



H.F.R.I.
Hellenic Foundation for
Research & Innovation

Description of the funded research project
**1st Call for H.F.R.I. Research Projects to Support Faculty
Members & Researchers and Procure High-Value
Research Equipment**

Title of the research project: Biotransformation of glycerol into high pharmaceutical-value poly-unsaturated fatty acids (PUFAs)

Principal Investigator:
Professor Seraphim Papanikolaou

Reader-friendly title:
Glycerol2PUFAs

Scientific Area:
Agricultural Sciences – Food Science and Technology

Institution and Country:
Agricultural University of Athens (AUA), Greece

Host Institution:
Agricultural University of Athens (AUA), Department of Food Science and Human Nutrition (DFSHN)

Collaborating Institutions:
1) University of Patras (UP), Department of Biology, Division of Genetics, Cell and Development Biology
2) Laboratory of Edible Fungi of Institute of Technology of Agricultural Products (LEF), ELGO – DEMETER



Professor Seraphim Papanikolaou



Professor Panagiotis Katsoris



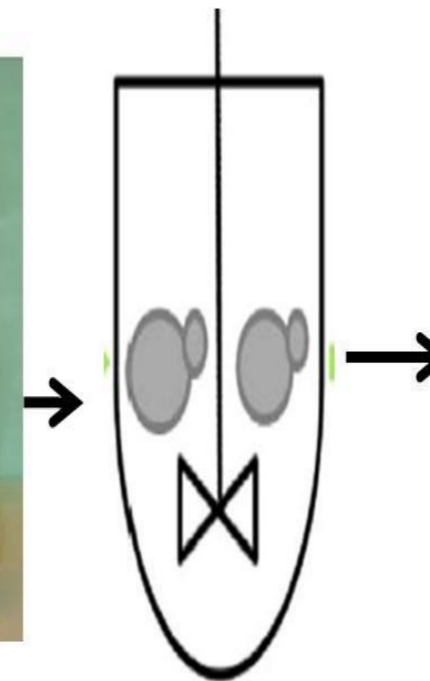
Associate Researcher Panagiota Diamantopoulou



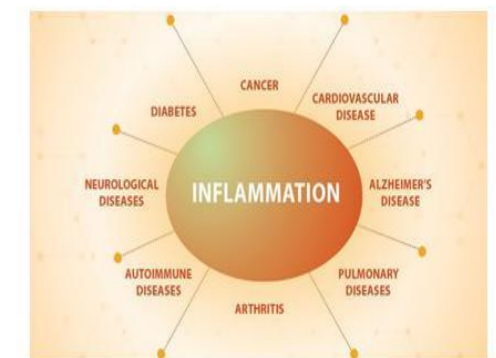
Assistant Professor Chrysavgi Gardeli



Biodiesel-derived glycerol



Zygomycetes fermentations



Fungal oil:

Functional lipid containing γ -linolenic acid, presenting various therapeutic properties

Budget: 168.000,00€
Duration: 36 months

Research Project Synopsis

Crude glycerol (a side-product of several agro-industrial activities including biodiesel production) is a renewable carbon source generated in continuously increasing quantities worldwide. This low-cost material will be employed as substrate for the production of microbial oil (called single-cell oil, SCO) containing important polyunsaturated fatty acids (PUFAs), like γ -linolenic acid (GLA). Here, we focus on the production of SCO containing GLA by Zygomycetes (i.e. *Mortierella* sp., *Cunninghamella echinulata*, etc) with the investigation ranging from fundamental understanding and optimization of SCO synthesis of selected microorganisms in shake-flasks to scale-up in laboratory-scale bioreactors of relatively high volume (10 L). Trials will be carried out in nitrogen-limited and nitrogen-excess conditions and the biosynthesis and analysis of other storage compounds (i.e. cellular polysaccharides) will also be investigated. Kinetic models that simulate the microbial behavior will also be developed. Lipids produced will be extensively analyzed [i.e. analysis of lipid fractions in neutral lipids, polar lipids (glycolipids + sphingolipids, phospholipids), etc] and will be thereafter transformed into fatty acid lithium and/or potassium salts (FALS/FAPS) that present anti-cancer activities. The effect of FALS/FAPS deriving from several types of Zygomycete oil will be studied against various types of cancer lines (i.e. PC3, DU145, LNCap, MCF-7, HL-60, etc). Finally, in the frame of bio-economy, waste-waters deriving from the previously mentioned submerged fermentations of Zygomycetes will be valorized through mushroom cultivations where solid wastes deriving from biodiesel and oleochemical production (e.g. sunflower meal, stalks, etc) will be used as alternative substrates in various solid-state fermentations for the production of edible and medicinal fungal carposomes and mushroom nutra-ceutical compounds (i.e. bio-active mycelia, polysaccharides, etc). Previous successful outcome of several projects in which the academic partners have participated will ensure the success again in this collaborative project on a timely and important topic with long-range impact.

Project originality

- 1) The discovery and study of efficient strains of oleaginous Zygomycetes that could transform raw glycerol into high-added value PUFA-rich SCOs, constitutes *per se* a remarkable originality given the inability of most Zygomycetes to efficiently grow on this substrate.
- 2) The advanced lipidome analysis of the produced SCOs that will be carried out (i.e. detailed analysis of lipid fractions i.e. neutral lipids, polar lipids, etc) constitutes another original approach of the project, since in a scarce number of reports, detailed lipidome analysis of Zygomycete-deriving SCOs has appeared.
- 3) The perspective of the development of numerical models simulating the kinetic behavior of SCO-producing fungi cultivated on crude glycerol is another originality; while modeling approaches quantifying the production of SCOs by yeasts and fungi have been applied in a scarce number of reports, yet studies modeling growth and SCO production from glycerol-based substrates, have not appeared.
- 4) In the proposed proposal, several bioreactor experiments in laboratory-scale bioreactors (active fermentation volume 1.0-4.0 L) are envisaged to be realized while effort will be done in order to further scale-up the process (i.e. in a 10-L laboratory-scale bioreactor), whereas only in a restricted number of publications, cultures of oleaginous Zygomycetes were carried out in laboratory-scale reactors.
- 5) The approach concerning the employment of SCOs that will be transformed into FALS/FAPS (and not the salts of the purified or pure GLA) in order anti-cancer activities to be studied is another interesting and original point of Glycerol2PUFAS project.
- 6) Tap water will (partially or completely) be replaced by waste-waters deriving from the Zygomycetes fermentations, in a zero-waste release concept, in which solid wastes deriving from biodiesel and oleochemical production will be used as alternative substrates for the production of edible and medicinal fungal carposomes and mushroom nutra-ceuticals, constituting another originality of the project.

Expected results & Research Project Impact

The use of synergies, collaborations and innovative ideas from a wide range of fields and topics, as well as the interplay between several scientific areas and scientists involved in a project, represents the most important prerequisite in order to produce advanced, high-level and original scientific knowledge. Under this optic, through Glycerol2PUFAs, various synergies are envisaged since the collaborating researchers and their groups cover a wide range of fields including White Biotechnology, Advanced Chemistry, Fermentation Technology, Fungal Biotechnology, Cell Biology and Applied Biochemistry.

In the current proposal, crude glycerol, a carbon source of low cost and relatively high purity, is envisaged to be used as substrate in order for Zygomycete strains to produce SCOs which contain the very important GLA. This fatty acid (FA) presents anti-cancer activities, which will be deeply studied and assessed. The (few) plants that produce lipids containing this FA are particularly expensive (ranging between 15-35 US \$ / kg). Moreover, with a view to sustainability and bio-economy, attention will be focused on the fact that waste-waters deriving from the submerged fermentations realized by Zygomycetes will be used as maceration waters in innovative and environmentally friendly solid-state fermentations, in which solid wastes originated from biodiesel or oleochemical facilities will be employed as substrates by edible and pharmaceutical mushrooms. Myco-products of high biological and pharmacological importance (i.e. bio-active mycelia, polysaccharides, etc) will be produced, whereas the anti-oxidant, pharmacological and anti-tumor properties of these compounds will be thoroughly studied.

The biotechnological production of high added-value compounds (functional lipids, bio-active myco-products, etc) through microbial transformation is a state-of-the-art technology given that these products can constitute the workhorse of new industries, in both Hellenic and international level, in a new, rapidly growing market, that of the Applied Biotechnology. Such types of industries will probably be interested by the Glycerol2PUFAs approach.

The importance of this funding

The acquisition of the funding concerning the project Glycerol2PUFAs, given the high level of competition concerning the HFRI projects, constitutes a great honor and a great responsibility of the P.I. of the project, Professor S. Papanikolaou, as well as for the participating members of the team (Professor Katsoris, Dr Diamantopoulou and Dr Gardeli). This honor is also an obligation for the participating scientists, in order to correctly and satisfactorily implement the project.

Concerning the P.I., the honor concerning the leadership of the project Glycerol2PUFAs, demonstrates that it has been fully recognized that most of his scientific activities deal with high quality research related to the Bioprocesses of Glycerol as well as the Biotechnology of Zygomycetes. In this Glycerol2PUFAs project, the above-mentioned activities will be fruitfully combined into a completely multidisciplinary approach, in which all participating scientists will present the most innovative elements of their research achievements and areas.



H.F.R.I.
Hellenic Foundation for
Research & Innovation

COMMUNICATION

185 Syggrou Ave. & 2 Sardeon St. 2
171 21, N. Smyrni, Greece
+30 210 64 12 410, 420
communication@elidek.gr
www.elidek.gr