

## Analysis of the Ecological Role and Biotechnology Potential of Marine Yeast *Rhodotorula* sp. in the Mangrove Forest of Pari Island, Jakarta

Andri Hutari(\*), Devi Anugrah, Husnin Nahry Yarza, Hilman Faruq,  
Muhammad Luqman Ar Rasyid

Department of Biology Education, Muhammadiyah University of Prof. Dr. Hamka,  
Jl. Tanah Merdeka No.20, RT.11/RW.2, Rambutan, Jakarta Timur,  
Daerah Khusus Ibukota Jakarta 13830, Indonesia

\*Corresponding author: andrihutari@uhamka.ac.id

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### Abstract

Mangrove forests are home to a diverse microbial population, which includes species that can convert organic waste from the mangrove forest into useful nutrients, thereby supporting the sustainability of the ecosystem. One such species is the marine yeast *Rhodotorula* sp. This yeast not only plays an important ecological role but also has tremendous biotechnological potential. It can be used to produce lipid omega-3, carotenoids, anti-bacterial agents, plant growth promoters, anti-cancer drugs, biosurfactants, and other high-value strategic products. Despite having the largest mangrove forest in the world, Indonesia has not yet fully exploited the biotechnological potential of marine yeast *Rhodotorula* sp. isolated from its mangrove forests. To address this, a study is being conducted to isolate the marine yeast *Rhodotorula* sp. from the mangrove forest of Pari island, Jakarta, and to review its ecological role and biotechnological potential. The ultimate goal of this study is to cultivate and utilize the marine yeast *Rhodotorula* sp. for its biotechnological potential in the future.

**Keywords:** *Rhodotorula* sp., Mangrove forest, Pari island, Biotechnology potential, Ecological roles.



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### INTRODUCTION

Yeast strains are in high demand by the biotechnology industry globally (Li *et al.*, 2022). One of the oleaginous yeasts that is known for its ability to produce valuable metabolites such as lipids, enzymes, and carotenoids is *Rhodotorula* sp. (Park *et al.*, 2018; Elfeky *et al.*, 2020; Li *et al.*, 2022). This yeast is a unicellular fungus that is commonly found in the open sea as well as in various other environments (Lane *et al.*, 2023). This particular type of yeast belongs to the kingdom Fungi, phylum Basidiomycota, class Microbotryomycetes, and order Sporidiobolales (Kot *et al.*, 2016)

. It is known for producing versatile biomass that contains a wide range of metabolites that are highly beneficial for the biotech industry (Byrtusová *et al.*, 2021). Some species of *Rhodotorula* sp. are oleaginous yeasts that can accumulate neutral lipids, mostly triglycerides (TAG), under certain culture conditions (Maza *et al.*, 2020).

The *Rhodotorula* genus is not a single group but rather a collection of species with different characteristics. Some of these species, called *Rhodotorula*, are single-celled and reproduce by budding or fission. Others, called *Rhodospiridium*, reproduce sexually and alternate between a yeast phase and a filamentous phase. Many of these species are known for their ability to produce valuable industrial products such as biofuel feedstocks, carotenoids, enzymes, and biosurfactants (Lyman *et al.*, 2019).

Many potential sources of marine yeast *Rhodotorula* sp. are found in mangrove forest areas, which are used in the nutraceutical, food, fisheries, and bioenergy industries (Miranda *et al.*, 2020). Indonesia, being home to the world's largest mangrove forests (Giri *et al.*, 2011), should have isolates of marine yeast *Rhodotorula* sp. from mangrove forests for widespread application in the biotechnology industry. The purpose of this study was to isolate the marine yeast *Rhodotorula* sp. from the mangrove forests of Pari Island, Jakarta, Indonesia, and analyze its potential for biotechnology. The isolate *Rhodotorula* sp. obtained will be preserved in a culture collection for future exploration of its biotechnological potential.

## **METHODS**

### **Time and Location of Research**

The research was conducted between June and September 2022 in the mangrove forest area of Pari Island, Jakarta, as well as in the Biology Education laboratory of the Faculty of Teacher Training and Education at Muhammadiyah University, Prof. Dr. Hamka (UHAMKA) Jakarta. The study involved isolating marine yeast *Rhodotorula* sp. from orange-colored mangrove leaves that had settled at the bottom of the mangrove waters.

### **Tools and Materials**

The tools required consist of plastic clips measuring 10x15 cm, Laminar Air Flow Cabinet, UV lamp, orbital shaker (DLAB), petri dish, tube needle, Bunsen, Erlenmeyer flask, autoclave, light microscope (Olympus BX43), USB microscope camera, and software (AmScope). The materials used consist of sea water, bacteriological agar (Oxoid), glucose (Himedia), yeast extract (Himedia), distilled water, Reef Salt (Aquaforest), aluminum foil, cotton gauze, cryovial (Corning) glycerol (Merck), and parafilm (M).

### **Sampling and Direct Plating**

In this research, samples of mangrove leaves that were submerged on the forest floor of Pari Island were collected. Only the leaves that were soft in texture and had an orange color were taken for further analysis. The yeast *Rhodotorula* sp. was isolated using the direct plating method, which was previously used by Hutari *et al.*, (2022).

### Microscopic Observation

Pure isolate of *Rhodotorula* sp. observed with a light microscope (Olympus BX43) and images taken using software (AmScope) after 2 days of direct plating.

### Identification of *Rhodotorula* sp.

To confirm that the obtained pure isolate belonged to the *Rhodotorula* genus, morphological identification was performed using the method described by Indratmi (2012).

### Short-term cultivation and easy maintenance of *Rhodotorula* sp.

A pure isolate of *Rhodotorula* sp. PP17 was successfully obtained and cultured in a 250 mL Erlenmeyer flask using AYA broth medium, as described by Hutari *et al.*, (2022). The culture was grown for 2 days at room temperature with an agitation speed of 200 rpm. After that, a master cell bank was established. A total of 1.2 mL of *Rhodotorula* sp. culture was mixed with 300  $\mu$ L of glycerol (Merck) and stored at  $-20^{\circ}\text{C}$  or lower in a 2 mL sterile cryovial (Corning).

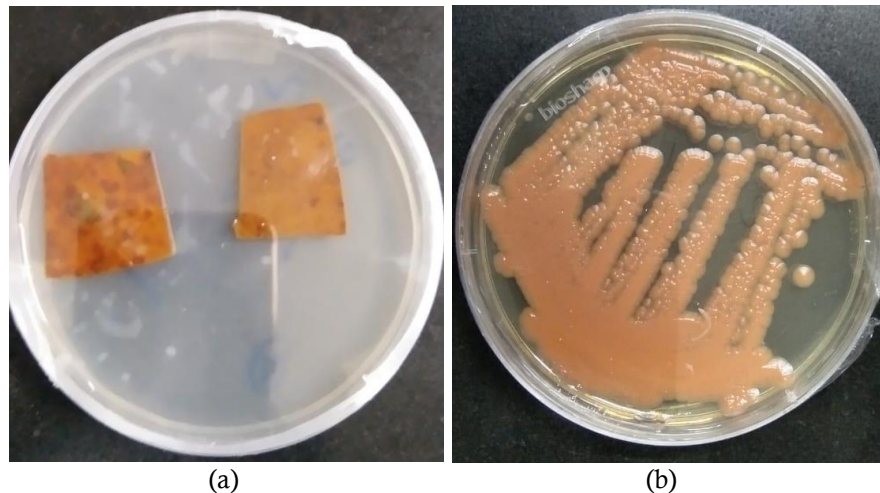
### Analyze the Role of Ecology and the Potential of Biotechnology

Analysis of the ecological role and biotechnological potential of *Rhodotorula* sp. carried out through a review of the latest relevant publications.

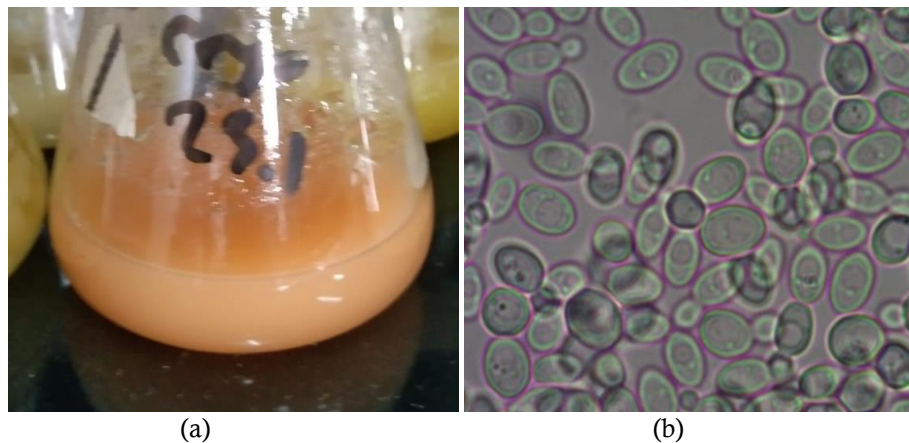
## RESULTS AND DISCUSSION

A total of 3 pure isolates of *Rhodotorula* sp. obtained from 20 mangrove leaves planted directly onto AYA agar (Figure 1a), namely PP16, PP17, and PP18. Next, PP17 isolate was used for short cultivation to obtain biomass for 2 days of cultivation and also to preserve it in the form of cryopreservation using 20% glycerol. Pure isolate of *Rhodotorula* sp. PP17 was then grown in AYA agar medium (Figure 1b) and in a 250 ml Erlenmeyer containing 100 ml AYA broth (Figure 2a). Micrograph of *Rhodotorula* sp. PP17 was documented using an Olympus BX43 microscope at 1000x magnification (Figure 2b).

*Rhodotorula* sp. PP17, which had been stored in a cryovial at  $-20^{\circ}\text{C}$ , was successfully grown again even after being stored for 12 months. This remarkable adaptation is noteworthy, given that cryopreservation is typically carried out at  $-80^{\circ}\text{C}$ . The ability of this species to regrow after being stored at non-ideal temperatures makes it a promising candidate for use as a workhorse in biotechnological applications that are becoming increasingly widespread (Park *et al.*, 2018). Generally, other microbes that are stored at  $-20^{\circ}\text{C}$ , such as the microalgae *Aurantiochytrium* sp. (although data was not shown), are unable to regrow after being stored at that temperature for one month.



**Figure 1.** Mangrove leaves planted directly onto agar medium (a); pure isolate of *Rhodotorula* sp. PP17 growing on AYA agar medium (b).



**Figure 2.** Culture of *Rhodotorula* sp. PP17 in AYA broth medium (a); Micrograph of *Rhodotorula* sp. PP17 with 1000x magnification (b)

The species plays an important ecological role as a decomposer, breaking down organic waste in mangrove forests into simpler substances that benefit other species in the ecosystem (Miranda *et al.*, 2020). Recently, Bogusławska-Wąs *et al.* (2019) found that *Rhodotorula* species naturally form symbiotic relationships with various marine fish by colonizing their digestive tracts. The *Rhodotorula* species exhibit a specific trait of biosynthesizing  $\beta$ -carotene, torulene, torularhodin, and exopolysaccharide (EPS) under fish digestive tract conditions. The interaction of *Rhodotorula* species with fish was concluded as a symbiotic relationship mechanism based on stimulation of nonspecific fish immunoprotection mechanisms and the yeast's antioxidant properties.

The genus *Rhodotorula* is known for its biotechnological potential, particularly as a carotenogenic yeast that can produce carotenoids (Byrtusová *et al.*, 2021; Elfeky *et al.*, 2020; Yoo *et al.*, 2016; Sharma & Ghoshal, 2020) such as beta-carotene, torulene, and torularhodin. This yeast can also produce a range of valuable metabolites, including pigments, ergosterol, lipids, and polysaccharides (Byrtusová *et al.*, 2021). Besides, *Rhodotorula* yeast is capable of producing isobutene (Fujii *et al.*, 1987), anti-bacterial pathogens (Hartati *et al.*, 2017; Vidya *et al.*, 2022), valuable enzymes (Kot *et*

*al.*, 2016); Zhang *et al.*, 2019; Li, *et al.*, 2022; Maza *et al.*, 2020), bio-emulsifier (Oloke & Glick, 2005), plant growth promoters (Hartati *et al.*, 2019), omega-3 fatty acids (Pino-Maureira *et al.*, 2021), exopolysaccharides (Silambarasan *et al.*, 2019), as well as biosurfactants (Gharaghani *et al.*, 2019).

The carotenoids produced by *Rhodotorula* yeast are increasingly being utilized as a replacement for synthetic carotenoids as supplements in poultry feed to increase poultry productivity (Grigore *et al.*, 2023). However, we must be aware of potential diseases that may be caused by the genus *Rhodotorula* sp. (Wirth & Goldani, 2012).

## CONCLUSION

The *Rhodotorula* sp. species can be found in Indonesian mangrove forests. It has huge potential and can be utilized as raw material in the strategic biotechnology industry. The *Rhodotorula* sp. PP17 species from the Pari Island mangrove forest has the ability to withstand cryopreservation storage at -20°C for at least 12 months, and possibly longer. To confirm its relationship to other *Rhodotorula* species, molecular identification is necessary. Additionally, analysis of the multifunctional metabolites produced by this species needs to be conducted before exploring it extensively in the livestock, food, cosmetics, and pharmaceutical industries.

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