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Oil Bodies and Their Associated Protein In Diatoms

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Abstract: Being the most dominant group of phytoplankton, diatoms contribute to 40% of primary production in ocean. These accumulate neutral lipids (i.e., triacylglycerol) in distinct inner compartment of cell known as oil bodies. Diatoms are found to have high lipid amount than other micro algae especially in nutrient limiting conditions. Oil bodies arise from Endoplasmic Reticulum and these have a hydrophobic core surrounded by a polar lipid monolayer and consists of specific proteins on their surface. Triacylglycerol (TAG) is converted to biofuels via transesterification. In this process, triglycerides are esterified with alcohol in the presence of catalyst. The changing environment conditions is in dire need of petroleum alternative, as TAG has shown its significance in biodiesel production, so a comprehensive study of TAG degradation could be useful to unravel the hurdles in biofuel production. The oil-associated proteins might be helpful in TAG degradation. However, only few species have been studied to understand micro algae proteomics. Proteomic analysis is harder due to several factors for instance, translocation of oil associated proteins within a cell. Techniques such as fluorophore tagging could be used to localize proteins but it has limitations. Oil bodies has direct contact with other cellular membrane that's why it is difficult to isolate pure, intact oil bodies. The extracted oil associated proteins are identified with SDS PAGE and LCMS.

Keywords **- Diatoms, TAG, Biofuel, Oil-associated proteins, Flurophore-tagging, SDS, LCMS**

I.INTRODUCTION

The vast biodiversity of phytoplankton ranges from cyanobacteria to photosynthetic eukaryotes which either have a chloroplast originated from primary symbiosis, for instance green algae or a photosynthetic organelle originated from secondary symbiosis, such as heterokonts (Lupette et al., 2019). Sometimes these can grow in dense accumulations in nature known as spring blossom (Tokushima et al., 2016). Diatoms are heterokonts (Benoiston et al., 2017), these are responsible for 40% of total primary production in ocean (Sarthou et al., 2005). Primary production occurs by fixing carbon dioxide via photosynthesis. The basic principle of this process is conversion of inorganic carbon dioxide into organic biomacromolecule, which are utilized to construct cellular compartments or are stored as reserved material. The main carbon storage is tryacylgylcerol (TAG) (Maeda et al., 2017). Diatoms along with many other organisms accumulate neutral lipids in well-defined intracellular compartments known as oil bodies. The characteristics of oil bodies are: oil bodies arise from Endoplasmic Reticulum and they accumulate neutral lipids surrounded by phospholipid monolayer surrounding them. These consists of specific proteins on their surface (Yoneda et al., 2016). The functions of oil associated protein are intracellular membrane trafficking, act as a structural protein to stabilize oil bodies and metabolizing lipids (Murphy et al. 2012). TAG accumulates in oil bodies, so it is possible that oil associated protein could help in metabolizing it (Nojima et al. 2013). Under favorable conditions diatoms can produce up to 60% of their cellular mass as TAGs (Sanjay et al. 2013). Climate change due to exhaustion of fossil fuel is a critical issue to be addressed in 21st century. Earth needs sustainable and environment friendly alternative, like biodiesel. Oil bodies in diatoms are the most promising group for biodiesel production (Tanaka et al., 2015). Along with biofuel production, diatoms have shown their importance in other applications such as human health, food and green chemistry. In spite of their imperative role in eukaryote's evolution, ecosystem and biotechnology, limited studies have been conducted on diatoms cell biology and physiology (Lupette et al. 2019). The composition and characteristic of diatom species are influenced by geographical, climatic and anthropogenic factors. These are sensitive to the slightest variation in their surroundings**.** The microalgal growth rate and TAG accumulation increase under stressing conditions (Chu et al., 2019). It's imperative to understand the cellular variations during oil body synthesis for unveiling the changes in lipids homeostasis and its accumulation mechanism for biotechnological applications (Liang et al. 2015). Diatoms in natural environment are continuously exposed to environmental deviations and stress such as rise or fall in temperature, high light, nitric oxide or hydrogen peroxide exposure or to various chemicals. These variations activate intense lipid remodeling marked by an accumulation of oil droplets within cells (Lupette et al. 2019). This review paper summarizes ecology and structure diatoms, their oil bodies and associated proteins and identification of oil associated proteins.

1.1 Diatom ecology and structure

In the past decade sustainable progress has been made to understand the diversity and taxonomy of the diatom flora Antarctic region (Kopalova, K., & van de Vijver, B. 2013). Bacillariophyta are known to be one of the most abundant algal group on Earth

which occur in freshwater, marine and semi- terrestrial habitats globally (Pinseel et al 2017). According to the previous studies, limited number of diatom taxes were found in Antarctic ecosystem (Jones 1996, Van de Vijver & Beyens 1999, Sabbe et al. 2003). Factors such as nutrient availability and latitude influence the species richness (Hansson & Ha˚kansson,1994). Nutrient gradient and salinity and species abundance are closely related (Jones *et al*., 1993). Diatoms are unicellular micro algae, range from 5-200mm in length or width. These vary in size and shape (Round *et al*., 1990). Diatoms store essential micronutrients, such as Co, Fe, Zn, etc., that are present in trace concentration. These micronutrients are essential for diatom growth, structure and distribution. Diatoms uptake Silica which is required for frustule formation. Silica uptake is affected by the other nutrient presence in the environment and diatom cell size. The cell wall of diatoms is made up of silica and is called as frustules. The outer part of frustule is called epitheca and the inner part is called hypotheca (Round *et al*., 1990). Under natural conditions, frustules are covered by mucopolysaccharide material (Sabater, 2010). In some pennate diatoms, a longitudinal slot in the theca is present, which are called raphe (Sabater, 2010). Frustules has different functions, for example acts a mechanical defense against grazers, protect cell from photo inhibition, which means silica content varies in species based on the environmental factors and growth phase (Zhang *et al*., 2017). To determine the taxonomy of diatoms it's important to specify shape of the body, length to breadth ratio, raphe structure and position, forms of central, apical and axial areas of the cell. These are classified in two orders i.e., centrale and pennales (Sabater, 2010).

2. Oil bodies in diatoms

Lipids are present in higher concentration in chloroplast or chromatophore structure of photosynthetic organisms. These are highly surface-active and plays a vital role in the structural organization of photosynthetic components (Kates et al., 1966). The structure of lipid bodies is composed of a hydrophobic core, mostly triacylglycerol (TAG), containing neutral lipids surrounded by a polar lipid monolayer (Lupette et al., 2019). The inert, hydrophobic, stable molecules with three fatty acid esterified to a glycerol backbone are called triacylglycerol (TAG) (Leyland et al., 2020). TAGs are the renewable source of biofuel production. Transesterification process is used to convert oil bodies to biofuels. This process involves the esterification of vegetable oils, micro algal oils and animal fats with alcohol in the presence of catalyst (Vasudevan and Briggs, 2008). For the efficient production of biofuel, the first step is to analyses the lipid productivity in different diatoms species to select substantial biofuel producers. Table1 shows the lipid body size and number among different species (Madea et al. 2017). The changing climate conditions are the evidence of dire need of alternative to petroleum (Sanjay et al. 2013). Lipid bodies have various names used in different studies such as lipid particles, lipid droplets, adipose, oleosome, oil bodies, oil globules and cytoplasmic inclusions (Fujimoto et al. 2011). Lipid bodies can minimize the effects of stress caused due to increase in carbon, lipid or protein aggregates accumulation. These act as an energy reservoir during nutrient scarcity, imbalance in electron flow, and also helps to maintain redox homeostasis (Murphy, and D.J 2012). The constant exposure to environmental stress conditions can result in lipid remodeling marked by oil bodies aggregation (Lupette et al. 2019). Nutrient deficiencies such as silicon, nitrogen and phosphorous leads to TAG accumulation. Diatoms show different metabolic changes to each nutrient stress condition which results in variations in lipid profile, enantiomers and positional isomers (Leyland et al. 2020) (Table 1)

Table 1: Oil bodies shape, size and number in diatoms (Maeda et al., 2017).

Shape	Species	Number	Size (µm)
Centrales	*Cyclotella cryptica*	1-10	1.1-3.9
	Chaetoceros calitrans	3-4	1.0-2.0
	Skeletonema costatum	3-5	1.1-2.3
	Thalassiosira pseudonana	3-5	0.3-1.5
Pennales	*Fistulifera solaris*	2	1.3-5.6
	Pseudo-nitzchia multiseries	6-15	0.7-1.4
	Nitzschia closterium	3-4	0.8-1.3
	Phaeodactylum tricornutum	1-5	0.4-2.6

3. Oil body associated proteins

Functions of most of the organelle are coordinated by different proteins (Lum, P. Y., & Wright, R. 1995). In plants and animals, some protein in oil bodies, such as oleosin, Perilipins, steroleosins and caleosins, are considered to play a crucial role in determining the stability and size of lipid droplets. In comparison to plants and animals, investigation of oil-associated proteins in microalgae are hindered due to less genetic information. Microalgae has Major Lipid Droplet Proteins (MLDP) and Lipid Droplet Surface Proteins (LDSP) (Nojima et al., 2013). In C. *reinhardtii,* MLDP was identified as the most abundant protein. Repression in the MLDP gene expression cause increase in oil body size which suggests MLDP might regulates the oil body size (Leyland et al., 2020). The second most abundant Lipid protein in higher plants is caleosin (Maeda et al., 2017). The stability of oil bodies isolated from micro algae is less than isolated from seeds. Hence, it's hard to extract oil associated proteins from oil bodies without destructing the integrity of these organelles (Lin et al. 2012). The first proteomic study of oil associated protein in diatoms was conducted on *fistulifera solaris.* Nutrient starvation induced oil-associated formation, cells were disrupted using multi-bead and extracted by centrifugation. Oil associated protein fractions were precipitated and run on SDS PAGE. This experiment identified 41 oil-associated proteins (Nojima et al., 2013). Stramenopile-type lipid droplet proteins (StLDP) are the most abundant Lipid droplet proteins in phaeodactylum tricornutum (Yoneda et al 2018).

3. Identification of oil-associated proteins

Several factors obfuscate the unravel of oil body proteome. For instance, oil body proteome differs between cell type, growth condition, species, etc. and has different location of oil body protein within a cell. Flourophore-tagging, such as green fluorescent protein (GFP), can be used to localise oil bodies. However, it has limitation, optical microscopy has lower resolution to differentiate the physically associated protein with oil bodies and protein present nearby (Soni et al., 2009). Immunogold labelling

on the other hand gives finer resolution via electron microscopy (Huynh et al., 2009). To identify oil body associated proteins following steps are used (i) Cell disruption (ii) oil body isolation (iii) protein extraction from oil bodies (iv) Protein identification. Osmotic stress or homogenisation are the methods to disrupt cell wall (Maeda et al., 2017). The diatom *fistulifera solaris* was disrupted by multi bead shocker. Lipid extraction from diatom biomass can be carried out by sanitation and soxhlation method (Nojima et al., 2013). Oil bodies has direct contact with other cellular membrane, this makes the pure and intact extraction (Leyland et al., 2020). It is difficult to extract pure, intact proteins from oil bodies as these contaminate with other cellular compartments (Maeda et al., 2017). To my knowledge, *Fistulifera solaris* (Nojima et al., 2013) and *phaeodactylum tricornutum* (Yoenda et al.,2016) are the only diatom species on which proteomic analysis has been done. SDS PAGE (Sodium dodecyl sulphate-polyacrylamide gel electrophoresis) and Liquid Chromatography coupled with mass spectrometry (LC/MS) can be used to identify proteins (Huynh et al., 2009). However, it has several drawbacks such as modification artefacts, poor peptide recovery and it depend on the effective stained protein band visibility. Also, the hydrophobic properties of some oil associated proteins and their high lipid content might interfere with SDS PAGE (Brasaemle et al., 2005).

II.CONCLUSION

Diatoms uptake nutrients from the environment and these nutrients helps to determine their structure, growth and distribution. The call wall of diatoms, known as frustule, is made up of Silica. Uptake of silica is affected by the availability of other nutrients in the environment and diatom cell size. Frustule has an outer and inner part called epitheca and hypotheca, respectively. Diatoms have shown their significance in biotechnological development, in biofuel production, pharmaceuticals, etc. With all these climatic changes, biodiesel to replace petroleum is the need of the hour. TAGs are a renewable source of biodiesel. But the molecular mechanism of diatoms is still unknown. Only few species have been explored to identify and analyses the oil associated protein in diatoms, these might play a useful role in TAG metabolism. Diatom stores TAG in the inner compartment of cell called oil bodies. Structure of oil bodies consist of a hydrophobic core surrounded by a polar lipid monolayer. During nutrient limiting conditions or imbalance in electron flow, oil bodies act as an energy reservoir. Moreover, these helps to maintain the redox homeostasis. Oil bodies has proteins on their surface. Less information is available about these proteins due to several factors e.g., different location of proteins within the cell. Few techniques such as fluorophore tagging is available to locate proteins but all these has limitations. Cell disruption is an important step to isolate oil bodies. Oil bodies isolated from micro algae are less stable than seeds which makes the extraction of oil associated proteins difficult without destructing their integrity. The isolated oil bodies associated proteins are identified using SDS PAGE and LCMS. However, the hydrophobic properties of some oil associated proteins and their high lipid content might interfere with SDS PAGE.

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