http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2023.567.575

Research Article
Isolation and Characterization of *Thraustochytrium* Trk-23 Producing Docosahexaenoic Acid from North Kalimantan, Indonesia

[1,2]Witono Basuki, [1]Rofiq Sunaryanto, [1]Andri Frediansyah, [3]Ika Rahmatul Layly, [4]Yusnitati, [5]Reni Giarni and [6]Abdul Wakhid Shodiq

[1]Research Centre for Food Technology and Processing, National Research and Innovation Agency, Jl. Jogya-Wonosari Km 31,5, Gading, Playen, Gunung Kidul 55861, Yogyakarta, Indonesia
[2]Faculty of Pharmacy, Universitas Pancasila, Lenteng Agung, Jakarta 12640, Indonesia
[3]Research Center for Applied Microbiology, National Research and Innovation Agency, Cibinong Science Center, Jl. Raya Jakarta-Bogor, Cibinong, Bogor 16915, Indonesia
[4]Research Center for Process and Manufacturing Industry Technology, National Research and Innovation Technology, Energy Building 625, KST BJ Habibie, Puspiptek, Serpong Tangerang Selatan 15314, Indonesia
[5]Research Center for Agroindustry, National Research and Innovation Agency, LAPTIAB, Building 610, KST BJ Habibie, Puspiptek, Serpong, Tangerang Selatan 15314, Indonesia
[6]Research Center for Horticulture and Estate Crops, National Research and Innovation Agency, Cibinong Science Center, Jl. Raya Jakarta-Bogor, Cibinong, Bogor 16915, Indonesia

Abstract

Background and Objective: The mangrove forest located in Tarakan Bay, North Kalimantan Province, Indonesia is geographically distant from human settlement and industrial activities. Thus, it remains unaffected by the presence of human-generated waste and industrial pollutants. This study aims to isolate and characterize the microalgae Thraustochytrids from this location, which can produce docosahexaenoic acid (DHA, C22:6, n-3). It is anticipated that these microalgae possess the potential for commercial production of DHA. **Materials and Methods:** The fallen leaf sample was collected from the mangrove forest, then isolated and purified by scratching technique until a single colony state. The pure isolate then be identified by 18S rDNA. The sequences were then analyzed for similarities using the Basic Local Alignment Search Tool (BLAST). The phylogenetic trees were carried out using the MEGA 6 program. The choice of phylogenetic trees was based on maximum likelihood. **Results:** The identification 18S rDNA gene, a strain namely Trk-23, was identified as *Thraustochytrium* sp. In the optimization of the cell growth of this strain, it was found that *Thraustochytrium* Trk-23 has maximum growth at 6.0% glucose, 1.0% yeast extract and 50% natural seawater, at a pH of 5.0 and a temperature of 30°C. The maximum lipid content is 6.0 g and the DHA proportion is 41.6% of the total fatty acid content with a DHA yield is 2.5 g L^{-1}. **Conclusion:** Some places in North Kalimantan are still free from industrial pollution and rich with *Thraustochytrium* sp., which is why it can find *Thraustochytrium* Trk-23. Due to its potency, *Thraustochytrium* Trk-23 is the promising candidate microalgae strain for producing DHA commercially.

Key words: Characterization, docosahexaenoic acid, mangrove forest, North Kalimantan-Indonesia, pollution, *Thraustochytrium* Trk-23

Citation: Basuki, W., R. Sunaryanto, A. Frediansyah, I.R. Layly, Yusnitati, R. Giarni and A.W. Shodiq, 2023. Isolation and characterization of *Thraustochytrium* Trk-23 producing docosahexaenoic acid from North Kalimantan, Indonesia. Pak. J. Biol. Sci., 26: 567-575.

Corresponding Author: Witono Basuki, Research Center for Food Technology and Processing, Jl. Jogya-Wonosari Km 31.5, Gading, Playen, Gunung Kidul 55861, Yogyakarta, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Docosahexaenoic acid (DHA, 22:6, n-3), as a class of Polyunsaturated Fatty Acids (PUFAs), could prevent coronary disease as observed in the Inuit population of Greenland, which has low cardiovascular disease cases. The PUFAs are also important in preventing other diseases like stroke, diabetes, Alzheimer's, inflammatory bowel disease, depression and cancer. The DHA is an important nutritional element for the normal development of the eyes and brain[1]. Recently, DHA has become an important component in many kinds of healthy foods, not only for infants but also for children, adults and the elderly.

Conventionally, fish oil is used to produce DHA[2]. However, global stocks of fish have gradually declined since the 1980s because of overfishing some decades ago[3]. Moreover, mercury contaminants in fish become worrying to consumers. In addition, certain consumers dislike the fish odor. Recently, research has explored bacteria, fungi, microalgae and plants as new sources of DHA[4]. Thraustochytrids have gained much interest due to their accumulation of a high amount of DHA which can be used to enrich various healthy foods. Biomass cells of microalgae can also be used in aquaculture[5] and poultry[6].

The microalgae in the Class Labyrinthulomycetes live in the marine ecosystem and mangrove habitats, in which they are decaying mangrove leaves[1,7]. Recently, thraustochytrids have been found in the Antarctic[8,9], Vancouver Island, British Columbia[10], Iceland[11], Britain[12], a mangrove of Kerala Coast, India[13], Shenzhen province, China[14], Tasmania and Queensland, Australia[15], Subic Bay, the Philippines[16], the mangrove areas in Malaysia[17] and the mangrove area in Lampung, Indonesia[18].

This research aims to focus on the isolation and characterization of *Thraustochytrium* sp., from Tarakan Bay, North Kalimantan Province, Indonesia, which could produce DHA. It is anticipated that these microalgae can be used in commercial DHA production.

MATERIALS AND METHODS

Study area: This research was conducted at the Laboratory for Bioindustrial Technology for Agro and Biomedics (LAPTIAB), National Research and Innovation Agency (BRIN). Puspiptek, Serpong, Tangerang. The research began in March, 2021 to September, 2021.

Sampling location of thraustochytrids: The fallen leaves samples were taken from the mangrove forest area in Tarakan Bay, North Kalimantan Province, Indonesia. This particular site was selected based on its distance from industrial operations, ensuring a relatively pristine environment free from the contamination associated with industrial waste. It is anticipated that viable thraustochytrids can be observed within this particular geographical setting. The fallen foliage was collected from the ocean floor and subjected to a thorough cleansing process using sterile seawater to mitigate the presence of pollutants[19]. Around 100 pieces of fallen leaves from the seabed were taken. The samples were then put in an ice box for the isolation of microalgae in the laboratory. From 100 pieces of fallen leaves, it selected the good ones around 50 pieces. Then, each piece was cut into small pieces 2×2 cm^2 and then used for direct plating in the 50 pieces of agar plate containing culture media[20].

Isolation of microalgae: Microalgae were isolated by slightly modifying the method of Perveen *et al.*[20]. The plates were then incubated at 28°C for 3 to 5 days. The isolated microalga was then regrown in a new isolation medium. The cultivated microalgae were then separated into a single colony. The stock culture was produced according to Yokochi *et al.*[21]. The natural seawater was filtered before being used in the experiment.

Production of DHA: The Trk-23 microalga strain colony was inoculated with 10 mL of a culture medium described by Basuki[18]. The pH was set to 6. The microalga was previously precultured for 24 hrs on a shaker at 180 rpm and 30°C. After that, more or less 0.1 mL of culture broth was taken and put into 10 mL of culture media and, the microorganism was then grown with the procedure as described by Basuki[18]. The lipid was determined using the method described by Bligh and Dyer[22].

Cell growth and lipid analysis: The growth of the cell was monitored by measuring the increasing dry cell weight during fermentation. Two milliliters of culture broth were taken and washed to separate another component of the cell in the broth, then dried at 105°C for 3 hrs to remove the water and finally weighed. Sample preparation for gas chromatography analysis was done with the procedure described by Basuki[18]. The type of Gas Chromatography-Mass Spectra (GC-MS) equipment, type of column, gas carrier, speed, operating temperature and identification of fatty acids were described in Basuki[18]. The identification of strains also was done with the procedure as described by Basuki[18].

DHA production at various glucose concentrations: *Thraustochytrium* Trk-23 was put into 10 mL media with various glucose concentrations, which were 0, 1, 2, 3, 4, 5, 6, 8, 10, 12 and 15% and another ingredient was the same with described by Basuki[18]. The microorganism preparation and fermentation were carried out as mentioned above. The data were obtained in triplicates.

Effect of various nutrition and culture conditions: To determine the effects of various carbon sources, nitrogen sources, seawater concentration, pH and temperature, *Thraustochytrium* Trp-23 was grown in media containing various carbon sources (D-glucose, D-fructose, glycerol, molasses, olive oil and palm oil), various nitrogen sources (yeast extract, peptone, sodium glutamate, ammonium acetate and tempeh flour), various seawater concentrations (0, 25, 50, 75 and 100%), various temperatures (0, 10, 20, 30 and 40°C) and various pH values (0.0, 3.0, 4.0, 5.0, 6.0 and 7.0). The basic composition media and fermentation condition were the same as the procedure described above. Dry cell weight, lipid and DHA were determined by the methods mentioned above. The data were obtained in triplicates.

RESULTS

Isolation of thraustochytrids: In the isolation of microalgae strains, from 50 plates there were 13 isolates were grown and 7 isolates of them can produce DHA. From GC-MS chromatography analysis, Trk-23 was chosen as the best isolate because of its high DHA production. In the next experiment, Trk-23 was also the selected strain for identification and characterization. Figure 1 shows that, the Trk-23 strain has a spherical form and is a single-cell organism. This picture agrees with the description of thraustochytrids[1]. The 18S rDNA sequence of Trk-23 has 95% similarity to *Thraustochytrium* sp. The phylogenetic relationship of *Thraustochytrium* Trk-23 with 18S rDNA sequences was shown in Fig. 2. Therefore, the strain Trk-23 was named *Thraustochytrium* Trk-23.

Composition and profile fatty acid of *Thraustochytrium* Trk-23: The fatty acids profile was shown in Fig. 3 and the fatty acid composition of *Thraustochytrium* Trk-23 was shown in Table 1. The most abundant fatty acid produced by *Thraustochytrium* Trk-23 was DHA (22:6, n-3) amounting to 41.60%. The other fatty acids were tetradecanoic acid (14:0) 2.45%, pentadecanoic acid (15:0) 5.67%, hexadecenoic acid (16:0) 25.15%, heptadecanoic acid (17:0) 20.30%, 9-octadecenoic acid (19:1) 0.48%, octadecenoic acid (18:2) 1.78%, nonadecanoic acid (19:0) 0.83%, 5,8,11,14-eicosatetraenoic acid (20:4) 0.55%, eicosenoic acid (20:0) 0.42%, hexanediol acid (22:0) 0.17% and tetracosanoic 2,9-dimethyl methyl ester (22:0) 0.61.

DHA production at various glucose concentrations: To examine the DHA production at various glucose concentrations, various glucose concentrations were applied to know its effect on cell growth, lipid and DHA production (Fig. 4). Until 6% glucose concentration this nutrition increases cell growth, lipid and DHA production and at the 6.0% glucose concentration resulting in the highest level of dry cell weight (16.0 g L^{-1}) and DHA production (2.5 g L^{-1}). Increasing the glucose concentration by more than 6% did not increase the DHA concentration in *Thraustochytrium* Trk-23 (Fig. 4).

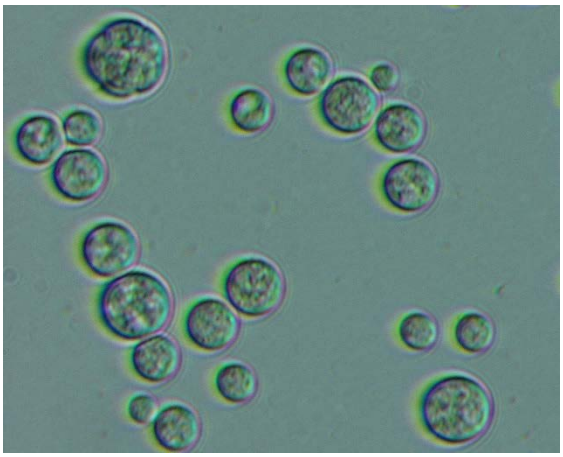

Fig. 1: Micrograph of *Thraustochytrium* Trk-23

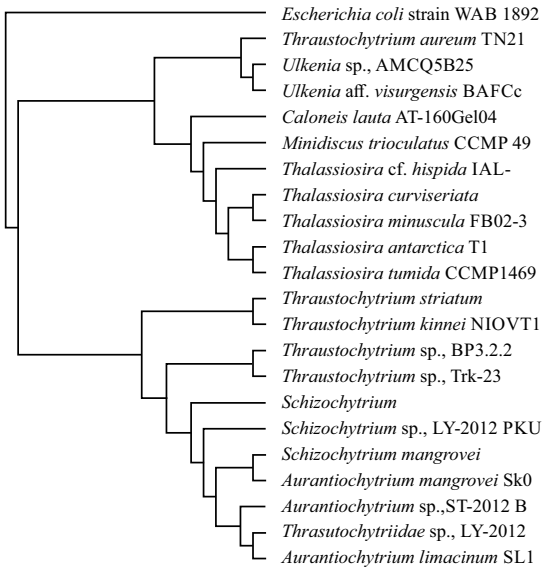

Fig. 2: Phylogenetic of *Thraustochytrium* Trk-23

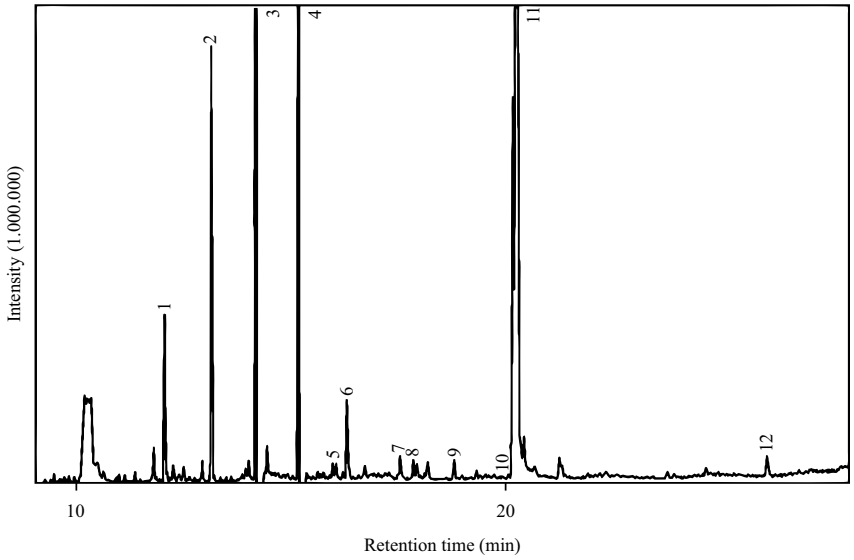

Fig. 3: Fatty acid profile of *Thraustochytrium* Trk-23

Table 1: Composition of fatty acid of the *Thraustochytrium* Trk-23

Peak No.	Retention time	Area (%)	Component
1	12.06	2.45	(14:0) Tetradecanoic acid, methyl ester
2	13.14	5.67	(15:0) Pentadecanoic acid, methyl ester
3	14.18	25.15	(16:0) Hexadecanoic acid, methyl ester
4	15.17	20.30	(17:0) Heptadecanoic acid, methyl ester
5	16.03	0.48	(19:1) 9-Octadecenoic acid, methyl ester
6	16.29	1.78	(18:2) Octadecenoic acid, methyl ester
7	17.54	0.83	(19:0) Nonadecanoic acid, methyl ester
8	17.84	0.55	(20:4) 5,8,11,14-Eicosatetraenoic acid, methyl ester
9	18.80	0.42	(20:0) Eicosanoic acid, methyl ester
10	19.54	0.17	(22:0) Hexanedioic acid, mono (2-ethylhexyl)
11	20.26	41.60	(22:6) 4,7,10.13,16,19-Docosahexaenoic acid, methyl ester
12	26.07	0.61	(24:0)Tetracosanoic 2,9-dimethyl-, methyl ester
		100.00	

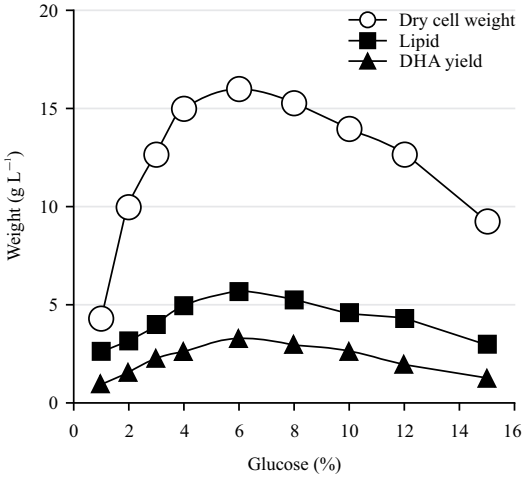

Fig. 4: DHA production at various glucose concentrations

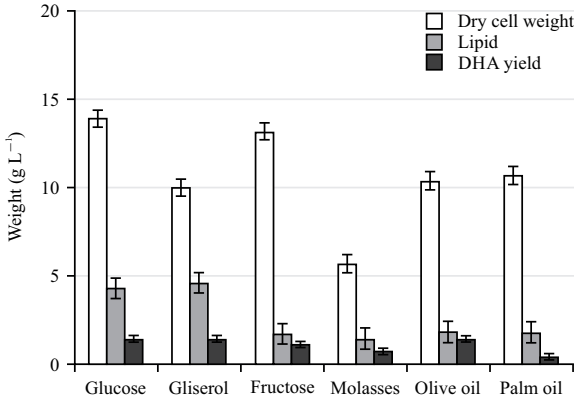

Fig. 5: Influence of various carbon sources on dry cell weight, lipid and DHA content

Influence of various carbon sources: *Thraustochytrium* Trk-23 was able to grow well in glucose, glycerol and fructose (Fig. 5). It means that these carbon sources supported good cell growth and DHA yield. *Thraustochytrium* Trk-23 was also able to grow well in molasses, olive oil and palm oil (Fig. 5), but gave low DHA production. The reason for this result may be because *Thraustochytrium* Trk-23 synthesizes DHA not through the elongase-desaturase pathway but using another pathway.

Influence of nitrogen sources on cell growth, lipid and DHA production: The most common source of nitrogen for the growth of *Thraustochytrium* Trk-23 is yeast extract. Figure 6 showed that the highest growth of *Thraustochytrium* Trk-23 and the highest DHA production was obtained with yeast extract, peptone and tempeh flour. *Thraustochytrium* Trk-23 can grow well when using tempeh flour but produces low DHA. Generally, this microalga can grow well in organic nitrogen sources but not inorganic nitrogen sources.

Influence of seawater concentration on cell growth: In general, *Thraustochytrium* Trk-23 can grow in 25 to 100% seawater concentrations (Fig. 7). These microalgae also can grow at zero salinity, but their cell growth is low. When seawater concentration was increased from 50 to 100%, there was almost no significant difference in cell growth, to avoid the effect of corrosion by high salt concentration on the metal instrument, 100% salinity was not used but 50% salinity was usually chosen.

Influence of temperature on cell growth: Interestingly, *Thraustochytrium* Trk-23 grows well at 30°C (Fig. 8) and when the temperature was increased to 40 the cell growth decreased drastically. The ability to grow in high temperatures

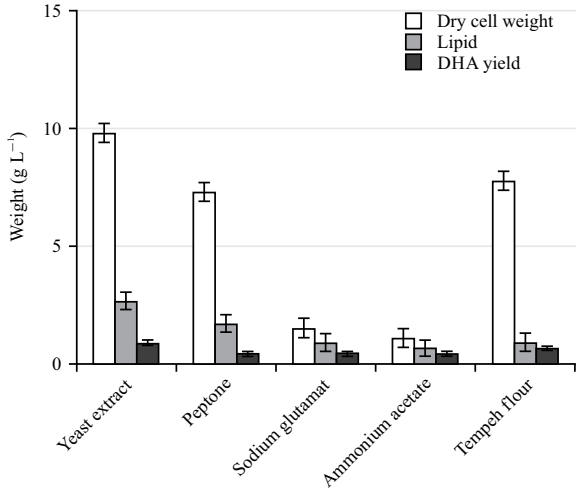

Fig. 6: Influence of nitrogen sources on cell growth, lipid and DHA production

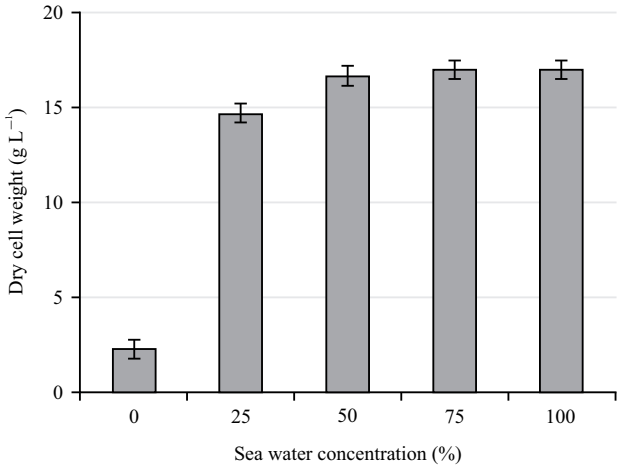

Fig. 7: Influence of seawater concentration on cell growth

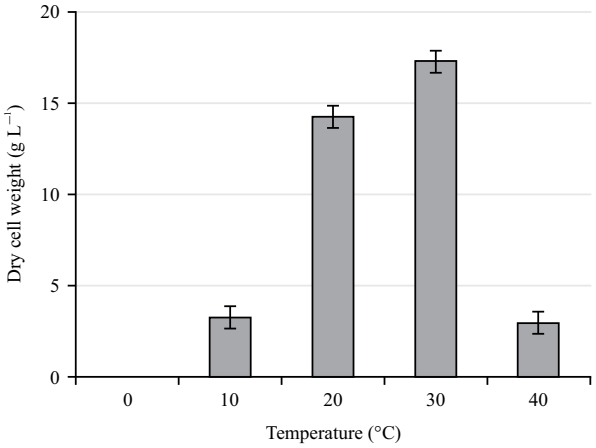

Fig. 8: Influence of temperature on cell growth

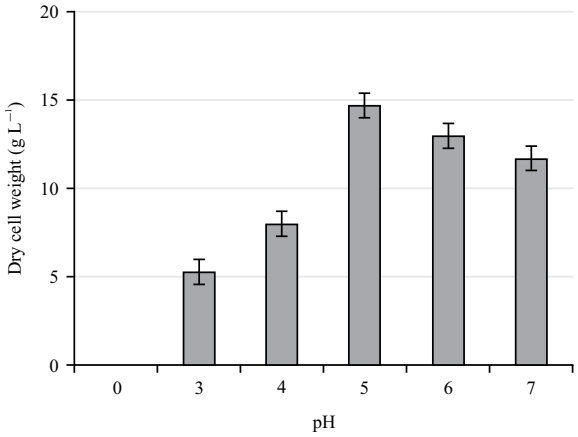

Fig. 9: Effect of pH on cell growth

because this microalga was isolated from a location that is very close to the equator which is known for its high temperatures. It may become a specific characteristic of tropical *Thraustochytrium* and its growth decreases drastically when the temperature becomes lower at 10°C. This optimum temperature is an advantage for Indonesia because it means that *Thraustochytrium* Trk-23 can be grown at room temperature. In contrast to *Thraustochytrium* strain that was isolated from Antarctica had an optimum temperature of 10°C and decreased its growth when the temperature was increased to 25°C.

Influence of pH on cell growth: The pH influences cell metabolism and product biosynthesis[23]. *Thraustochytrium* sp., was tried to grow medium in pH 3.0 to 7.0 in this experiment. Figure 9 showed that, *Thraustochytrium* Trk-23 grew best at pH 5 and growth was a little lower at pH 6.0 and 7.0. Even though *Thraustochytrium* Trk-23 has an optimum pH of 5.0, due to corrosion avoidance it is recommended to use pH 6.0 or pH 7.0 in growing this microalga.

DISCUSSION

The results section illustrated the micrograph of *Thraustochytrium* Trk-23, the phylogenetic of *Thraustochytrium* Trk-23, fatty acid profile of *Thraustochytrium* Trk-23, composition of fatty acid of the *Thraustochytrium* Trk-23, DHA production at various glucose concentrations; the Influence of various carbon sources on dry cell weight, lipid and DHA content; the influence of nitrogen sources on cell growth, lipid and DHA production; the influence of seawater concentration on cell growth; the influence of temperature on cell growth and effect of pH on cell growth.

The profile of fatty acid of *Thraustochytrium* Trk-23 (Fig. 3) is close to thraustochytrid from the mangrove area in Lampung, Indonesia[18], which has no DPA (docosapentaenoic acid, 22:5 n-6), a component fatty acid that is usually available in thraustochytrids from temperate countries, such as British thraustochytrids[12], China *Aurantiochytrium* SD 116[23] and Japan *Schizochytrium limacinum* SR12[21].

From the composition of fatty acid of the *Thraustochytrium* Trk-23, it can be found that total saturated fatty acid (SFA) is 55.60%, total unsaturated fatty acid (UFA) is 44.40% and SFA/UFA is 1.25. The composition that SFA is higher than UFA is typical for tropical microalgae because usually saturated fat is used as an energy reserve.

The content of DHA (22:6, n-3) 41.60% in *Thraustochytrium* Trk-23 is relatively higher than the other tropical *Thraustochytrium*, such as *Thraustochytrium aureum* isolated from Jawa Island Indonesia with DHA content is 9.2%[24]. The *Thraustochytrium* isolated from Malaysian mangroves has DHA content is around 1.5-38.4%[25] and *Thraustochytrium* isolated from Philippines mangroves has DHA content around 24-35% of total fatty acids[26]. In general, PUFA content in tropical microalgae is lower than in microalgae from Antarctica and temperate countries, because of the high-temperature stress[27].

Lipid synthesis of microalgae was affected by the C/N ratio[23]. A high C/N ratio will increase the lipid content of microorganisms[28]. However, increasing the glucose concentration by more than 6% did not increase the DHA concentration in *Thraustochytrium* Trk-23, most likely because *Thraustochytrium* Trk-23 produces DHA did not use the elongase-desaturase pathway in the production of the fatty acids[23].

The growing temperature at 30 may affect the metabolism of *Thraustochytrium* Trk-23, because of high-temperature stress. According to Teoh *et al.*[27], temperature stress may effect on lipid and fatty acid composition of microalgae. Jiang and Gao[29] found that reducing the temperature growth of *Phaeodactylum tricornutum* from 25 to 20°C, 15 or 10°C can increase the production of PUFA. The same result was reported by Hu and Gao[30] that cultures grown at lower temperatures can increase the PUFA and EPA content. In general, unsaturated fatty acids tend to increase at low temperatures because they increase membrane fluidity[31]. In future experiments, those findings may become a way to increase DHA content in tropical *Thraustochytrium* Trk-23.

CONCLUSION

This research shows that *Thraustochytrium* Trk-23, isolated from Tarakan, North Kalimantan Province, Indonesia, is a promising microalga for DHA production with good productivity. In the optimization of the cell growth of this strain, it was found that *Thraustochytrium* Trk-23 has maximum growth at 6.0% glucose, 1.0% yeast extract and 50% natural seawater, at a pH of 5.0 and a temperature of 30°C. The maximum lipid content is 6 g L^{-1} and the DHA is 41.6 % of the total fatty acid content with a DHA yield of 2.5 g L^{-1}. Due to its potency, *Thraustochytrium* Trk-23 is a promising candidate microalga strain for producing DHA Commercially.

SIGNIFICANCE STATEMENT

This study is important because it focuses on the isolation and characterization of *Thraustochytrium* sp., from Tarakan Bay, North Kalimantan Province, Indonesia, which could produce DHA. The results show that *Thraustochytrium* Trk-23, the best isolated from that place is a promising microalga for DHA production. The optimization found that *Thraustochytrium* Trk-23 has maximum growth at 6.0% glucose, 1.0% yeast extract and 50% natural seawater, at a pH of 5.0 and a temperature of 30°C. The maximum lipid content is 6 g L^{-1} and the DHA is 41.6 % of the total fatty acid content with a DHA yield of 2.5 g L^{-1}. Due to its potency, *Thraustochytrium* Trk-23 is a promising candidate microalga strain for producing DHA commercially.

ACKNOWLEDGMENT

The authors would like to acknowledge the people who assisted in this work become realized. This experiment was financed by the Agency for the Assessment and Application of Technology (BPPT) with Grant No: SP DIPA 081.01-0/2021.

REFERENCES

1. Jaseera, K.V. and P. Kaladharan, 2020. An overview of systematics, morphology, biodiversity and potential utilisation of thraustochytrids. J. Mar. Biol. Assoc. India, 62: 13-21.
2. Ganesan, B., C. Brothersen and D.J. McMahon, 2014. Fortification of foods with omega-3 polyunsaturated fatty acids. Crit. Rev. Food Sci. Nutr., 54: 98-114.
3. FAO., 2018. The State of World Fisheries and Aquaculture 2018: Meeting the Sustainable Development Goals. Food and Agriculture Organization, Rome, Italy, ISBN-13: 9789251305621, Pages: 227.
4. Adarme-Vega, T.C., D.K.Y. Lim, M. Timmins, F. Vernen, Y. Li and P.M. Schenk, 2012. Microalgal biofactories: A promising approach towards sustainable omega-3 fatty acid production. Microb. Cell Fact., Vol. 11. 10.1186/1475-2859-11-96.
5. Chang, K.J.L., C.C. Parrish, C.J. Simon, A.T. Revill and P.D. Nichols, 2020. Feeding whole thraustochytrid biomass to cultured atlantic salmon (*Salmo salar*) fingerlings: Culture performance and fatty acid incorporation. J. Mar. Sci. Eng., Vol. 8. 10.3390/jmse8030207.
6. Cherian, G., 2015. Nutrition and metabolism in poultry: Role of lipids in early diet. J. Anim. Sci. Biotechnol., Vol. 6. 10.1186/s40104-015-0029-9.
7. Morabito, C., C. Bournaud, C. Maës, M. Schuler and R.A. Cigliano *et al.*, 2019. The lipid metabolism in thraustochytrids. Progress Lipid Res., Vol. 76. 10.1016/j.plipres.2019.101007.
8. Caamaño, E., L. Loperena, I. Hinzpeter, P. Pradel and F. Gordillo *et al.*, 2017. Isolation and molecular characterization of *Thraustochytrium* strain isolated from Antarctic Peninsula and its biotechnological potential in the production of fatty acids. Braz. J. Microbiol., 48: 671-679.
9. Shene, C., P. Paredes, D. Vergara, A. Leyton and M. Garcés *et al.*, 2020. Antarctic thraustochytrids: Producers of long chain omega 3 polyunsaturated fatty acids. MicrobiologyOpen, Vol. 9. 10.1002/mbo3.950.
10. FioRito, R., C. Leander and B. Leander, 2016. Characterization of three novel species of Labyrinthulomycota isolated from ochre sea stars (*Pisaster ochraceus*). Mar. Biol., Vol. 163. 10.1007/s00227-016-2944-5.
11. Stefánsson, M.Ö., S. Baldursson, K.P. Magnússon, A. Eyþórsdóttir and H. Einarsson, 2019. Isolation, characterization and biotechnological potentials of thraustochytrids from icelandic waters. Mar. Drugs, Vol. 17. 10.3390/md17080449.
12. Marchan, L.F., K.J.L. Chang, P.D. Nichols, J.L. Polglase, W.J. Mitchell and T. Gutierrez, 2017. Screening of new British thraustochytrids isolates for docosahexaenoic acid (DHA) production. J. Appl. Phycol., 29: 2831-2843.

13. Jaseera, K.V., P. Kaladharan, K.K. Vijayan, S.V. Sandhya, M.L. Antony and M.A. Pradeep, 2019. Isolation and phylogenetic identification of heterotrophic thraustochytrids from mangrove habitats along the Southwest Coast of India and prospecting their PUFA accumulation. J. Appl. Phycol., 31: 1057-1068.
14. Wang, Q., H. Ye, B. Sen, Y. Xie, Y. He, S. Park and G. Wang, 2018. Improved production of docosahexaenoic acid in batch fermentation by newly-isolated strains of *Schizochytrium* sp. and *Thraustochytriidae* sp. through bioprocess optimization. Synth. Syst. Biotechnol., 3: 121-129.
15. Chang, K.J.L., G.A. Dunstan, G.C. J. Abell, L.A. Clementson, S.I. Blackburn, P.D. Nichols and A. Koutoulis, 2012. Biodiscovery of new Australian thraustochytrids for production of biodiesel and long-chain omega-3 oils. Appl. Microbiol. Biotechnol., 93: 2215-2231.
16. Arafiles, K.H.V., J.C.O. Alcantara, J.A.L. Batoon, F.S. Galura, P.R.F. Cordero, E.M. Leaño and G.R. Dedeles, 2011. Cultural optimization of thraustochytrids for biomass and fatty acid production. Mycosphere, 2: 521-531.
17. Hong, W.K., D. Rairakhwada, P.S. Seo, S.Y. Park, B.K. Hur, C.H. Kim and J.W. Seo, 2011. Production of lipids containing high levels of docosahexaenoic acid by a newly isolated microalga, *Aurantiochytrium* sp. KRS101. Appl. Biochem. Biotechnol., 164: 1468-1480.
18. Basuki, W., 2019. Bioconversion of glycerol to docosahexaenoic acid by *Thraustochytrium* WB-02 an indigenous Indonesian Microalga strain. Makara J. Sci., 23: 97-102.
19. Gupta, A., C.J. Barrow and M. Puri, 2012. Omega-3 biotechnology: Thraustochytrids as a novel source of omega-3 oils. Biotechnol. Adv., 30: 1733-1745.
20. Perveen, Z., H. Ando, A. Ueno, Y. Ito and Y. Yamamoto *et al*., 2006. Isolation and characterization of a novel thraustochytrid-like microorganism that efficiently produces docosahexaenoic acid. Biotechnol. Lett., 28: 197-202.
21. Yokochi, T., D. Honda, T. Higashihara and T. Nakahara, 1998. Optimization of docosahexaenoic acid production by *Schizochytrium limacinum* SR21. Appl. Microbiol. Biotechnol., 49: 72-76.
22. Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.
23. Gao, M., X. Song, Y. Feng, W. Li and Q. Cui, 2013. Isolation and characterization of *Aurantiochytrium* species: High docosahexaenoic acid (DHA) production by the newly isolated microalga, *Aurantiochytrium* sp. SD116. J. Oleo Sci., 62: 143-151.
24. Bajpai, P.K., P. Bajpai and O.P. Ward, 1991. Optimization of production of docosahexaenoic acid (DHA) by *Thraustochytrium aureum* ATCC 34304. J. Am. Oil Chem. Soc., 68: 509-514.
25. Ou, M.C., H.Y. Yeong, K.L. Pang and S.M. Phang, 2016. Fatty acid production of tropical thraustochytrids from Malaysian mangroves. Botan. Mar., 59: 321-338.
26. Kalidasan, K., N.V. Vinithkumar, D.M. Peter, G. Dharani and L. Dufossé, 2021. Thraustochytrids of mangrove habitats from Andaman Islands: Species diversity, PUFA profiles and biotechnological potential. Mar. Drugs, Vol. 19. 10.3390/md19100571.
27. Teoh, M.L., S.M. Phang and W.L. Chu, 2013. Response of antarctic, temperate, and tropical microalgae to temperature stress. J. Appl. Phycol., 25: 285-297.
28. Converti, A., A.A. Casazza, E.Y. Ortiz, P. Perego and M. del Borghi, 2009. Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. Chem. Eng. Process.: Process Intensif., 48: 1146-1151.
29. Jiang, H. and K. Gao, 2004. Effects of lowering temperature during culture on the production of polyunsaturated fatty acids in the marine diatom *Phaeodactylum tricornutum* (Bacillariophyceae). J. Phycol., 40: 651-654.
30. Hu, H. and K. Gao, 2006. Response of growth and fatty acid compositions of *Nannochloropsis* sp. to environmental factors under elevated CO_2 concentration. Biotechnol. Lett., 28: 987-992.
31. Bidigare, R.R., M.E. Ondrusek, M.C. Kennicutt, R. Iturriaga, H.R. Harvey, R.W. Haham and S.A. Macko, 1993. Evidence a photoprotective for secondary carotenoids of snow algae. J. Phycol., 29: 427-434.