

I. INTRODUCTION

1.1 Background

PUFA (Polyunsaturated Fatty Acid) is a biomolecule with more than one double bond. PUFAs have a methyl group (Omega, "ω" or "n") at one end of the molecule and a carboxylic acid group at the other end. Omega-3 is a type of polyunsaturated fatty acid (PUFA) that plays an essential role in human health. The main types of Omega-3s include α -linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Kapoor et al., 2021). Based on their nutritional and pharmacological properties, DHA and EPA can promote brain and retinal development, anti-cancer, and cardiovascular activity (Kalidasan et al., 2021a). According to Aranceta and Pérez-Rodrigo (2012), the daily intake for total EPA and DHA is 250 mg/day for adults and 100 mg/day of DHA for children aged 7-24 months. During pregnancy and lactation, the recommended adult daily intake of DHA and EPA should be supplemented with 100-200 mg/day of DHA. The global demand for omega-3 continues to increase in line with the growing awareness of its benefits for brain, heart, and nervous system health (Tocher et al., 2019).

Since omega-3 fatty acids cannot be synthesized endogenously because the human body lacks the desaturase enzymes required to modify double bonds, omega-3 must be obtained from external dietary sources (Ponnampalam et al., 2021). Since the 1990s, global fishing capacity has reached maximum levels,

resulting in a limited supply of fish and Omega-3s (Marchan et al., 2017). Several studies have reported that the omega-3 content in fish commonly consumed by humans is generally not very high, as observed in lemuru fish oil (*Sardinella lemuru*), which contains only 14.36% EPA and 4.6% DHA (Suseno et al., 2014). A similar pattern is found in mackerel tuna fish oil (*Euthynnus affinis*), where the omega-3 content is nearly balanced, at 3% EPA and 5% DHA (Mohanty et al., 2016). In addition, fatty acids obtained from fish oil have a fishy odor and pose a risk of contamination with chemicals such as PCBs (polychlorinated biphenyls), mercury, and dioxins. Therefore, a renewable and sustainable alternative source of DHA is needed (Chandrasekaran et al., 2018).

Thraustochytrids are heterotrophic, osmo-heterotrophic, non-photosynthetic, eukaryotic stamenopiles, and oleaginous unicellular organisms that are classified in the kingdom Chromista (Morabito et al., 2019). Thraustochytrids are found in various habitats, such as mangrove forest areas in seagrasses, algae, sediment, and fallen mangrove leaves (Kalidasan et al., 2021b). They also showed that Thraustochytrids have the potential to be used as an alternative source of Omega-3. Thraustochytrids strain TSKK1 (Genbank No. KT716334) produced 13.53 g-L⁻¹ biomass with total lipids of 41.33% and DHA of 39.16% of total fatty acids (Kalidasan et al., 2021a).

Indonesia has the largest mangrove area in the world, accounting for approximately 21% of the global mangrove cover, with a total area of about 3.3 million hectares (Spalding and Leal, 2024). Indonesia has several

conservation areas with extensive mangrove ecosystems, including Glagah Wangi Demak, which covers an area of 225.17 hectares (Rohmawati et al., 2022). The mangrove vegetation in Glagah Wangi Demak is dominated by *Avicennia marina*, *Avicennia alba*, and *Rhizophora mucronata* Lam. This area is characterized by salinity levels ranging from 34‰ to 35‰, pH values between 6 and 9, and temperatures ranging from 27°C to 28°C (Hutama et al., 2016).

So far, research on Thraustochytrids has been conducted in several mangrove areas across Indonesia. Suhendra (2022) carried out research in the mangrove ecosystem of Raja Ampat, West Papua, using the direct plating isolation method from decayed mangrove leaf samples. Hutari et al. (2022) conducted research in the mangrove area of Pari Island, Seribu Islands, Jakarta, using the direct plating method from decayed mangrove leaves. Meanwhile, Suhendra et al. (2023) conducted research in the mangrove forest of Kulon Progo, Yogyakarta, using the direct plating method from decayed mangrove leaves. However, there is a lack of studies that performed direct lipid confirmation through specific staining methods, such as Sudan Black B, nor did they include molecular identification based on the 18S rRNA gene or phylogenetic analysis to accurately determine species-level classification, as well as less similar research in Glagah Wangi Demak. Therefore, the present research offers added value and novelty by not only relying on morphological identification but also incorporating lipid detection using Sudan Black B staining, as well as conducting molecular and phylogenetic analyses on lipid-

positive isolates. The scope of this research is limited to the macroscopic and microscopic characterization, confirmation of lipid presence through Sudan Black B staining, and molecular identification of isolates that tested positive for high-lipid content. Mangrove leaves were selected in this research, specifically those that had fallen and decayed, with yellow, brownish yellow, brown, and black colors (Jaritkhuan and Suanjit, 2018). Mangrove leaves were chosen as the isolation substrate because, during the decomposition process, they release compounds such as glutamic acid and pectin, which are known to elicit a chemotactic response and attract Thraustochytrids in the surrounding environment (Kalidasan et al., 2021b). The isolation of Thraustochytrids was carried out using the pollen baiting and direct plating methods (Rosa et al., 2011; Gupta et al., 2013; Bagul and Annapure, 2021a; Lyu et al., 2021). The diversity was observed through macroscopic and microscopic characteristics. To determine the lipid potential, Sudan Black B staining was performed, in which lipid-positive cells appear blue-black, while lipid-negative cells appear pink (Manikan et al., 2015). Molecular identification of Thraustochytrids was conducted using the 18S rRNA genetic marker, as it provides a strong phylogenetic signal and can distinguish up to the genus level. Research by Cavalier-Smith et al. (1994) demonstrated that 18S rRNA is effective in grouping Thraustochytrids, which are phylogenetically closely related to heterokont algae (Ochromista), but not to true fungi or Mycetozoa. Analysis was conducted through sequencing, then compared with data in GenBank using BLAST, followed by phylogenetic analysis using MEGA XII to examine the

relationship of isolates with other Thraustochytrids isolates (Kumar et al., 2024).

1.2 Problem Formulation

- 1.2.1 How are the macroscopic and microscopic differences of Thraustochytrids present in decayed mangrove leaf samples from Glagah Wangi Demak?
- 1.2.2 What are the results of lipid detection in Thraustochytrids cells using the Sudan Black B staining method?
- 1.2.3 What are the molecular identities of Thraustochytrid isolates indicated as potential high lipid producers based on qualitative screening using Sudan Black B staining?

1.3 Research Objectives

- 1.3.1 Examine the macroscopic and microscopic differences of Thraustochytrids isolates obtained from decayed mangrove leaf litter collected from Glagah Wangi Demak.
- 1.3.2 Detect the presence or absence of lipid content in the Thraustochytrids isolates using the Sudan Black B staining method.
- 1.3.3 Identify the molecular characteristics of Thraustochytrids isolates indicated as potential high lipid producers based on qualitative Sudan Black B staining, using 18S rRNA gene sequencing and phylogenetic analysis

1.4 Research Benefit

- 1.4.1 Providing new data on the macroscopic and microscopic differences of Thraustochytrids from the Indonesian mangrove environment, especially from decaying mangrove leaves at Glagah Wangi Demak.
- 1.4.2 Support the exploration of Thraustochytrids as a source of natural lipids that have the potential to be utilized in further research in the fields of marine biotechnology, pharmaceuticals, and the food industry.
- 1.4.3 Provide the results of molecular identification and phylogenetic analysis of Thraustochytrid isolates indicated as potential high lipid producers based on qualitative Sudan Black B screening, as a basis for further research in the development of Thraustochytrid-based applications.