**Substrate specificity of LPCATs from photosynthetic oleaginous microalgae in forward and reverse reactions and characterisation of their functions in acyl editing of phosphatidylcholine**

Omega-3 very long-chain polyunsaturated fatty acids (VLC-PUFA) are essential for human health and are supplied to human bodies with the food. The main source of these fatty acids in our diet, are fish. However, fish themselves cannot synthesise VLC-PUFA. Similarly, they obtain these fatty acids from their diet, mainly from algae. Recently, the search for new VLC-PUFA sources which could enrich our diet with them has been started. As an alternative to fish, vegetable oils enriched with VLC-PUFA are proposed. The production of this type of oilseeds requires, however, dip knowledge of the mechanisms of VLC-PUFA biosynthesis and their transfer to triacylglycerols (the main component of oils).

Many algae species can efficiently synthesize VLC-PUFAs, making them perfect model organisms to study VLC-PUFA biosynthetic pathways. This group includes, among others, diatoms, algae, which will be the subject of current research.The model diatom *Phaeodactylum tricornutum* synthesises high levels of eicosapentaenoic acid (EPA, 20:5), an omega-3 VLC-PUFA, which is most beneficial for our health. In this organism the majority of EPA is incorporated into membrane galactoglycerolipids despite the fact that EPA is synthesised on the membrane polar lipid phosphatidylcholine (PC). These findings indicate not only that a mechanism of EPA biosynthesis evolved in Phaeodactylum, but that a mechanism of selective channelling of this VLC-PUFA out of PC and into the chloroplast galactoglycerolipids (and in minor amounts to triacylgylcerols) following their synthesis on PC, has evolved as well. In contrast to the elucidation of the biosynthetic pathway of EPA, the mechanisms of how fatty acids enter PC and how they are removed from PC after being modified to participate in the assembly of galactoglycerolipids and TAGs remain unknown for now, as well as the identities of the key enzymes/genes that control these fatty acid fluxes.

It has been demonstrated that in higher plants fatty acid exchange between PC and the pool of acyl-CoA (fatty acid connected with coenzyme A) is catalysed by LPCAT type of enzymes (acyl-CoA:lysophosphatidylcholine acyltransferases). The aforementioned exchange plays an important role in channelling of plant PUFAs (18:2 and 18:3) from PC to TAGs. Accordingly, we assume that similar mechanism could exist in algae, and thus we are planning to study the role of LPCAT type of enzymes in VLC-PUFA biosynthesis in diatoms.

In order to reveal the mechanisms controlling the flux of VLC-PUFA from PC into galactoglycerolipids and TAGs in Phaeodactylum, we will start with cloning LPCAT candidate genes and selecting these presenting real LPCAT activities. Next, these selected genes will be introduced to yeast mutant deficient in LPCAT activity and substrate specificity of tested genes will be studied. We will especially focus on the ability of EPA intermediates: 18:3 and 20:4 as well as EPA (20:5) itself, transfer from PC (were the subsequent double bonds are inserted) to acyl-CoA pool (where the elongation of fatty acids occurs and from where 18:3, 20:4 and EPA are transferred to other complex lipids) by these cloned ptLPCATs (LPCAT from *Phaeodactylum tricornutum*). We will also reconstruct EPA biosynthetic pathway in an oilseed model plant *Arabidopsis thaliana* and study the effect of ptLPCATs on omega-3 VLC-PUFA accumulation in this plant. Additionally ptLPCATs will be knocked-out in model diatom *Phaeodactylum tricornutum* and the effects on VLC-PUFA accumulation will be investigated. All of this planned research will deepen our understanding of the mechanism of VLC-PUFA biosynthesis and the mechanism of channelling them to their place of storage. The study will also increase our metabolic toolbox for bioengineering the production of designer oils, especially those containing omega-3 VLC-PUFA. The project can prove to be a pioneering investigation into algae LPCATs.

The proposed study will also boost and deepen bilateral academic exchange and cooperation in the field of lipid metabolism in photosynthetic organisms between Polish and Chinese groups involved in the project. Joint implementation of the planned research may strengthen the proficiency of the Polish partner in the field of molecular biology of plant lipids and the proficiency of the Chinese partner in the field of plant lipid biochemistry.