Spatial diversity of microalgae in Simeulue Island, Indonesia

D C Purbani*, Y Yuliani and I N Sumerta
Laboratory of Microbial Systematic, Research Center for Biology, Indonesian Institute of Sciences, Cibinong, 16911, Indonesia
*Email: debora.christin.purbani@lipi.go.id, deborachristin22@gmail.com

Abstract. Microalgae naturally occur as valuable genetic resources for many industrial interests. However, the study on their diversity and potential as local strain resources, especially in Indonesia are limited. To assess the spatial diversity as basic information on exploring their further potential, we isolated microalgae from outer island-Simeulue, the Province of Aceh in four different habitats and locations. Seventeen cultures of microalgae were selected, purified, and maintained to the preferable conditions in IMK and AF6 media under light-based culture. Generally, the selected microalgae were referred to eukaryotic group based on morphological observation. BLASTn and phylogenetic analysis of 18S rRNA gene revealed 12 closely related species which are Chlorella kessleri, Chlorella sorokiniana, Oocystis heteromucosa, Stichococcus bacillaris, Coccomyxa melkonianii, Scenedesmus acuminatus, Scenedesmus bijugus, Coelastrella oocystiformis, Ankistrodesmus falcatus, Chlorococcum oleofaciens, Stigeoclonium tenue, and Planophila laetevirens. These species inhabit the spatial habitat and location of natural aquatic sources of Simeulue island. This study is expected to provide basic information for uncovering patterns of microalgae diversity and obtain new genetic resources for industrial uses as well as for taxonomic studies.

1. Introduction
Microalgae are a vast group of prokaryotic and eukaryotic phototrophic microorganisms which present in unicellular or simple multi-cellular and occur in all-natural water bodies such as ponds, dams, rivers, seas, and oceans [1]. They play a major role as producer of aquatic ecosystems which contain rich source of proteins, carbohydrates, and essential fatty acids [2]. Therefore, microalgae become important aquatic microorganisms for energy, livestock, health, food, and cosmetics sectors [3]. Many countries have been utilizing microalgae for various purposes such as algae producing oil for biodiesel, omega 3 sources and cosmetics; algae’s carbohydrates for bio-ethanol purposes [4,5]; algae’s protein for animal feed and natural pigments; algae’s biomass for aquaculture feed and organic fertilizer [6,7]; and waste decomposer/bioremediation agents [8]. In addition, they are also source of antioxidants, carrageenan, biopolymers, bio-hydrogen, sources of vitamins, antifungal agents, antimicrobials, and antivirals [9–11].

Indonesia, as a tropical maritime country with high sunlight intensity, has the potential to have high diversity of microalgae. However, the information regarding microalgae diversity in Indonesia is very limited. Therefore, the exploration of microalgae from Indonesian resources needs to be continued in spatial scales. Conducting spatial isolation is essential that may imply the microalgae community structure and biodiversity sustainability, which also beneficial for the future in energy, livestock, health, food, and cosmetics sectors.
The present study was carried out to isolate and identified microalgae from one of the outer islands—Simeulue which is located in the Indian Ocean, 105 nautical miles (194.46 km) from Meulaboh-Aceh, Sumatera mainland. This region has a wide natural aquatic ecosystem to view the pattern for spatial microalgae diversity in fresh and sea water. By isolating this microorganism, we expected to obtain microalgae’s cultures as a national treasure of biological resources and promising use in the forthcoming.

2. Materials and Methods

2.1. Sample sites and isolation of microalgae
Water samples were collected from four different locations in Simeulue Island, Indonesia, namely Langi Beach (02°49'10.4''N 95°46'20.1''E), Batu Alafan Beach (02°49'36.3''N 95°45'12.0''E), Kuala Makmur Rivers (02°33'16.8''N 96°15'13.1''E), and Lake Laut Tawar (02°50'25.64''N 95°48'54.2''E). For the microalgae collection procedure, we applied random sampling method and measured several physio-chemical parameters including water temperature and pH from the surface and bottom of the water on each sampling site. Microalgae were then isolated using the capillary micro-pipetting method under the Olympus CKX41 light microscope, purified, and cultivated under light conditions in AF6 (freshwater) and IMK (marine) liquid medium. The microalgae culture selection was based on their growth, morphology, and homogeneity [12].

2.2. Morphological analysis
We performed microscopic observation under Olympus BX5 light microscope to select and identify the microalgae culture. To figure the appearance of the culture, the microphotography was conducted to the attached Olympus DP26. Taxa identification referred to the standard taxonomic manual books by observing the morphological forms of chloroplasts, filaments, shape and size of the cells [13,14].

2.3. Molecular and phylogenetic analysis
Molecular identification was performed by preparing microalgae from freshly grown cells on the liquid medium. DNA template was extracted by using the Genomic DNA mini kit (Plant) Geneaid, following the manufacture protocol. One µl of lysed microalgae cell was used for PCR amplification of the partial 18S rRNA gene with primer 18SF (5’ CCA ACC TGG TTG ATC CTG CCA GTA 3’) and 18SR (5’ CCT TGT TAC GAC TTC ACC TTC CTC T 3’) using the GoTaq master mix. The PCR program ran at pre denaturation 94°C for five minutes, 35 cycles of denaturation 94°C for one minute, annealing 63°C for one minute, and extension 72°C for one minute, then final extension at 72°C for 10 minutes, and storage at 4°C [15]. The PCR amplicons were then visualized on 2% agarose for confirmation and sequenced to 1stBASE (Malaysia). The trace of the amplicons from forward and reverse primers were aligned using ChromasPro version 1.6 to determine the 18S rRNA gene sequences. Subsequently, Basic Local Alignment Search Tool (BLAST) for nucleotide was carried out to compare the homology among other type species on public databases (GenBank/ENA/DDBJ). Meanwhile, to figure the phylogenetic tree, all sequences were aligned using Clustal W and the distance matrix was calculated using the two-parameter of kimura methods on MEGA X software. Finally, the tree was constructed by applying the Neighbor-Joining (NJ) with 1000 bootstrap welding methods [16] with Chilomonas paramecium (L28811) as an outer group.

3. Result and Discussion
A total of 17 strains of microalgae were isolated and identified from water samples in Simeulue Island, Indonesia and grouped into green algae based on the morphological observation (figure 1). This was the first step for grouping microalgae which the taxonomic placement was listed on table 1.
Figure 1. Representative morphology of isolated microalgae from Simeulue Island: (A) Chlorella kessleri, (B) Chlorella sorokiniana, (C) Oocystis heteromucosa, (D) Stichococcus bacillaris, (E) Coccomyxa melkonianii, (F) Scenedesmus acuminatus, (G) Scenedesmus bijugus, (H) Coelastrella oocystiformis, (I) Ankistrodesmus falcatus, (J) Chlorococcum oleofaciens, (K) Stigeoclonium tenue, and (L) Planophila laetevirens.
Table 1. Diversity of microalgae species isolated from different habitats in Simeulue Island, Indonesia.

| Taxonomic placement | Species                        | Origin               | Organism ID |
|---------------------|--------------------------------|----------------------|-------------|
| Chlorophyta,        | *Chlorella kessleri*          | Langi Beach          | M17-S22     |
| Trebouxiophyceae,   |                                |                      |             |
| Chlorellales,       |                                |                      |             |
| Chlorellaceae       |                                |                      |             |
| Chlorophyta,        | *Chlorella sorokiniana*        | Batu Alafan Beach    | M17-S24     |
| Trebouxiophyceae,   |                                |                      |             |
| Chlorellales,       |                                |                      |             |
| Oocystaceae         |                                |                      |             |
| Chlorophyta,        | *Oocystis heteromucosa*        | Kuala Makmur Rivers  | M17-S14     |
| Trebouxiophyceae,   |                                |                      |             |
| Chlorellales,       |                                |                      |             |
| Oocystaceae         |                                |                      |             |
| Chlorophyta,        | *Stichococcus bacillaris*      | Kuala Makmur Rivers  | M17-S11     |
| Trebouxiophyceae,   |                                |                      |             |
| Trebouxiophyceae or| *Coccomyxa melkonianii*        | Kuala Makmur Rivers  | M17-S15     |
| incertae sedis,     |                                |                      |             |
| Coelastrellaceae    |                                |                      |             |
| Chlorophyta,        | *Scenedesmus acuminatus*       | Lake laut Tawar      | M17-S23     |
| Chlorophyceae,      |                                |                      |             |
| Shaeropleales,      | *Scenedesmus bijugus*          | Langi Beach          | M17-S21     |
| Scenedesmaceae      |                                |                      |             |
| Coelastrella        | *Coelastrella oocystiformis*   | Batu Alafan Beach    | M17-S07     |
| Chlorophyta,        | *Ankistrodesmus falcatus*      | Kuala Makmur Rivers  | M17-S12     |
| Chlorophyceae,      |                                |                      |             |
| Shaeropleales,      |                                |                      |             |
| Selenastraceae      |                                |                      |             |
| Chlorophyta,        | *Chlorococcum oleofaciens*     | Kuala Makmur Rivers  | M17-S10     |
| Chlorophyceae,      |                                |                      |             |
| Chlamydomonadaceae  |                                |                      |             |
| Chlorococaceae      |                                |                      |             |
| Chlorophyta,        | *Stigeoclonium tenue*          | Kuala Makmur Rivers  | M17-S17     |
| Chlorophyceae,      |                                |                      |             |
| Chaetophorales,     |                                |                      |             |
| Chaetophoraceae     |                                |                      |             |
| Chlorophyta,        | *Planophila laetevirens*       | Langi Beach          | M17-S13     |
| Ulvopyceae,         |                                |                      |             |
| Ulotrichales,       |                                |                      |             |
| Planophilahecemaceae|                                |                      |             |

Molecular approach supported the certainty in lower-level taxon. Taxonomically, they belong to *Chlorella kessleri*, *Chlorella sorokiniana*, *Oocystis heteromucosa*, *Stichococcus bacillaris*, *Coccomyxa melkonianii*, *Scenedesmus acuminatus*, *Scenedesmus bijugus*, *Coelastrella oocystiformis*, *Ankistrodesmus falcatus*, *Chlorococcum oleofaciens*, *Stigeoclonium tenue*, and *Planophila laetevirens*. They had high homology consensus in range of 99-100% compared to the type strain in the public databases. The phylogenetic tree emphasized the taxonomic placement of the obtained microalgae cultures which were related to 12 species in eight clades (figure 2). Clade *Coelastrella* consisted of three closely related genera which are *Coelastrella* (3 strains), *Scenedesmus* (2 strains), and *Ankistrodesmus* (2 strains); clade *Stigeoclonium* (1 strain); *Chlorococcum* (1 strain); *Chlorella* (3 strains); *Stichococcus* (1 strain); *Coccomyxa* (1 strain); *Oocystis* (2 strains); and clade *Planophila* (1 strain).
Figure 2. Phylogram of microalgae isolated from different habitats in Simeulue Island, Indonesia, based on 18S rDNA sequences.
Each of the isolated microalgae has unique characteristic and function. The Chlorophyceae which consisted of Shaeropleales, Chlamydomonadales, and Chaetophorales have vegetative nonmotile unicellular or colonial taxa with biflagellate zoospores and DO (directly opposed) basal body orientation [17]. Scenedesmus and Ankistrodesmus are Shaeropleales genera which are capable of producing biofuels and high-value antibacterial compounds [18,19]. Meanwhile, Coelastrella is a carotenoids producer (including β-carotene, lutein, free astaxanthin, canthaxanthin, and phytofluene) [20]. The order Chlamydomonadales mostly have characteristic unicellular or colonial flagellates, with basal body orientation CW (displaced in a “clockwise”), and have two or four flagella [17]. Chlorococcum of the Chlamydomonadales group may be used for the flocculation of lipids from wastewater [21]. Another order, the Chaetophorales are semiterrestrial and freshwater filamentous algae with various morphological complexity of an upright, a prostrate system and development of branching. They are distributed worldwide and can be found either as an individual organism or as a part of biofilms [22]. Only one genus of this class which is Stigeoclonium that may be a prospective candidate for biodiesel [23].

Next genera of Chlorella, Oocystis, Coccomyxa, and Stichococcus belonged to Trebouxiophyceae which mostly represented in coccoid cells in freshwater and coastal marine environments in Simeulue Island. Their characteristics are found in unicellular or colonial algae that are propagated asexually by autosores or zoospores with CCW (rotates in counterclockwise) basal body orientation of flagellated stage. Many species of this group are known to form autosores which can only be classified through molecular data and live in symbiosis with fungi in lichen thallia [17]. Chlorella is known for food source as it could produce a high protein content and beneficial for health by reducing cholesterol and producing antioxidants [24]. Oocystis and Coccomyxa are also known to have high lipid content and can produce bio-oil and antioxidants [25–27]. Lastly, isolated freshwater Ulvophyceae from Simeulue Island is represented in one genus Planophila, which morphologically consists of filamentous and thallous green algae with bi- or quadriflagellated zooids from CCW basal body orientation [17]. There is not much information about the characteristics and utilization of Planophila.

Members of Chlorophyceae were predominantly found among all cultured microalgae which no single species possessed a higher density than others meaning that they presented in equal density throughout the water samples. This result reflects that Simeulue aquatic ecosystems were in the healthy condition providing resources to many microalgae. Meanwhile, almost all species were found in different habitats and locations meaning that each species was found in fresh water or sea water (table 1). Interestingly, Chlorella sorokiniana, Ankistrodesmus falcatus, and Coelastrella oocystiformis were found in both fresh and sea water. Generally, microalgae family of Trebouxiophyceae, Chlorophyceae, Ulvophyceae are often found in both of fresh and sea water [28].

The growth of microalgae on aquatic ecosystem is supported by the physio-chemical properties, such as temperature and pH. The water temperature on site locations was in range of 25-30°C and pH from 6.9 to 8.1. These conditions can support the life processes of the aquatic living organisms with optimal temperature for microalgae generally between 24°C and 28°C although this may vary depending on species type and strain. On the other hand, the pH range for most microalgae is between 7 and 9, with the optimum between 6.5-9.5 [29]. The pH was slightly higher in Kuala Makmur Rivers, which may be attributed to the increased of photosynthetic activity in the aquatic body and the demand of more CO₂ compare to the result from respiration and decomposition [30]. Therefore, pH is an important quality parameter that could affect the survival and nutrition of biological life in water community.

This exploration, isolation, and preservation of microalgae are essential to be conducted to unravel the biological resources of Indonesian archipelago which has many potential benefits in various life aspects. Microalgae could provide another perspective for answering the future challenge in science, innovation, and technology development. Furthermore, delineating the microalgae biodiversity could impact on conserving natural resources in Indonesia which become a big challenge for sustainable development.
4. Conclusion
Microalgae isolated from Simeulu Island, Indonesia, increased the number of spatial diversity in fresh and sea water from various habitats and locations. They consisted of three major classes Shaeropleales, Chlamydomonadales, and Chaeotophorales which were closely related to Chlorella kessleri, Chlorella sorokiniana, Oocystis heteromucosa, Stichococcus bacillaris, Coccomyxa melkonianii, Scenedesmus acuminatus, Scenedesmus bijugus, Coelastrella oocystiformis, Ankistrodesmus falcatus, Chlorococcum oleofaciens, Stigeoclonium tenue and Planophila laetevirens. This information supports the importance of Indonesia as biodiversity hot spot for taxonomic study based on genetic resources and bioprospecting in the future.

Acknowledgments
Microalgae in this study were isolated and identified as a part of the Research Center for Biology-LIPI fund on DIPA Project in 2017. The authors also acknowledge to Gita Azizah Putri for her laboratory assistance.

References
[1] Van Den Hoek C, Mann D G and Jahns H M 1995 Algae: an Introduction to Phycology (England: Cambridge University Press) p 623
[2] Plaza M, Santoyo S, Jaime L, García-Blairsy Reina G, Herrero M, Señoráns FJ, and Ibanez E 2010 J Pharm Biomed Anal 51(2) 450-455
[3] Khan M I, Shin J H and Kim J D 2018 Microb Cell Fact 17(36) 1-21
[4] Chen J, Li J, Dong W, Zhang X, Tyagi RD, Drogui P, and Surampalli R. Renew Sustain Energy Rev 90 336-346
[5] Wang H M D, Chen C C, Huynh P and Chang J S 2015 Bioresour Technol 184 355-362
[6] Shields R and Lupatsch I 2012 TATuP - Zeitschrift Für Tech Theor Und Prax 21 23-37
[7] Alam T 2019 J Agric Mar Sci 23 81-91
[8] Kshirsagar AD 2013 Int J LifeSc Bi Pharm Res 2(3) 2250-3137
[9] Shalaby E A, Atta M B, Sleem I A, Mohamed M A, Lightfoot DA and El-Shemy H A 2019 Waste and Biomass Valorization 10 1419-1431
[10] Khetkorn W, Rastogi R P, Incharoensakdi A, Lindblad P, Madamwar D, Pandey A, and Larroche C 2017 Bioresour Technol 243 1194-1206
[11] Batista A P, Nunes M C, Raymundo A, Gouveia L, Sousa I, Cordóbés F, Guerrero A and Franco J 2011 Food Hydrocoll 25(4) 817-825
[12] Barsanti L and Gualtieri P 2014 Algal Culturing (Italy: CRC Press) p 221–66
[13] Naselli-Flores L and Barone R 2010 Green Algae. 2010 (Boston, London: Academic) p. 166–73
[14] Bellinger E G, Sigee DC 2015 Freshwater Algae: Identification, Enumeration and Use as Bioindicators 2nd Ed (New Jersey: Wiley-Blackwell) p 296
[15] Tale M, Ghosh S, Kapadnis B and Kale S 2014 Bioresour Technol 169 328–35
[16] Kumar S, Stecher G, Li M, Knyaz C and Tamura K 2018 Mol Biol Evol 35(6) 1547–1549
[17] Krienitz L 2009 Algae (Encycl. Inl. Waters) Reference Module in Earth Systems and Environmental Sciences Ed (Cambridge, MA: Academic Press) pp 103-113
[18] Ishaq A G, Matias-Peralta H M and Basri H 2016 Pertanika J Trop Agric Sci 29(1) 1-16
[19] Nascimento I A, Marques S SI, Cabanelas I T D, Pereira S A, Druzian J I, de Souza C O, Vich D, de Carvalho G, and Nascimento M 2013 Bioenergy Res 6 1-13
[20] Dimitrova P, Marinova G and Pilarski P 2016 Nat Math Sci 6(3) 139-49
[21] Aravantinou A F, Theodorakopoulos M A and Manariotis I D 2013 Bioresour Technol 147 130-134
[22] Michetti K M, Leonardi P I and Cáceres E J 2010 Phycol Res 58(1) 35-43
[23] Kim B H, Kim D H, Choi J W, Kang Z, Cho D H, Kim J Y, et al. 2015 J Microbiol Biotechnol 25(9) 1547-1554
[24] Silva J, Alves C, Pinteus S, Reboleira J, Pedrosa R and Bernardino S 2019 Chlorella in
Nonvitamin and Nonmineral Nutritional Supplements (Cambridge, MA: Academic Press) pp 187–193

[25] Ruiz-Domínguez M C, Vaquero I, Obregón V, de la Morena B, Vílchez C and Vega J M 2015 *JAppl Phycol* **27**(3) 1099-1108

[26] Csavina J L, Stuart B J, Guy Riefler R and Vis M L 2011 *J Appl Microbiol* **111**(2) 312-318

[27] Gargano I, Marotta R, Andreozzi R, Olivieri G, Marzocchella A, Spasiano D, Pinto G and Pollio A 2016 *N Biotechnol* **33**(6) 797-806

[28] Borowitzka M A, John B and John A R 2016 *The Physiology of Microalgae* (Switzerland: Springer International Publishing) p 673

[29] Lee E, Jalalizadeh M and Zhang Q 2015 *Algal Res* **12** 497-512

[30] Besson A and Guiraud P 2013 *Bioresour Technol* **147** 464-470