MOLECULAR IDENTIFICATION AND PHYLOGENY OF FRESHWATER MICROALGAE IN NABAO LAKE, CABIAO, NUEVA ECIJA

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An Undergraduate Thesis Submitted to the Faculty of the Department of Biological Sciences, College of Arts and Science, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines
In Partial Fulfillment of the Requirements
for the Degree of

BACHELOR OF SCIENCE IN BIOLOGY
(Major in Biotechnology)

JUNE 2019

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ABSTRACT

MORENO, LOUISE KATHREEN V., Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, **JUNE 2019, MOLECULAR IDENTIFICATION AND PHYLOGENY OF FRESHWATER MICROALGAE IN NABAO LAKE, CABIAO, NUEVA ECIJA**

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Microalgae are unicellular photosynthetic organisms which belong to a diverse group. This study focused on identification of microalgae in Nabao Lake, Cabiao, Nueva Ecija. Microalgae were isolated through serial dilution and cultures were stored in room temperature with continuous light and aeration. Urea was used to promote the growth of microalgae. Morphological features were observed and used for identification. The gene marker, rbc*L* was used for molecular identification and it was also used to infer phylogenetic tree using MEGA. Phytochemical components of the isolated microalgae were also determined through TLC.

Morphological and molecular identification revealed microalgal isolate NA as *Desertella yichangensis*. NB was identified as *Monoraphidium contortum* based on morphological characteristics. Phylogenetic analysis constructed for *D. yichangensis* showed genetic divergence of the species. Additionally, phytochemical screening suggested the presence of fatty acids, sugars, triterpenes, coumarins, alkaloids, tannins, phenols flavonoids, essential oils, and steroids for both microalgal extract. Aside from the mentioned secondary metabolites, *D. yichangensis* contains sugar while *M. contortum* contains anthrones.

LITERATURE CITED

Adhoni, S. A., Thimmappa, S. C., Kaliwal, B. B. & District, D. (2016). Phytochemical analysis and antimicrobial activity of Chorella vulgaris isolated from Unkal Lake. *Journal of Coastal Life Medicine*, 4(5), 368–373.

Amir, A. & Singh, S. (2018). Microalgae as promising and renewable energy source: a review. *Journal of Fundamentals of Renewable Energy and Applications*, 8(4), 8–11.

Arguelles, E. D. L. R. & Monsalud, R. G. (2018). Morphotaxonomy and Diversity of Terrestrial Microalgae and Cyanobacteria in Biological Crusts of Soil from Paddy Fields of, 11(2), 1-12.

Bhagavathy S., Sumathi P. & Bell., J. S .I. (2011). Green algae *Chlorococcum humicola*-a new source of bioactive compounds with antimicrobial activity. *Asian Pacific Journal of Tropical Biomedicine*, 1(1), 1–7.

Becker, E. W. (2007). Micro-algae as a source of protein. *Biotechnology Advances*, 25(2), 207–210.

Behera S., Singh R., Arora R., Kumar Sharma N., Shukla M. & Kumar S. (2014). Scope of algae as third generation biofuels. *Frontiers in Bioengineering and Biotechnology*, 2(1), 13-15.

Bell, K. L., Loeffler, V. M. & Brosi, B. J. (2017). An rbcL reference library to aid in the identification of plant species mixtures by DNA metabarcoding. Applications. *Application in Plant Science*, 5(3), 3–9.

Bellinger, E.G. & Sigee, D. C. (2015). Freshwater Algae: Identification and Use as Bioindicators. New Jersey, USA: John Wiley and Sons Ltd.

Benemann, J.R., & Oswald, W.J. Systems and Economic Analysis of Microalgae Ponds for Conversion of CO{Sub 2} To Biomass. United States: US Department of Energy.

Bhadury, P. & Wright, C. P. (2004). Exploitation of marine algae: biogenic compounds for potential antifouling application. *Planta*, 219(4), 561–578.

Bishop, M. & Zubeck, M. (2012). Evaluation of microalgae for use as nutraceuticals and nutritional supplements. *Journal of Nutrition & Food Sciences*, 2(5), 1067-1071.

Boyer, S. L., Flechtner, V. R. & Johansen, J. R. (2001). Is the 16S–23S rRNA internal transcribed spacer region a good tool for use in molecular systematics and

population genetics? A case study in cyanobacteria. *Molecular Biology and Evolution*, 18(6), 1057-69.

Brennan, L. & Owende, P. (2010). Biofuels from microalgae review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable Sustainable Energy Reviews*, 14(2), 557-77.

Chacón-Lee T. L. & González-Mariño G. E. (2010). Microalgae for ``healthy" foods_ possibilities and challenges. *Comprehensive Reviews in Food Science and Food Safety*, 9(6), 655-675 .

Dai, J. & Mumper, R. J. (2010). Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. *Molecules*, 15(10), 7313–7352.

Danesi, E.D.G., Rangel, C.O., Carvalho, J.C.M. & Sato, S. (2002). An investigation of effect of replacing nitrate by urea in the growth and production of chlorophyll by Spirulina platensis. *Biomass and Bioenergy*, 23(4), 261–269.

Darienko, T., Gustavs, L., Mudimu, O., Menendez, C. R., Schumann, R., Karsten, U. & Menendez, C. R. A. D. (2010). Chloroidium , a common terrestrial coccoid green alga previously assigned to Chlorella (Trebouxiophyceae , Chlorophyta). *Europeaan Journal of Phycology*, 45(1), 75-95.

Dayong, S., Jing, L., Shuju, G. & Lijun, H. (2008). Antithrombotic effect of bromophenol, the alga-derived thrombin inhibitor. *Journal of Biotechnology*, 136(8), 577–588.

Devi, G.K., Manivannan, K., Thirumaran, G., Rajathi, F.A.A. & Anantharaman, P. (2011). *Invitro* antioxidant activities of selected seaweeds from Southeast coast of India. *Asian Pacific Journal of Tropical Medicine*, 4(3), 205– 211.

Dhanalakshmi, M. & Angayarkanni, J. (2013). Phytochemistry And Antibacterial Activity Of Chlorosarcinopsis Species. *International Journal of Scientific & Technology Research*, 2(10), 315–321.

Dragone G., Fernandes B. D., Vicente A. A. & Teixeira J. A. (2010). Third Generation Biofuels from Microalgae. Badajoz, Spain: Formatex Research Center.

Duong, V. T., Li, Y., Nowak, E. & Schenk, P. M. (2012). Microalgae Isolation and Selection for Prospective Biodiesel Production, *Energies*, 5(6), 1835–1849.

Edeoga, H. O., Okwu, D. E. & Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 4(7), 685-8.

Evangelista, V., Barsanti, L., Frassanito, A. M., Passarelli, V. & Gualtieri, P. (2008). Algal Toxins: Nature, Occurrence, Effect and Detection. Netherlands: Springer.

Felício, R. D., Albuquerque, S. D., Young, M. C. M., Yokoya, N. S. & Debonsi, H. M. (2010). Trypanocidal, leishmanicidal and antifungal potential from marine red alga *Bostrychiatenella* J. *Agardh* (Rhodomelaceae, Ceramiales). *Journal of Pharmacy Biomedical Analysis*, 52(5), 763–769.

Ferreira, C. R. S., S., Silva da Gama, F., Faustino, S. M. M. & Almeida, S. (2017). Research Article Study of the Secondary Metabolites , Cytotoxic and Antioxidant Activity of the Methanolic Monoraphidium contortum Crude Extract. *Journal of Chemical and Pharmaceutical Research*, 9(6), 327–331.

Fucikova, K., Lewis, P. O. & Lewis, L. A. (2014). Widespread desert affiliation of trebouxiophycean algae (Trebouxiophyceae , Chlorophyta) including discovery of three new desert genera. *Phycological Research*, 62(4), 294–305.

Gangl, D., Zedler, J. A. Z., Rajakumar, P. D., Martinez, E. M. R., Riseley, A., Włodarczyk, A. & Robinson, C. (2015). Biotechnological exploitation of microalgae. *Journal of Experimental Botany*, 66(22), 6975–6990.

Ghazala, B., Shameel, M., Choudhary, M. I., Shahzad, S. & Leghari, S. M. (2004). Phycochemistry and bioactivity of Tetraspora (volvocophyta) from Sindh. Pakistan *Journal of Botany*, 36(3), 531-548

Guedes, A., Amaro, M. & Malcata, F. (2011). Microalgae as Sources of Carotenoids. *Marine Drugs*, 9(4),625-644

Guevarra, B. Q. (2005*)*. A Guidebook to Plant Screening: Phytochemical and Biological. Manila: UST Publishing House.

Guindon, S., Dufayard, J. F., Lefort, V., Anisimova, M., Hordijk, W. & Gascuel, O. (2010). New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Systematic Biology*, 59(3), 307-321.

Guiry, M. D. (2012). How many species of algae are there?. *Journal of Phycology*, 48(5), 1057–63.

Gouveia L., Batista A.P., Sousa I., Raymundo A. & Bandarra N.M. (2008). Food Chemistry Research Developments. New York, U.S.A.: Nova Publishers.

Hadi, S. I. I. A., Santana, H., Brunale, P. P. M. & Gomes, T. G. (2016). DNA Barcoding Green Microalgae Isolated from Neotropical Inland Waters. *Public Library of Science ONE*, 11,(2), 1–18.

Hannon M., Gimpel J., Tran M., Rasala B. & Mayfield S. (2010). Biofuels from algae: challenges and potential. *Biofuels*, 1(5), 763-784.

Horiike, T. (2017). An introduction to molecular phylogenetic analysis. *Reviews in Agricultural Science*, 4(1), 36- 45

Hoshina, R., Iwataki, M. & Imamura, N. (2010). *Chlorella variabilis* and *Micractinium reisseri sp.* nov. (*Chlorellaceae, trebouxiophyceae*): redescription of the endosymbiotic green algae of paramecium Bursaria (Peniculia, Oligohymenophorea) in the 120th year. *Phycological Research*, 58 (3), 188-201.

Khan, M. I., Shin, J. H., & Kim, J. D. (2018). The promising future of microalgae: Current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial Cell Factories*, 17(1), 1–21.

Kim, S. K., Thomas, N. V. & Li, X. (2011). Anticancer compounds from marine macroalgae and their application as medicinal foods. *Advancement Food Nutrient Resources*, 64(1), 213–224.

Kim, G., Mujtaba, G., & Lee, K. (2016). Effects of nitrogen sources on cell growth and biochemical composition of marine chlorophyte Tetraselmis sp. for lipid production. *Algae*, 31(3), 257–266.

Krienitz, L., Friedl, T., Ustinova, J. & Huss, V. (2001). Traditional generic concepts versus 18S rRNA gene phylogeny in the green algal family Selenastraceae (Chlorophyceae, Chlorophyta). *Journal of Phycology*, 37(5), 852–865.

Kumar, K., Dasgupta, C. N. & Das, D. (2014). Cell growth kinetics of Chlorella sorokiniana and nutritional values of its biomass. *Bioresource Technology*, 167(1), 358-366

Lee, R.E. (2008). Phycology. USA: Cambridge University Press.

Lemieux, C., Otis, C. & Turmel, M. (2014). Chloroplast phylogenomic analysis resolves deep-level relationships within the green algal class Trebouxiophyceae. *BMC Evolutionary Biology*, 14(1), 1–15.

Lewis, L. A. & McCourt, R. M. (2004). Green algae and the origin of land plants. *American Journal of Botany*, 91(10), 1535-1556.

Martínez-Francés, E. & Escudero-Oñate, C. (2018). Cyanobacteria and Microalgae of [illegible] Production of Valuable Bioactive Compounds, Microalgal Biotechnology. London, United Kingdom: IntechOpen.

Masih, N. & Singh, B. (2012). Phytochemical Screening of Some Plants Used in [illegible] Based Cosmetic Preparations. Berlin, Heidelberg: Springer.

Mata, T. M., Martins, A. A., Caetano, N. S. (2010). Microalgae for biodiesel production and other applications: A review. *Renewable. Sustainable. Energy. Revision*, 14(1), 217–232.

Milledge J. J. (2011). Commercial application of microalgae other than as biofuels: a brief review. *Review of Environmental Science and Biotechnology*, 10(1), 31-41.

Mostafa, S. S. (2012). Microalgal biotechnology: prospects and applications. *Plant Science*, 10(1), 5772-5779 .

Na, H. J., Moon, P. D., Lee, H. J., Kim, H. R., Chae, H. .J., Shin, T., Seo, Y., Hong S. H. & Kim H. M. (2005). Regulatory effect of atopic allergic reaction of *Carpopeltisaffinis. Journal of Ethnopharmacology*, 101(27), 43–48.

Nigam, P. S. & Singh, A. (2011). Production of liquid biofuels from renewable resources. *Progress in Energy and Combustion Science*, 37(1), 52-68

Nedbalova, L., Mihal, M., Kvíderová, J., Procházková1, L., Rezanka, T. & Elster, J. (2016). Identity , ecology and ecophysiology of planktic green algae dominating in ice-covered lakes on James Ross Island (Northeastern Antarctic Peninsula). *Extremophiles*, 21(1), 187-200.

Novis, P. M., Beer, T. & Vallance, J. (2008). New records of microalgae from the New Zealand alpine zone, and their distribution and dispersal. *New Zealand Journal of Botany*, 46, 347–366.

Novis, P. M. & Visnovsky, G. (2012). Novel alpine algae from New Zealand: Chlorophyta. *Phytotaxa*, 36(3), 347–366

Ozkurt, I. (2009). Qualifying of safflower and algae for energy. *Energy Education Science and Technology*, 23(1), 145–51

Perumal, S., Thirunavukkarasu, A. R. & Pachiappan, P. (2015). Advances in Marine and Brackishwater Aquaculture. New Delhi; New York: Springer.

Priyadarshani, I. & Rath, B. (2012). Commercial and industrial applications of micro algae: A review. *Journal of Algal Biomass Utilization*, 3(4), 89-100.

Pröschold, T. & Leliaert, F. (2007). Systematics of the Green Algae: Conflict of Classic and Modern Approaches. Boca Raton, FL (USA): CRC Press.

Pröschold, T., Marin B., Schlösser U. G. & Melkonian M. (2001). Molecular phylogeny and taxonomic revision of Chlamydomonas (Chlorophyta). Emendation of Chlamydomonas Ehrenberg and Chloromonas Gobi,and description of Oogamochlamys gen. nov. and Lobochlamys gen. nov. *Protist*, 152(4), 265-300.

Rajendran, N., Logeswari, V., Kathiresan, E. & Tamilselvi, A. (2014). Phytochemicals , antimicrobial and antioxidant screening from five different marine microalgae. *Journal of Chemical and Pharmaceutical Sciences*, 2(2), 78–85.

Rajvanshi, S. & Sharma M. P. (2012). Microalgae: a potential source of biodiesel. *Journal of Sustainable Bioenergy Systems*, 2(3), 49-59.

Rindi, F., Allali, H. A., Lam, D. W. & López-Bautista, J. M. (2009). Biodiversity Hotspots. New York: Nova Science Publishers Inc.

Ryu, B. M., Himaya, S. W. A., & Kim, S. K. (2015). Handbook of Marine Microalgae: Biotechnology Advances. London: Elsevier Inc.

Sarker, S. D. & Nahar, L. (2017). Application of Nanotechnology in Phytochemical Research. *Pharmaceutical Sciences*, 23(3), 170–171.

Shanab, S. M., Mostafa, S., Shalaby, E. & Mahmoud, G. (2012). Aqueous extracts of microalgae exhibit antioxidant and anticancer activities. *Asian Pacific Journal of Tropical Biomedicine*, 2(8), 608-615.

Singh, J. & Saxena, R. C. (2015).Handbook of Marine Microalgae: Biotechnology Advances. London: Elsevier Inc.

Sirakov, I., Velichkova, K., Stoyanova, S., Staykov, Y. & Katya Velichkova, C. (2015). The importance of microalgae for aquaculture industry. *International Journal of Fisheries and Aquatic Studies*, 2(4), 81-84.

Soletto, D., Binaghi, L., Lodi, A., Carvalho, J.C.M. & Converti, A. (2005). Batch and batch fed cultivation of *Spirulina platnensis* using ammonium sulphate and urea as nitrogen sources. *Aquaculture*, 243(1), 217–224.

Song, H., Hu, Y., Zhu, H., Wang, Q., Liu, G. & Hu, Z. (2016). Three novel species of coccoid green algae within the Watanabea clade (Trebouxiophyceae , Chlorophyta), *International Journal of Systematic and Evolutionary Microbiology*, 66(12), 5465–5477.

Song, H., Zhang, Q. I., Liu, G., & Hu, Z. (2016). Polulichloris henanensis gen. et sp. nov. (Trebouxiophyceae, Chlorophyta), a novel subaerial coccoid green alga. *Phytotaxa*, 218(2), 137–146.

Song, T., Martensson, L., Eriksson, T., Zheng, W. & Rasmussen, U. (2005). Biodiversity and seasonal variation of the cyanobacterial assemblage in a rice paddy field in Fujian, China. *The Federation of European Materials Societies Microbiology Ecology*, 54(1), 131-40.

Spolaore, P., Cassan, J.C., Duran, E. & Isambert, A. (2005). Commercial application of microalgae. *Journal of Bioscience and Bioengineering*, 2(2), 87-96.

Suh, S., Yang, E. J., Lee, S. G., Youn, U. J., Han, S. J., Kim, I. & Kim, S. (2017). Bioactivities of ethanol extract from the Antarctic freshwater microalga, Chloromonas sp. *International Journal of Medicinal Sciences*, 14(6), 560–569.

Thus, H., Muggia, L., Perez-Ortega, S., Favero-Longo, S. E., Joneson, S., O'Brien, H., Nelsen, M. P., Duque-Thus, R. & Grube, M. (2011). Revisiting photobiont diversity in the lichen family Verrucariaceae (Ascomycota). *European Journal of Phycology*, 46(4), 399–415

Van. Vuuren, S., Taylor, J., & Gerber, A.(2006). Easy Identification for Most Common Freshwater Algae. South Africa: North-West University and Department of Water Affairs and Forestry.

Yaakob, Z., Ali E., Zainal, A., Mohamad, M. & Takriff, M. S. (2014). An overview: biomolecules from microalgae for animal feed and aquaculture. *Journal of Biological Research*, 21(1), 6-15.