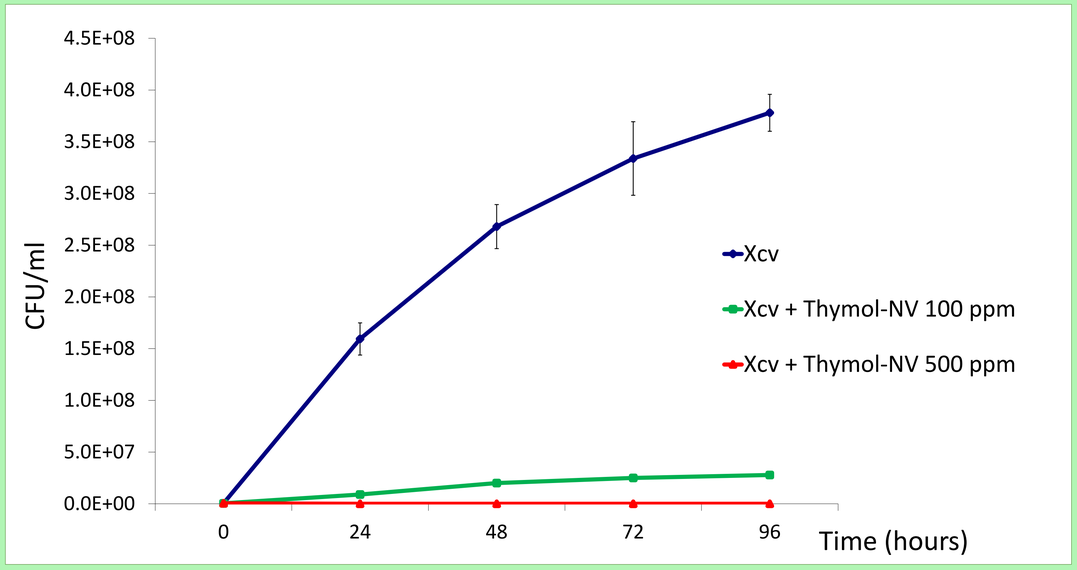


SMALL ANGLE X-RAYS SCATTERING



Saxs investigations: peaks pattern characteristic of cubic phases

XCV GROWTH INHIBITION TEST



We developed a *Nannochloropsis*-derived set of lipid nanoparticles using the entire lipid fraction extracted, minimizing the waste of raw material. An in depth physico-chemical characterization of the nanovectors was followed by *in vitro* tests against the gram-negative *Xanthomonas campestris* pv. *vesicatoria*, that causes bacterial leaf spot on tomatoes.

Our thymol-delivering nanosystem significantly broke down the growth of Xcv at the tested concentrations. Further *in vitro* investigations are ongoing, in the perspective of *in vivo* tests.

1) Bondioli, P., Della Bella, L., Rivolta, G., Zittelli, G. C., Bassi, N., Rodolfi, L., ... & Tredici, M. R. (2012). Oil production by the marine microalgae Nannochloropsis sp. F&M-M24 and Tetraselmis suecica F&M-M33. *Bioresource technology*, *114*, 567-572.
2) Lambert, R. J. W., Skandamis, P. N., Coote, P. J., & Nychas, G. J. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of applied microbiology*, *91*(3), 453-462.
3) Bonas, U., Schulte, R., Fenselau, S., Minsavage, G. V., Staskawicz, B. J., & Stall, R. E. (1991). Isolation of a gene cluster from Xanthomonas campestris pv. vesicatoria that determines pathogenicity and the hypersensitive response on pepper and tomato. *Mol. Plant-Microbe Interact*, *4*(1), 81-88.