

## ABSTRACT

Yufiarta Hapsari, 24020121120003. **Isolation and Molecular Identification of Thraustochytrids from Decayed Mangrove Leaves in Mangrove Forest Glagah Wangi Demak.** Under the guidance of Anto Budiharjo and Shumpei Iehata.

Thraustochytrids are unicellular eukaryotic organisms from the Chromista kingdom that are osmo-heterotrophic and non-photosynthetic. Thraustochytrids are known as oleaginous organisms that can produce polyunsaturated fatty acids (PUFAs), particularly docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). Thraustochytrids are found in various habitats, such as seagrasses, algae, sediments, and fallen mangrove leaves in the mangrove forest area. This research aimed to isolate and characterize Thraustochytrid from decaying mangrove leaves at Glagah Wangi Beach, Demak. Isolation was performed using two methods (direct plating and pollen baiting). For the pine pollen method, decaying mangrove leaf samples were incubated at 28°C for 24 hours with antibiotics, antifungals, and pine pollen, then spread on GYP agar. Morphological identification was performed macroscopically and microscopically, followed by Sudan Black B staining to detect lipid content. Subsequently, molecular identification was conducted using the 18S001 and 18S13 primer set. The results of the study indicated the presence of 9 Thraustochytrids isolates, 5 from site I estuary and 4 from site II coastal water. Sudan Black B staining showed that DK1, DK4, and DK9 isolates exhibited strong positive lipid staining, suggesting the presence of intracellular lipid accumulation. Molecular analysis revealed the presence of two genera, *Aurantiochytrium* and *Schizochytrium*. Phylogenetic analysis showed that DK1 was closely related to *Aurantiochytrium* sp. TF88 (86%), DK4 was closely related to *Schizochytrium* sp. PLU-D86 (98%), DK9 was closely related to *Schizochytrium* sp. PLU-D11 (84%), indicating varying levels of confidence in genus-level identification. This study documents the presence of multiple Thraustochytrid genera across the two sampling sites and provides preliminary insights into isolates that display lipid accumulation, which may serve as candidates for future PUFA-focused investigations.

**Keywords:** *Thraustochytrids, Mangrove, Sudan Black B, Lipid, 18S rRNA*