

## Installation of Operational Processes for the Establishment of Microalgal Culture Collection

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### ABSTRACT

Microalgae are the most common photosynthetic organisms which are available in all aquatic systems. Microalgae cultures are used in a wide variety of industrial areas due to their valuable chemical compounds. Today microalgae are widely used in scientific research, as learning resources for students and as raw materials for industry. The science world and the industry need cultures which are pure and identified with all of their characteristics in order to utilise in those areas. Therefore, the microalgae culture collections isolating and preserving microalgae cultures are needed worldwide. Considering these important functions of cultural collections, efforts to create the Algal Culture Collection started at Ahi Evran University (AEU-CCA). Our culture collection consists of totally 19 microalgae species belonging to the phylums of Cyanobacteria, Chlorophyta, Charophyta and Bacillariophyta. Collecting microalgae from fresh water resources, their identification, isolation and arrangement of culture conditions have begun to be carried out and it is a still-continuing process. While the microalgae are preserved in broth medium by sub-culturing, their long-term preservation studies through cryopreservation have begun. The present study mainly aims to put isolated microalgae species at the disposal of scientific communities, to conduct biotechnological studies and to arrange the microalgae culture collection in order to maintain biological diversity.

**KEY WORDS:** Cyanobacteria, Chlorophyta, Charophyta, Bacillariophyta, microalgae culture collection

**How to cite this article:** Acikgoz Erkaya, I., Yalcin Duygu, D., Ozer, T., (2018). Installation of Operational Processes for the Establishment of Microalgal Culture Collection, *MedFAR*, 2(2):49-62.

## 1. Introduction

The microalgae are the most common photosynthetic organisms which are available in all aquatic systems such as freshwater, sea water, deserts and polar ecosystems as well as being carbon and chemical energy sources for other organisms (Sirakov et al., 2015). They convert into biomass through use of luminous energy and carbon dioxide and they do that operation more efficiently in proportion to high plants (Park et al., 2011). On basis of that characteristic, they are named as primary producers.

The microalgae are cultured in order to be used in food (Borowitzka and Borowitzka, 1987; Colla et al., 2007), animal feed (Becker, 2007; Guedes & Malcata, 2012), production of useful compounds (Sajilata, 2008; Rangel-Yagui et al., 2004; Madhyastha & Vatsala, 2007), refinement of waste water (Velichkoca et al., 2014; Fraile et al., 2005), cosmetic (Stolz & Obermayer, 2005) and pharmaceutical (Rania & Hala, 2008) industries. Besides, the microalgae are potentially good sources for production of biofuel due to high fat content and fast biomass production (Sharma et al., 2013). Apart from these, they have fast rates of growth and stable towards possible changes in temperature, light and nutritional elements in culture systems (Sirakov et al., 2015; Guedes and Malcata, 2012). Taking all those characteristics into consideration, the studies on microalgae date back to a long time. The initial studies conducted upon the microalgae are on the natural sciences related to ecosystems. Nowadays, the research topics in microalgae sciences are

widely used as learning sources for students and as raw material for the industry.

The culture collections are biological sources which are of great significance for the maintenance of biological diversity. The first microalgae collection was constituted by Chodat and Pringsheim in 1920s (Gartner, 1958) and they are available in different areas of the world. Algae culture collections provide services not only to their customers but also in terms of maintaining biological diversity, identification, isolation, preservation of cultures, storage and training (Friedl & Lorenz, 2012). The researchers who carry out studies on microalgae and the industry areas using these microorganisms in their various production processes emphasize that there is a need for microalgae cultures whose characteristic specifications have been well preserved. Therefore, the algae culture collections are essential in terms of reliability of biological sources, that is, providing repeatability required for a scientific research and biotechnological application. The culture collections eliminate the necessity for isolating and identifying the species in question and the effort to do those processes as they already provide the researchers with certified organisms whose all characteristics are identified (OECD, 2007). The goal of the present study is to systematically arrange the species previously isolated and preserved in our laboratory, in addition to this, to isolate different species to form a microalgae culture collection for the purpose of making biotechnological studies and preserving biological diversity.

## 2. Materials and Methods

### 2.1. Sample Collection

The sampling information was recorded as date, location and habitat. The fresh water resources were identified as river, stream, lake, pond and reservoir. The water samples were taken from the surface.

### 2.2. Sterilization

All the equipments and nutrient media etc. were sterilised through microbiological methods so as not to cause any contamination and mistake in the processes of collecting samples, bringing to the laboratory, isolation and sub-culturing. The prepared nutrient media were taken into glass vessels for auto-claving process, their embouchures were closed with cotton plug and they were covered with aluminium foil so as not to cause any damage during sterilization. Sterilization is performed in an autoclave at 15 lb in<sup>-2</sup> pressure and 121°C during 15-30 mins. The sterilisation of glass materials was performed at for one hour 170°C (WHO, 2016).

### 2.3. Isolation

During isolation process, the collected samples were taken into pre-enrichment medium (MgSO<sub>4</sub>.7H<sub>2</sub>O-2.50 gr, KNO<sub>3</sub>-5.0 gr, KH<sub>2</sub>PO<sub>4</sub>-1.25 gr, FeSO<sub>4</sub>.7H<sub>2</sub>O-0.009 gr, distile water-1000 ml) (Andersen & Kawachi, 2005; Nichols, 1973). Particularly two methods consisting of dilution method and isolation from single cell were utilised

for isolation of species. In dilution technique, 1:10 serial dilution was conducted repeatedly five times supposing that single cell would remain in the last tube as approximate cell number was not known. Bearing in mind that single cells in the tube might die, some tubes might contain one or more species and none might exist in some of them, many tubes were cultivated from the last dilution. The isolation from the single cell was made through Pasteur pipette. The cells were taken from the sample and added into a sterilised droplet. Those cells were transferred into a second sterilised droplet, the droplet method was carried out until it was obtained purely, they were analyzed microscopically and they were taken into an appropriate nutrient media. Throughout this process, great effort was made so as not to cause any damages in cells (Andersen & Kawachi, 2005; CSIRO, 2017). The filamentous algae were purified by taking directly into nutrient media.

### 2.4. Identification of the Species

The species were identified on microscope through use of identification keys, which are presented in Table 1, 2 and 3 (Huber - Pestalozzi, 1938; Huber - Pestalozzi, 1955; Bourrelly, 1972; Prescott, 1975; Patrick & Reimer, 1975; Huber - Pestalozzi, 1982; Krammer & Lange-Bertalot, 1991a; Krammer & Lange-Bertalot, 1991b; Krammer & Lange-Bertalot, 1999a; Krammer & Lange-Bertalot, 1999b; Cox, 1996).

**Table 1.** The microalgae phylums at AEU-CCA culture collection

Phylum	Class	Number of Strains
Cyanobacteria	Cyanophyceae	7
Chlorophyta	Trebouxiophyceae	2
	Chlorophyceae	5
Charophyta	Klebsormidiophyceae	1
	Conjugatophyceae (Zygnematophyceae)	2
Bacillariophyta	Bacillariophyceae	2

Besides, the species were identified at molecular level through Fourier Transform Infrared (FTIR) spectroscopy (Figure 2). Infrared analysis was

carried out at Bilkent University (UNAM), Ankara (Turkey), using a Vertex 70 with Hyperion Microscope fitted with a Bruker Tensor 37 FTIR

spectrometer. A view on the microscope was chosen from the transmission region between 400 and 5000  $\text{cm}^{-1}$  wave number range, 4  $\text{cm}^{-1}$  resolution and aperture of 20x20  $\mu\text{m}$  square, and 128 scans were taken as spectra (Sigeo et al., 2002; Duygu et al., 2012).

## 2.5. Culture Conditions

Since choosing the appropriate nutrient media and concentration were of great significance for culturing, many experiments were attempted with different nutrient media. While isolating the microalgae, diluted nutrient media were utilised so as to enable weak cultures to thrive. Following that an intense culture was obtained, sub-culturing process was carried out through undiluted medium by using sufficient number of cells. While producing cultures, BG-11 and Allen media were utilised and the pH was adjusted as 6.8-7.0 (Andersen and Kawachi, 2005; CSIRO, 2017; UTEX, 2017). The cultures were cultivated into 30 ml nutrient media in 50 ml erlenmeyers. Those containing 30 ml medium were incubated at 25°C and under fluorescent lamps at photon flux density of 50  $\mu\text{mol photons m}^{-2} \text{s}^{-1}$  with a photoperiod of a light 16: dark 8 (Guillard, 2005).

## 2.6. Sub-culturing

When the new microalgae are isolated, they are added into culture list, sub-cultured and preserved in this way. The recorded pure strain is cultivated under culture conditions. The species are preserved in the broth medium. The sub-cultures are prepared by putting 30 ml nutrient media into 50 ml erlenmeyers and adding approximately 5-10% culture depending upon the intensity of cells (Hur et al., 2015). The sub-cultures are taken into fresh nutrient media every 15 days. After three sub-culturing process, the oldest sub-culture strain stored is discarded.

The studies on preservation of cultures through cryopreservation began and the cryopreservation of some species was made directly at -80°C with 5% DMSO, glycerol, skimmed milk and without cryoprotectant (Rastoll et al., 2013; Nakanishi et al., 2012; Salas-Leiva & Dupré, 2011; Day, 2007).

## 2.7. Explanatory Notes on the Information Related to the Strain

All the information regarding date, location, isolator, isolation method, medium, temperature, illumination intensity, photoperiod, cryopreservation and sub-culturing interval of each strain was recorded. The cultures were listed considering their scientific names and the codes given at AEU-CCA (The Culture Collection of Algae at University of Ahi Evran). A detailed description of the information given on a strain is as follows:

1. Genus name
2. Class name
3. Scientific name with nomenclator
4. Sampling: Sampling information is categorized by date and locality
5. Isolator: Isolator's name in the order of family name and first name
6. Identifier: Identifier's name in the order of family name and first name
7. State: The maintenance method such as liquid and cryopreserved
8. Culture: Media, temperature, photosynthetic photon flux density (PPFD,  $\mu\text{mol photons m}^{-2}\text{s}^{-2}$ ), light: dark cycle
9. AEU-CCA registration number: CCA, Phylum no, species (coded acronym), strain number.

## 3. Results

The microalgae species were isolated from fresh water samples collected from fresh water resources in Ankara and surroundings, identified and produced in a laboratory setting. While isolating the cultures, microbiological methods were utilised. The optimum conditions that would enable the best multiplication of species were investigated. As a result of experiments, it was realised that BG 11 and Allen medium gave the best result for preservation of cultures. The optimum values were determined as; pH 6.8-7.0, illumination light 16: 8 dark period and 22-25°C temperature. The catalogue numbers of cultures were prepared in accordance with systematics given in international culture collections and literature (Table 2). During maintenance of sub-

cultures, their multiplication is monitored and checked through microscope (Figure 1). The molecular identification of cultures were made through FTIR spectra and the results were evaluated (Figure2).

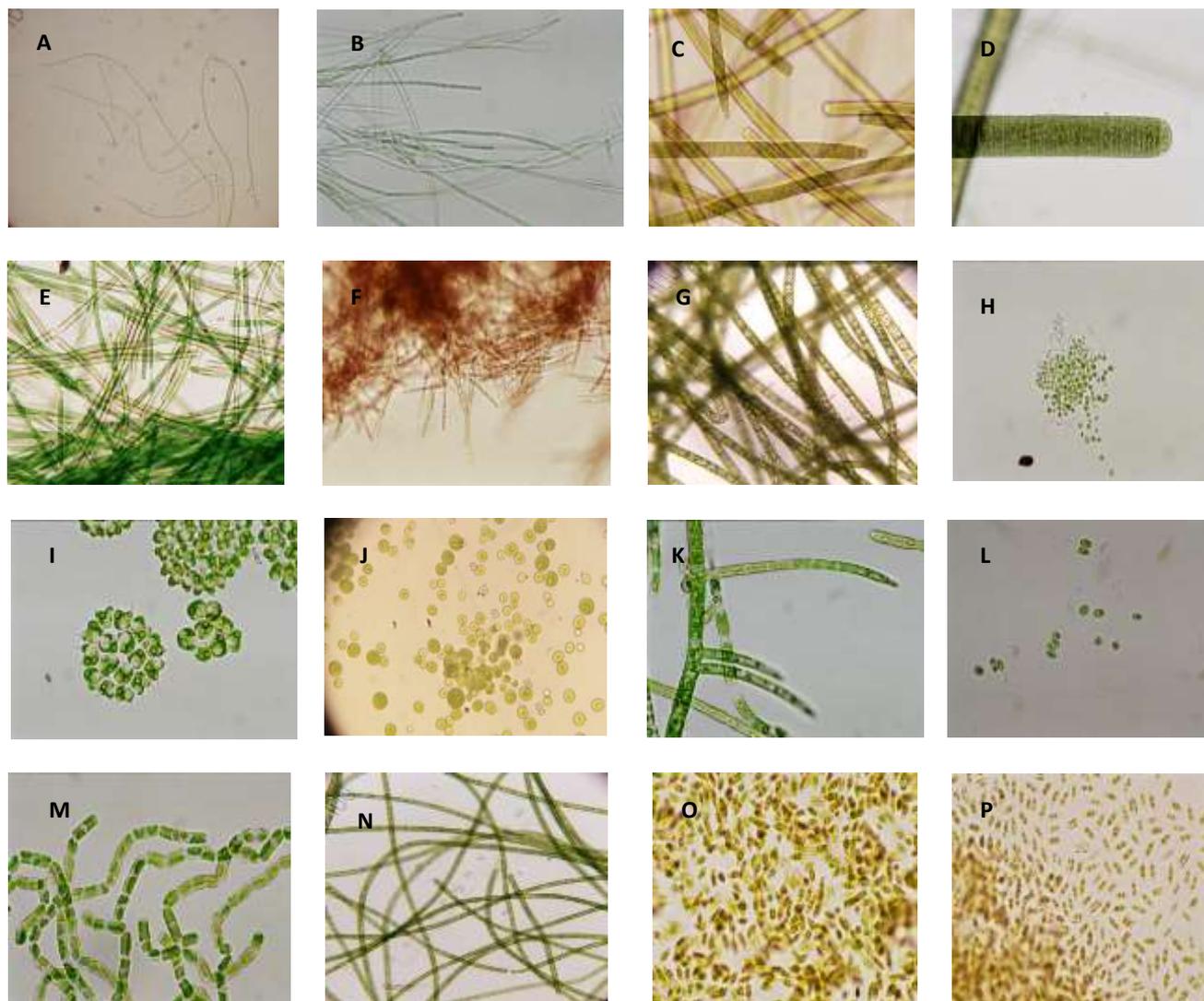
**Table 2.** The list of codes given to the species at Ahi Evran CCA

1	<b>DOLICHOSPERMUM:</b> Cyanophyceae <i>Dolichospermum affine</i> (Lemmermann) Wacklin, L.Hoffmann and Komárek <b>Sampling</b> 2007, Ankara, freshwater surface pond, <b>Isolator</b> Ozer T, <b>Identifier</b> Ozer T (2007), <b>States</b> liquid, <b>Culture</b> BG 11, Allen, 24°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA01Ana01
2	<b>ANAGNOSTIDINEMA:</b> Cyanophyceae <i>Anagnostidinema lemmermannii</i> (Woloszynska) Strunecky <i>et al.</i> <b>Sampling</b> 2007, Ankara, freshwater surface pond, <b>Isolator</b> Acikgoz Erkaya I, <b>Identifier</b> Acikgoz Erkaya I (2007), <b>States</b> liquid, <b>Culture</b> BG 11, Allen, 24°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA01Os01
3	<b>OSCILLATORIA:</b> Cyanophyceae <i>Oscillatoria</i> sp. <b>Sampling</b> 2007, Ankara, freshwater surface pond, <b>Isolator</b> Ozer T, <b>Identifier</b> Ozer T (2007), <b>States</b> liquid, <b>Culture</b> BG 11, Allen, 24°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA01Os03
4	<b>OSCILLATORIA:</b> Cyanophyceae <i>Oscillatoria princeps</i> Vaucher ex Gomont <b>Sampling</b> 2007, Ankara, freshwater surface pond, <b>Isolator</b> Acikgoz Erkaya I, <b>Identifier</b> Acikgoz Erkaya I (2007), <b>States</b> liquid, <b>Culture</b> BG 11, Allen, 24°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA01Os02
5	<b>MICROCOLEUS:</b> Cyanophyceae <i>Microcoleus autumnalis</i> (Gomont) Strunecky, Komárek and J.R.Johansen <b>Sampling</b> 2007, Ankara, freshwater surface pond, <b>Isolator</b> Acikgoz Erkaya I, <b>Identifier</b> Acikgoz Erkaya I (2007), <b>States</b> liquid, cryopreserved <b>Culture</b> BG 11, Allen, 24°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA01Ph02
6	<b>PSEUDANABAENA:</b> Cyanophyceae <i>Pseudanabaena mucicola</i> (Naumann and Huber-Pestalozzi) Schwabe <b>Sampling</b> 2007, Ankara, freshwater surface pond, <b>Isolator</b> Ozer T, <b>Identifier</b> Ozer T (2007), <b>States</b> liquid, <b>Culture</b> BG 11, Allen, 24°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA01Ph01
7	<b>ARTHROSPIRA:</b> Cyanophyceae <i>Arthrospira platensis</i> Gomont <b>Sampling</b> 2009, Ankara, freshwater surface pond, <b>Isolator</b> Acikgoz Erkaya I, <b>Identifier</b> Acikgoz Erkaya I (2009), <b>States</b> liquid, <b>Culture</b> BG 11, Allen, 24°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA01Spr01
8	<b>CHLORELLA:</b> Trebouxiophyceae <i>Chlorella vulgaris</i> Beyerinck [Beijerinck] <b>Sampling</b> 2007, Ankara, freshwater surface reservoir, <b>Isolator</b> Duygu Yalcin D, <b>Identifier</b> Duygu Yalcin D (2007), <b>States</b> liquid, cryopreserved <b>Culture</b> BG 11, Allen, 22°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA02Ch01
9	<b>CHLORELLA:</b> Trebouxiophyceae <i>Chlorella</i> sp. <b>Sampling</b> 2007, Ankara, freshwater surface reservoir, <b>Isolator</b> Duygu Yalcin D, <b>Identifier</b> Duygu Yalcin D (2007), <b>States</b> liquid, cryopreserved <b>Culture</b> BG 11, Allen, 22°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA02Ch02
10	<b>PSEUDOPEDIASTRUM:</b> Chlorophyceae <i>Pseudopediastrum boryanum</i> (Turpin) E.Hegewald <b>Sampling</b> 2007, Ankara, freshwater surface pond, <b>Isolator</b> Duygu Yalcin D, <b>Identifier</b> Duygu Yalcin D (2008), <b>States</b> liquid, <b>Culture</b> BG 11, Allen, 22°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA02Pdr01

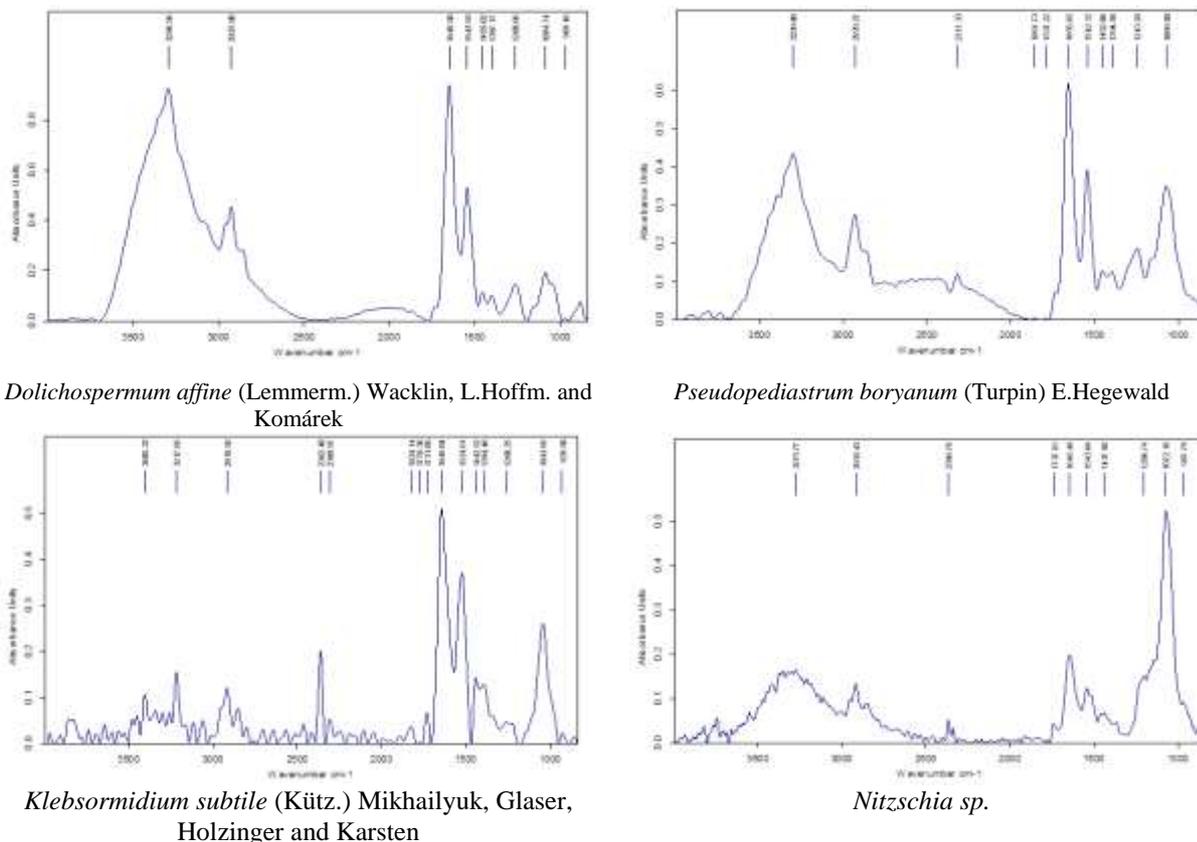
11	<p><b>CHLAMYDOMONAS:</b> Chlorophyceae <i>Chlamydomonas</i> sp.1</p> <p><b>Sampling</b> 2017, Ankara, freshwater surface reservoir, <b>Isolator</b> Duygu Yalcin D, <b>Identifier</b> Duygu Yalcin D (2017), <b>States</b> liquid, <b>Culture</b> BG 11, Allen, 22°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA02Chl01</p>
12	<p><b>CHLAMYDOMONAS:</b> Chlorophyceae <i>Chlamydomonas</i> sp.2</p> <p><b>Sampling</b> 2017, Ankara, freshwater surface reservoir, <b>Isolator</b> Duygu Yalcin D, <b>Identifier</b> Duygu Yalcin D (2017), <b>States</b> liquid, <b>Culture</b> BG 11, Allen, 22°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA02Chl02</p>
13	<p><b>STIGEOCLONIUM:</b> Chlorophyceae <i>Stigeoclonium nanum</i> (Dillwyn) Kützing</p> <p><b>Sampling</b> 2007, Ankara, freshwater surface pond, <b>Isolator</b> Acikgoz Erkaya I, <b>Identifier</b> Acikgoz Erkaya I (2008), <b>States</b> liquid, <b>Culture</b> BG 11, Allen, 22°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA02Stg01</p>
14	<p><b>TETRADESMUS:</b> Chlorophyceae <i>Tetradismus obliquus</i> (Turpin) M.J. Wynne</p> <p><b>Sampling</b> 2007, Ankara, freshwater surface reservoir, <b>Isolator</b> Duygu Yalcin D, <b>Identifier</b> Duygu Yalcin D (2007), <b>States</b> liquid, <b>Culture</b> BG 11, Allen, 22°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA02Sce01</p>
15	<p><b>KLEBSORMIDIUM:</b> Klebsormidiophyceae <i>Klebsormidium subtile</i> (Kützing) Mikhailyuk, Glaser, Holzinger and Karsten</p> <p><b>Sampling</b> 2008, Ankara, freshwater surface pond, <b>Isolator</b> Ozer T, <b>Identifier</b> Ozer T (2008), <b>States</b> liquid, cryopreserved <b>Culture</b> BG 11, Allen, 22°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA02Stc01</p>
16	<p><b>SPIROGYRA:</b> Conjugatophyceae (Zygnematophyceae) <i>Spirogyra</i> sp.1 Link</p> <p><b>Sampling</b> 2015, Ankara, freshwater surface reservoir, <b>Isolator</b> Acikgoz Erkaya I, <b>Identifier</b> Acikgoz Erkaya I (2015), <b>States</b> liquid, <b>Culture</b> BG 11, Allen, 22°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA03Spg01</p>
17	<p><b>SPIROGYRA:</b> Conjugatophyceae (Zygnematophyceae) <i>Spirogyra</i> sp.2 Link</p> <p><b>Sampling</b> 2016, Ankara, freshwater surface reservoir, <b>Isolator</b> Acikgoz Erkaya I, <b>Identifier</b> Acikgoz Erkaya I (2016), <b>States</b> liquid, <b>Culture</b> BG 11, Allen, 22°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA03Spg02</p>
18	<p><b>ACHNANTHES:</b> Bacillariophyceae <i>Achnanthes</i> sp. Bory</p> <p><b>Sampling</b> 2007, Ankara, freshwater surface pond, <b>Isolator</b> Acikgoz Erkaya I, <b>Identifier</b> Acikgoz Erkaya I (2007), <b>States</b> liquid, <b>Culture</b> BG 11, Allen, 22°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA04Ach01</p>
19	<p><b>NITZSCHIA:</b> Bacillariophyceae <i>Nitzschia</i> sp. Hassall</p> <p><b>Sampling</b> 2017, Ankara, freshwater surface pond, <b>Isolator</b> Ozer T, <b>Identifier</b> Ozer T (2017), <b>States</b> liquid, <b>Culture</b> BG 11, Allen, 22°C, 20 PPFD, 16L:8D, <b>Original No.</b> CCA04Nitz01</p>

**Table 3.** List of Synonyms

<b>Synonym</b>	<b>Current name</b>
<i>Anabaena catenula</i> var. <i>affinis</i> (Lemmermann) Geitler <i>Anabaena affinis</i> Lemmermann	<b><i>Dolichospermum affine</i></b> (Lemmermann) Wacklin, L.Hoffmann and Komárek
<i>Oscillatoria lemmermannii</i> Woloszynska <i>Jaaginema lemmermannii</i> (Woloszynska) Anagnostidis and Komárek	<b><i>Anagnostidinema</i></b> <b><i>lemmermannii (Woloszynska)</i></b> <b><i>Strunecky et al.</i></b>
<i>Trichophorus princeps</i> (Vaucher) Desvaux 1809 <i>Oscillatoriella princeps</i> (Vaucher) Gaillon 1833 <i>Lyngbya princeps</i> (Vaucher ex Gomont) Hansgirg 1893	<b><i>Oscillatoria princeps</i></b> Vaucher ex Gomont
<i>Phormidium autumnale</i> (C.Agardh) Trevisan ex Gomont <i>Lyngbya autumnalis</i> (Gomont) P.A.C.Senna <i>Oscillatoria autumnalis</i> C.Agardh <i>Oscillatoriella autumnalis</i> (C.Agardh) Gaillon	<b><i>Microcoleus autumnalis</i></b> (Gomont) Strunecky, Komárek and J.R.Johansen
<i>Phormidium mucicola</i> Nauman and Huber-Pestalozzi <i>Lyngbya naumannii</i> Iltis	<b><i>Pseudanabaena mucicola</i></b> (Naumann and Huber-Pestalozzi) Schwabe
<i>Oscillatoria platensis</i> (Gomont) Bourrelly <i>Spirulina jenneri</i> var. <i>platensis</i> Nordstedt <i>Spirulina platensis</i> (Gomont) Geitler	<b><i>Arthrospira platensis</i></b> Gomont
<i>Chlorella pyrenoidosa</i> var. <i>duplex</i> (Kützing) West <i>Pleurococcus beijerinckii</i> Artari 1892 <i>Chlorella communis</i> Artari 1906 <i>Chlorella candida</i> Shihira and R.W.Krauss 1965	<b><i>Chlorella vulgaris</i></b> Beyerinck [Beijerinck]
<i>Helierella boryana</i> Turpin <i>Pediastrum boryanum</i> (Turpin) Meneghini	<b><i>Pseudopediastrum boryanum</i></b> (Turpin) E.Hegewald
<i>Scenedesmus obliquus</i> (Turpin) Kützing <i>Acutodesmus obliquus</i> (Turpin) Hegewald and Hanagata <i>Scenedesmus acutus</i> Meyen <i>Scenedesmus bijugatus</i> Kützing <i>Scenedesmus acutus</i> f. <i>alternans</i> Hortobagyi	<b><i>Tetrademus obliquus</i></b> (Turpin) M.J.Wynne
<i>Hormidium subtile</i> (Kützing) Heering <i>Stichococcus subtilis</i> (Kützing) Klercker <i>Chlorhormidium subtile</i> (Kützing) Starmach <i>Ulothrix subtilis</i> var. <i>variabilis</i> Kirchner <i>Ulothrix subtilissima</i> Rabenhorst <i>Hormidium subtilissimum</i> (Rabenhorst) K.R.Mattox and Bold <i>Chlorhormidium subtilissimum</i> (Rabenhorst) Fott <i>Klebsormidium subtilissimum</i> (Rabenhorst) P.C.Silva, K.R.Mattox and W.H.Blackwell	<b><i>Klebsormidium subtile</i></b> (Kützing) Mikhailyuk, Glaser, Holzinger and Karsten



**Figure 1.** Micrographs of CCA strains. (A) *D. affine* (CCA01Ana01), (B) *G. lemmermanni* (CCA01Os01), (C) *Oscillatoria* sp. (CCA01Os03), (D) *O. princeps* (CCA01Os02), (E) *M. autumnalis* (CCA01Ph02), (F) *P. mucicola* (CCA01Ph01), (G) *Spirogyra* sp.2 (CCA03Spg02), (H) *C. vulgaris* (CCA02Ch01), (I) *P. boryanum* (CCA02Pdr01), (J) *Chlamydomonas* sp.1 (CCA02Chl01), (K) *S. nanum* (CCA02Stg01), (L) *T. obliquus* (CCA02Sce01), (M) *K. subtile* (CCA02Stc01), (N) *Spirogyra* sp.1 (CCA03Spg01), (O) *Achnanthes* sp. (CCA03Ach01), (P) *Nitzschia* sp. (CCA04Nitz01).



**Figure 2.** FTIR spectra of some species



**Figure 3.** Culture and management of strains at the AEU-CCA

**4. Discussion**

The efforts to constitute microalgae culture collection began following the Project to be conducted at Gazi University in 2007. After the Project, the number of isolated species was increased. The studies are still being carried on at universities of Kırşehir and Ankara. The research team firstly focused upon identification of the species through instrumental measurements.

Afterwards, studies to constitute a culture collection began to be conducted as the number of isolated species increased. The AEU-CCA microalgae collection consists of the microalgae isolated as a result of ecological and biotechnological studies conducted in Ankara and its surroundings. The species involved in the collection are economically of great significance, endemic and pave the way for

algae bloom; the studies are maintained upon that systematicity (Figure 3). The microalgae culture collection consists merely of the microalgae species isolated from the fresh water bodies by our research team. The collection contains 19 species belonging to different phylums and we keep studying in order to increase that number. Apart from identification of the species by microscope, their molecular identifications were made through FTIR spectra (Figure 2). All the cultures are preserved parallelly in BG 11 and Allen medium. Besides, in order to enable long-term preservation of the species, the cryopreservation was also made by using different cryoprotectants.

There are many culture collections constituted worldwide and those collections contain a great number of bio-algae species belonging to different families. The species in the collection are not only intended for sale but also they are used for research, education, development of biotechnology and for other worldwide projects. A major part of those collections execute quality management systems and thus, they provide reliability for their services.

Among the best-known culture collections, Culture Collection of Algae and Protozoa (CCAP) (UK), the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP) (USA), Sammlung von Algenku Huren Göttingen (SAG) (Germany), the Culture Collection of Algae at the University of Texas at Austin (UTEX) (USA), American Type Culture Collection (ATCC), the Culture Collection of the Centre of Algology (CCALA), the Culture Collection of Algae at the University of Coimbra (ACOI), the Microbial Culture Collection at the National Institute for Environmental Studies (NIES), Pasteur Culture Collection (PCC), and Korea Marine Microalgae Culture Center (KMMCC) can be considered (Hur et al., 2015; Friedl and Lorenz, 2012). The AEU-CCA microalgae collection we constituted is a very young collection which practices the methods and techniques exercised by large culture collections and keeps studying to increase the number of species.

The cultures in our collection enabled an opportunity for different studies to be carried upon and the results obtained from those studies were published in national and international journals and symposiums (Yalçın et al., 2007; Duygu et al.,

2008; Duygu et al., 2012; Baykal Özer et al., 2012; Yalçın et al., 2014; Udoh et al., 2014; Özer et al., 2016; Yalçın et al., 2016; Erkaya et al., 2016).

Our microalgae culture collection contains species belonging to the phylums of; Cyanobacteria (7), Chlorophyta (7), and Charophyta (3) (Table 1, 2 and 3). There are worldwide studies which keeps being conducted on characterization and biotechnology of the species belonging to those phylums. Cyanobacteria with reference to their microbial activity and in pharmaceutical aspects have been studied by many different researchers (Tiwari & Sharma, 2013; Bhateja et al., 2006; Kumar et al., 2006). They play a significant role in environmental management (i.e. as biofertilizers, soil conditioners, ameliorants of polluted water bodies, and scavengers of heavy metals etc.), in bioindustry (i.e. natural pigments, nutritional supplements, drugs, biofuel etc.), in food and feed (i.e. single cell protein, amino acids, vitamins and minerals) (Vijayakumar, 2012; Noue & Proulx, 1988; Shelef & Soeder, 1980; Vijayakumar, 2005). Chlorophyta are the most diverse and widespread group of algae (Norton et al., 1996). Many of their species are being cultivated with economic aims for a long time. They are also known by their use in the treatment of waste water (Ponnuwamy et al., 2013; Afkar et al., 2010; Ahmad et al., 2013), in production of biodiesel (Chisti, 2007; Gao et al., 2012; Makarevičienė et al., 2011), in production of electricity using microbial fuel cells (Klinthong et al., 2015), in animal food supplements (Fedler & Parker, 1993; Becker, 2004) and in providing valuable extracts for chemical products (Liang et al., 2004; Ördög et al., 2004). Charophytes are phylums which recently have drawn special attention from plant physiologists because of their evolutionary significance. They have become important models in order to comprehend basic facts such as biochemistry, cell biology, developmental biology, ecology and molecular biology, as the studies on species belonging to that phylum have increased (Domozych et al., 2016; Delwiche, 2016). Diatoms draw attention due to their biological characteristics (i.e. fast growth, short life cycle and simple nutritional requirements), their use and application in biofuel production, in medicine and fresh bait

(Caldwell, 2009). The diatoms are one of the appropriate raw material for production bioactive metabolites. The technological developments to identify those compounds have recently become easier (Armbrust et al., 2004; Li et al., 2014).

## 5. Conclusion

The microalgae have a significant value in economy based upon biology and in science world. There are many products that could be obtained from microalgae such as protein, pigmentary substance, raw material for bioplastics and biodiesel. The microalgae are the best biologic method of cleaning absorbable heavy metals and wastes containing undesired chemicals, which are recommended by the scientists. There are projects being conducted at present on microalgae mass culture in many countries such as India, China, the USA and European Union etc. and the biomass harvested is used for many applications. The science world and the industry need cultures which are pure and identified with all of their characteristics in order to utilise in those areas. Therefore, the microalgae culture collections isolating and preserving bio microalgae cultures are needed worldwide. To conclude, we hope to collaborate with other research groups in order to examine chemical compositions of the species which are available at AEU-CCA microalgae culture collection and will be added recently and to get a biotechnological benefit from them.

## Acknowledgements

The present study has started through support from Gazi University Scientific Research Project (G.U.BAP 04/2007-28) and many species were isolated within the scope of that Project.

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