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Short Communication

# Isolation and Morphological Identification of Some Indigenous Microalgae from Ethiopia for Phycoprospecting

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## Abstract

Bioprospecting of microalgae is one of the latest promising industries because of its high photosynthetic efficiency. Microalgae prospects are under limelight not only for its value added applications but also for no competition for food, water and arable land usage. However, the product is still costlier when compared to other organisms which obstruct large-scale phycoprospecting. Indigenous microalgae can improve phycoprospecting and it is on high demand. There is a need to isolate and identify the potential native microalgae for local application with ease. Besides, biodiversity in Ethiopia is intense and not investigated much on microalgae. Therefore, this research aims to isolate microalgae from eight different sites including Akaki pond, Akaki River, Kality pond, Kality Gidb pond, Tuludimtu ditch, Awash Lake, Koka Lake and Sumit Ditch. The samples were inoculated to Bold's Basal medium and incubated under natural Sun light at 25˚C for 15 days. Then microalgae were purified using agar plate and identified morphologically using light microscope. Eighteen species of algae were obtained from 12 genera. *Pediastrum* sp., *Chlorella* sp., *Chlamydomonas* sp., *Scenedesmus* sp., *Chlorogonium* sp., *Oscillatoria* sp., *Anabaena* sp., *Microcystis* sp., *Microspora* sp., *Closterium* sp., *Synechocystis* sp., and *Navicula* species were among the identified genera. Of these, 8 genera belong to eukaryotic protist and the other 4 comes under prokaryotic cyanobacteria. These Ethiopian native species of microalgae can be used effectively for its value added application locally.

**Keywords:** *Microalgae, Ethiopia, Cyanobacteria, Protista, Microscope, Phycoprospecting*.

## 1. Introduction

Microalgae are ubiquitous and have been evolving on Earth for billions of years and responsible for evolution of aerobic organisms including humans by using $CO_2$ from the primitive atmosphere and released $O_2$. Studies suggested that tiny algae have been producing 70% of atmospheric $O_2$ (Walker, 1980). Microalgae (2-200 µm) are organisms highly capable of utilizing solar energy and $CO_2$ to create biomass and they are the primary producer for majority life on the planet (Wilkie et al., 2011). Algal species were estimated between 250,000 to millions of which 35,000 species are scientifically recorded. Currently about 5,000 algal species are available through culture collections and only 10 to 20 species are cultivated industrially (https://subitec.com/en/fascination-algae-facts-on-microalgae; Raja et al., 2008). In addition, the estimated number of unknown species of algae is projected to be two orders of magnitude more than currently known species (Anderson, 1992; Norton et al., 1996). It is a stunning vision that how much potential remains waiting in undiscovered species because the group of discovered species is ever growing for its various applications. Many researchers recommend that microalgae may hold the key to solve many problems including pollution, hunger, energy, global warming, and diseases in sustainable manner (Wilkie et al., 2011).

In fact, Stuart & Hessami (2005) found that a 4000 $m^3$ pond under natural Sun light could sequester up to

2.2 kiloton of $CO_2$ per year with no competition with food crops, not restricted to arable land and portable water, can be grown in salt water and wastewater (Suresh et al., 2018), easily adapt conditions, low energy requirement (Oswald, 2003), carbon neutral, renewable, used as single cell protein, nutrient supplements, pigments production, biogas (Suresh et al., 2013) and antioxidants. In addition, Chisti (2007; 2008) found that microalgae can produce 23–55 $m^3$ oil per acre as compared to oil palm (high oil producing plant) which produces only 1.4 $m^3$/acre.

With these promising advantages, microalgae industry is not popular in many part of the world including Ethiopia due to lack of study on characterization and exploitation. Despite intense biodiversity in Ethiopia, its utilization is negligible due to inadequate studies. The existing few studies focused on the community structure and primary production of microalgae (Damtew Etisa et al., 2018; Adane Fenta & Almaz Kidanemariam, 2016) and for biodiesel (Abebe Girma et al., 2016) lately. A very low attention has been given to the indigenous microalgae and its potentials in Ethiopia. To propel algal biotechnological applications in any country, one should investigate the native phycological flora and its potential value to industrial scale.

Therefore, the objective of this study was to isolate microalgae from various sites in Ethiopia and identify the isolates based on morphological characteristics. The present study is part of ongoing efforts to screen efficient native microalgal strains for their phycoremediation of industrial pollutants in Ethiopia. Focusing on microalgae-based processes, the unexplored country of Ethiopia is awaiting the opportunity to play its role in phycoprospecting and to contribute to the economy of the country in general.

## 2. Materials and Methods

### 2.1. Microalgae Sample Collection and Growth

The microalgae water samples were collected from eight sites in Ethiopia (Akaki Pond-AKP, Akaki River-AKR, Kality Pond-KAP, Kality Gidb Pond-KGP (8°53′44.99″ N, 38°47′20.98″ E), Tuludimtu Ditch-TUD (8°51′00″ N, 38°48′59.98″ E), Awash Lake-AWL (8°58′59.99″ N, 40°10′0.01″ E), Koka Lake-KOL (8°21′20.86″ N, 39°0′3.74″ E) and Sumit Ditch-SUD (9°0′19.44″ N, 38°45′48.99″ E) and 1 mL was inoculated into a 250 mL flask counting 100 mL sterile Bold's Basal medium (BBM) and incubated in the lab under natural Sun light (day and night cycle) at 25˚C for 15 days. The culture flasks were manually shaken twice a day.

### 2.2. Microalgae Purification and Identification

BBM grown microalgae were purified using spread and streak plate method and then isolated colonies were inoculated in 100mL BBM and incubated same as mentioned above. Purified algae were identified by its morphology using light microscope (Labomed, USA) by wet slide mount method at 40x and 100x (oil immersion). The photomicrographs were taken with an iPhone camera via ocular lens and followed the Janse van Vuuren et al., (2006) manual to identify the microalgae genera.

## 3. Results and Discussion

In this preliminary study a total of 18 microlagal species were isolated using the standard plating techniques, and based on distinguishable morphological characters under light microscopic examination. These strains were preliminary ascribed to the 12 genera, namely, *Pediastrum* sp., KGP, *Chlorella* sp., AKR, TUD, *Chlamydomonas* sp., AWL, *Scenedesmus* sp., KAP, KGP, *Chlorogonium* sp., AKP, *Oscillatoria* sp., SUD, TUD, *Anabaena* sp., KGP, KAP, *Microcystis* sp., KOL, *Microspora* sp., KGP, KAP, *Closterium* sp., AKP, *Synechocystis* sp., KAP, AKR and *Navicula* sp., SUD. Among this, 4 genera belongs to cyanobacterial group (*Oscillatoria, Anabaena, Microcystis* and *Synechocystis*) which are prokaryotes and other 8 belongs to protist algae of eukaryotes. Moreover, 3 out of 18 species were identified as filamentous algae and they resemble the genera of *Oscillatoria*, *Anabaena* and *Microspora*. Most of the genera belong to division of Chlorophyta (*Pediastrum* sp., *Chlorella* sp., *Chlamydomonas* sp., *Scenedesmus* sp., *Chlorogonium* sp., *Microspora* sp.). Interestingly, a diatom (*Navicula* sp.) and charophyta (*Closterium* sp.) species also screened. In general all isolates have some potential application except *Microcystis* sp., which is a disease causing microalgae by producing neurotoxins. Figure 1 shows the photograph of isolated microalgal species.

Microalgae are available in all existing earth ecosystems, and representing a diverse polyphyletic group of species living in a wide range of environmental conditions and have potential to solve problems in the world. Yet they are one of the most poorly understood, characterized and exploited groups of microorganisms on earth (Wilkie et al., 2011; Raja et al., 2018). In addition, Wilkie et al., (2011) suggested that algae has great diversity, however indigenous species are potential candidates for bioprospecting because native species has advantage over type culture and genetically engineered organisms. Isolating native microalgae with desirable properties gives robust biological platform for phycoprospecting. Native strains come equipped with millions of years of adaptation to the local biotic and abiotic stress (naturally engineered species). Through optimization of native species may yield superior organisms for bioresource production. Consequently, isolation is a fundamental process to obtain pure cultures and is the first phase towards the screening and selection of microalgae strains with the potential for the value added applications.

Although morphological analysis is frequently used to identify microalgae, it is inaccurate and very difficult for the identification at the species level, because the relationship between diagnostic morphology and biological species boundaries are largely unknown in many micro-eukaryotes (Moniz & Kaczmarska 2010). Besides, according to Yu et al., (2012) the microalgae morphology for the same strain varies in relation to age and culture conditions. Recently in Ethiopia, Etista et al., (2018) found that the *Chlamydomonas* sp., was dominant in Lake Abaya in all seasons, therefore in our study expected the same species in all samples but observed only in Awash Lake sample. Abebe Girma Demissie et al., (2016) isolated very different genera such as *Oedogonium* sp., and *Cladophora* sp., from Lake Abaya and Chamo in Arab Minch, those genus were absent in our sampling sites in Addis Ababa. In addition of those unique genera some common genera of *Chlorella* sp., and diatoms also identified (Abebe Girma Demissie et al., 2016). In general, our observation disclosed that different sites shown one or two different microalgae species, whereas Kality Gidb pond sample was contained 4 different genera of microalgae such as *Pediastrum* sp., *Scenedesmus* sp., *Anabaena* sp., and *Microspora* sp. In case of Kality pond also shown 4 different genera namely *Scenedesmus* sp., *Anabaena* sp., *Microspora* sp., and *Synechocystis* sp. Attractively, the same genera of microalgae were noticed in different site with a little altered morphology, for example *Scenedesmus* sp., and *Anabaena* sp., from KAP and KGP sample (Figure 1).

The cells of *Scenedesmus sp.*, from Kality Pond (KAP) observed 4-celled coenobia, while Kality Gidb Pond (KGP) sample showed 8 celled coenobia which is in agreement with Van den Hoek et al., (1995) description about the *Scenedesmus* sp. In case of *Anabaena* sp. isolated from KAP sample shown rectangular cells in the filament while elliptical to sphere shaped cells in KGP sample. Adane Fenta and Almaz kidanemariam, et al., (2016) reported variation of microalgae between sites is due to the difference in water quality, changes in physico-chemical characteristics lead to concomitant qualitative and quantitative changes in microalgae. Therefore, the result shows that the sampling sites was characterized by eutrophic mainly as a result of high nutrient loading from surface runoff from domestic, industrial, agriculture and construction near the sampling sites. In another study, Metzger and Largeau (2005) reported that, within in each chemical race and for the same strain, the morphology of the alga could vary in relation to age and culture conditions. The morphological heterogeneity of the alga makes the microscopic examination difficult and in support this point, Trainor (1998) observed *Scenedesmus* species shown diverse morphology under different environmental conditions in accordance with our identification. Identification of microalgae is supported by molecular tools would enhance the authentication of classification (Moniz & Kaczmarska, 2010), therefore, further analysis on its molecular identification under process.

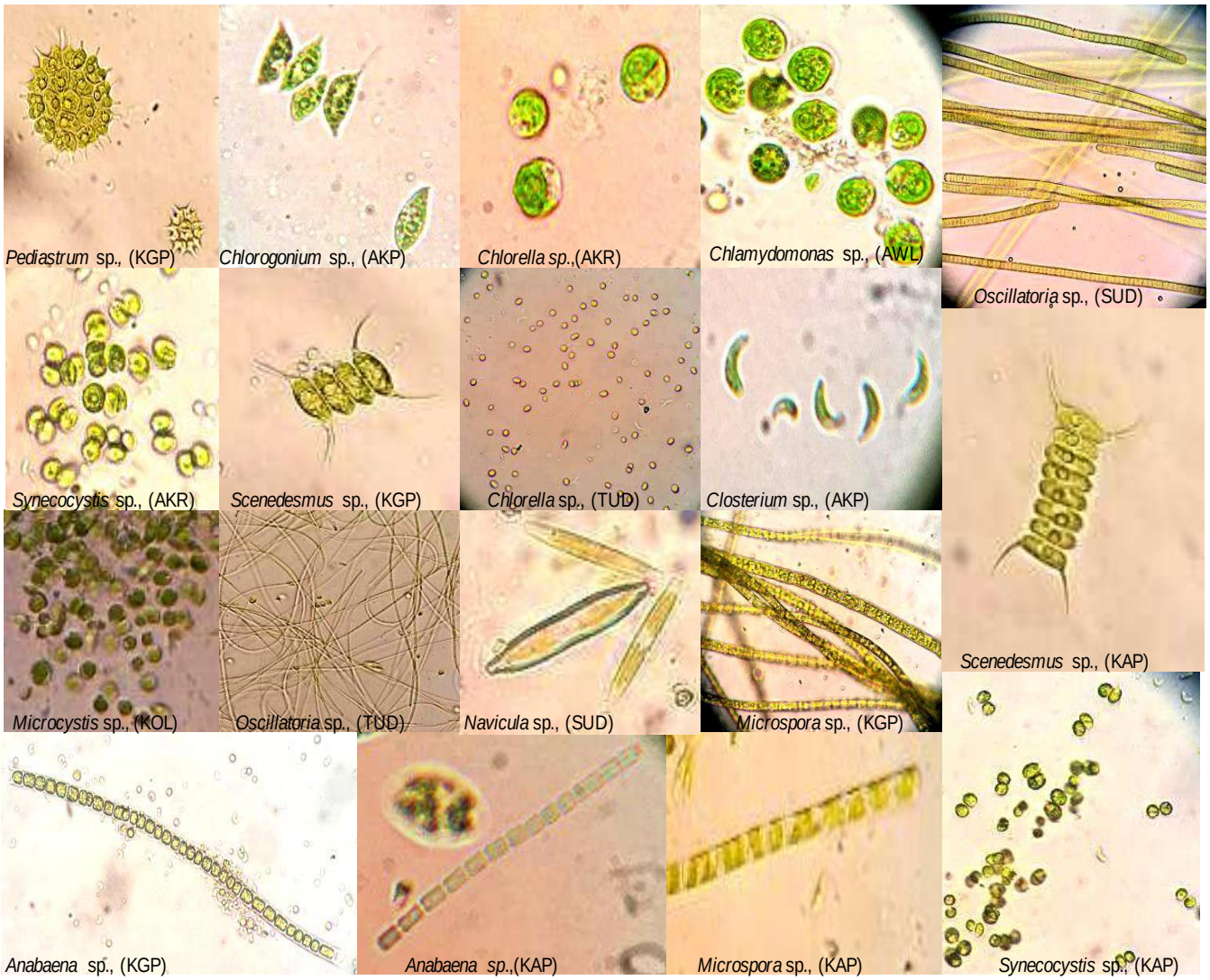


Figure 1. Microscopic images of microalgae isolated from the different water samples

In these isolates, some algae have lot of potential on value added products like single cell proteins (*Chlorella* sp), feed (*Chlorella* sp., *Navicula* sp.), oil producers (*Chlorella* sp., *Scenedesmus* sp., and diatom), wastewater treatment (*Chlorogonium* sp., *Scenedesmus* sp., *Oscillatoria sp.,)* nitrogen fixer as biofertilizer *(Anabaena sp),* pollutants removal, bioindicator (*Closterium* sp.,) and model organisms for study (*Synechocystis* sp., and *Chlamydomonas* sp.). These Ethiopian native genera of microalgae can be used effectively for its value added application locally and it's under investigation.

## 5. Conclusion

In this study, eighteen microalgal species were isolated from eight different sites from Ethiopia and identified by morphological features and noticed those belong to 12 different genera. Among these, 8 genera belong to eukaryotic protist microalgae and other 4 comes under prokaryotic cyanobacteria it includes 3 filamentous algae. Some isolates are industrially important such as *Chlorella*, *Anabaena*, diatom, *Scenedesmus*. Given the great diversity of microalgae, it is suggested that many native species are potential candidates for local application. Further analysis on its molecular identification is needed and its potential phycoprospecting are under process.

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