

# I. INTRODUCTION

## 1.1. Background

Marine microbes are essential to global nutrient cycling, playing critical roles in biogeochemical cycles such as nitrogen, carbon, phosphorous cycling, as well as the formation of organic matter (Castillo & Villafania, 2024). Marine microbes also provide valuable resources, producing a range of bioactive compounds, including peptides, surfactants, enzymes, exopolysaccharides, and various metabolites, that are useful for marine organisms and humans (Barzkar et al., 2023). Microbial communities comprising bacteria, fungi, protists, archaea, and viruses play distinct roles in marine ecosystems (He et al., 2024). They inhabit a wide array of marine environments, from open water and estuaries to sediment and the bodies of marine organisms (Hunter-Cevera et al., 2005). Among these diverse marine microorganisms, Thraustochytrids have garnered attention due to their significant biotechnological potential.

Thraustochytrids are unicellular and eukaryotic protists that are classified within the heterokont lineage, specifically within the class Labyrinthulomycetes. This organism is characterized by morphological characteristics, molecular sequences, and the production of specific compounds such as PUFA and pigments. Thraustochytrids are found in diverse aquatic environments, including estuarine, marine, detrital water, sediment, and mangrove ecosystems (Kaliyamoorthy et al., 2023). A defining feature of Thraustochytrids are their ectoplasmic network, a web-like organelle crucial for nutrient uptake. This network enables them to

function as significant decomposers in marine ecosystems. The ectoplasmic network facilitates both adherence and penetration of substrates, while hydrolytic enzymes secreted by Thraustochytrids solubilize nutrients for subsequent absorption by the cells (Kalidasan, Vinithkumar, et al., 2021).

Thraustochytrids exhibit significant potential for producing valuable components, especially omega-3 and omega-6 PUFA like EPA and DHA. Thraustochytrids possess 50 – 70% lipid content in dried biomass, with 30 – 50% DHA (Chauhan, Patel, et al., 2023). Among various sources of DHA, Thraustochytrids exhibit the highest DHA content. The DHA levels vary significantly among different organisms. For instance, salmon contain approximately 13,000 *mg/100g*, *Camelina sativa* (a plant source) has about 13,680 *mg/100g* and the microalgae *Cryptocodinium cohnii* offers up to 30,000 *mg/100g*. Notably, *Shizochytrium* provides the highest concentration, around 49000 *mg/100g* DHA content (Chauhan, Patel, et al., 2023; DeFilippis et al., 2010; Stramarkou et al., 2021)

While fish oil has traditionally served as a primary source of polyunsaturated fatty acids (PUFAs), its supply is insufficient to satisfy global demand. There are also concerns about fish oil, as fish can accumulate contaminants such as heavy metals, antibiotics, mercury, and organochlorine pollutants like dichloro diphenyl trichloroethane (DDT). Additionally, fish oil can have undesirable odors, flavors, and taste, is prone to instability, and can be costly and difficult to purify (Abedi &

Sahari, 2014). Consequently, Thraustochytrids are being explored as promising for commercial PUFA production due to their high lipid yields and rapid growth rate. PUFA are essential components of a balanced diet, and deficiencies in these compounds can have negative health consequences (Stefánsson et al., 2019).

Besides PUFAs, Thraustochytrids also known for synthesizing carotenoid pigment, such as  $\beta$ -carotene, canthaxanthin, and astaxanthin, under certain culture condition (Leyton et al., 2021). Colony color, comprising yellow and orange is frequently used as a preliminary indicator of carotenoid production. For example, *Aurantiochytrium* from Hong Kong mangroves showed distinct fatty acid profiles in addition to color differences, suggesting correlation between color, carotenoid presence, and metabolic capacity. Moreover, carotenoid content is sensitive to growth conditions, such as light, carbon sources, and nutrient availability (King-Wai et al., 2009)

Mangroves are woody plant communities predominantly found in tropical and subtropical coastal intertidal zones (Lifeng et al., 2024). In 2020, mangrove forests covered an estimated 145,068 km<sup>2</sup> globally, with Asia holding the largest areas (39.2%) (Jia et al., 2023). These vital ecosystems provide crucial biological services to coastal areas, including coastline protection, sediment regulation, water quality improvement, seawater intrusion control, carbon storage, and microclimate stabilization. Furthermore, mangrove ecosystems support diverse biological functions, serving as food resources, breeding grounds, nesting sites, and habitats for

a wide range of marine organisms, including fish, shrimp, and microbial communities (Sahputra et al., 2022).

Thraustochytrids represent major components of microbial communities within mangrove ecosystems and predominantly plays a role in litter decomposition, food web enhancement, and nutrient enrichment (Kalidasan, Vinithkumar, et al., 2021). A close relationship exists between Thraustochytrids and mangroves, with Thraustochytrids capable of utilizing both dissolved organic matter (DOM) and particulate organic matter (POM) as energy sources for growth (Liu et al., 2017). This interaction contributes to enhanced nutrition cycling and supports ecosystem productivity.

Thraustochytrid strains have been predominantly isolated from subtropical and temperate regions, including the coastal areas of Malaysia. Manikan et al. (2015) have isolated *Aurantiochytrium* strain from a seawater sample collected from Port Dickson, Malaysia. On the other hand, Ou et al. (2016a) have isolated Thraustochytrids from mangrove leaves from Port Dickson and Morib, Selangor, on the west coast of Peninsular Malaysia. They reported a total of 33 Thraustochytrid isolates, with 32 isolates identified as *Aurantiochytrium* and one isolate identified as *Parietichytrium*. So far, Thraustochytrids from many mangrove habitats of global mangroves have still been poorly studied (Kalidasan, Vinithkumar, et al., 2021). While Malaysia possesses extensive mangrove ecosystems, reports on Thraustochytrids isolated from these areas are limited. Notably, no studies have yet explored the Thraustochytrids

community within the mangrove ecosystem of Setiu Wetland, Malaysia. The prominence of mangroves in this wetland suggests a high potential for discovering a diverse Thraustochytrids population.

### **1.2. Problem Formulation**

1. How are the diversity of Thraustochytrids presence within the mangrove ecosystem of Setiu Wetland, Malaysia?
2. How are the morphological and molecular characteristics of Thraustochytrids isolates obtained from the mangrove leaves and sediment of Setiu Wetland?

### **1.3. Research Objective**

This research aims to comprehensively explore and identify the diversity of Thraustochytrids collected from mangrove leaves and sediment of Setiu Wetland, Malaysia using molecular analysis.

### **1.4. Research Benefits**

1. This research contributes in investigating the variation of Thraustochytrids living in the Malaysian mangrove ecosystem.
2. This research expands the current scientific understanding of Thraustochytrids' variation and where they are found.