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Review

Production and collection of microalgae isolated from freshwater reserves in Central Anatolia, Turkey

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Abstract

Algae samples were collected from the Central Anatolian Region, including lakes and dam lakes. The algae, which were isolated and grown in a BG-11 medium, were later identified. A total of 17 microalga species have been isolated and cultured from the Central Anatolian Region freshwater resources. The Gazi University Microalgae Culture Collection (GAZI-MACC) contains diverse groups and strains of algae. In this collection, some species are used for toleration against heavy metals and morphological changes and some are used in a variety of experimental studies, such as in determining microalgae toxins and in systematic research studies. The GAZI-MACC is responsible for the following tasks: preservation of the national algae resources, provision of living resources for scientific studies, uses as resources for scientific publications and provision of reliable, confidential, and patented resource services. Collections are in a unique position as the custodians of genetic resources and therefore play a key role in the conservation of genetic resources. This is done through purification, expert preparation, authoritative identification, description, determination of biochemical and other characteristics, comparison with related material, reliable and effective preservation, evaluation of value for biological control uses, and indication of the importance of beneficial and detrimental attributes.

Keywords: Isolation, microalgae, culture collection, Central Anatolia, Turkey

Orta Anadolu (Türkiye) tatlısu kaynaklarından izole edilmiş mikroalglerin üretimi ve koleksiyonu

Özet

Orta Anadolu Bölgesindeki göl ve barajlardan toplanan algler, BG-11 besi ortamında yetiştirilmiş ve izole edildikten sonra teşhis edilmiştir. Orta Anadolu Bölgesi tatlısu kaynaklarından 17 mikroalg türü izole edilmiş ve kültüre alınmıştır. Gazi Üniversitesi Mikroalg Kültürü Koleksiyonu (GAZI-MACC) çeşitli alg grup ve suşlarını içerir. Bu koleksiyondaki bazı türler sistematik araştırmalar ve mikroalg toksinlerinin belirlenmesi gibi değişik deneysel çalışmalarda kullanılmıştır. GAZI-MACC şu birkaç görevle yükümlüdür: ulusal alg kaynaklarını koruma, bilimsel çalışmalar için canlı kaynak sağlama, bilimsel yayınlar için kaynak olarak kullanılma ve güvenli, gizli ve patentli kaynak hizmeti sağlama. Koleksiyonlar genetik kaynakların koruyucusu olarak eşsiz bir konumdadır ve bu nedenle genetik kaynakların korunmasında önemli bir rol oynamaktadır. Koleksiyonlar; saflaştırma, uzman hazırlığı, yetkili teşhis, betimleme, biyokimyasal ve diğer özelliklerin belirlenmesi, ilgili malzeme ile karşılaştırma, güvenli ve etkili koruma, biyolojik kontrol kullanımları için kıymetin değerlendirilmesi ve faydalı ve zararlı özelliklerin öneminin gösterilmesi yoluyla yapılır.

Anahtar kelimeler: İzolasyon, mikroalg, kültür koleksiyonu, Orta Anadolu, Türkiye

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INTRODUCTION

One of the important functions that algae perform outside of photosynthesis is that they increase in large amounts the organic matter of water. In this way, they increase the nutrients of aquatic organisms. Therefore, waters with algae are highly efficient and very suitable for other living beings to live. They can convert solar energy into chemical energy through photosynthesis. Microalgae consist of prokaryotic cyanobacteria and eukaryotic algae. As a group, microalgae consist largely of single-celled forms, but some are multicellular (Thompson 2002; Hosikian et al. 2010). Algae also play a cleansing role for water renewal. Those with filling, even constructive properties, change the shape and quality of the shore and the water bottoms. They make food for aquatic animals, produce food for them and form the basis of the feeding chain in an oceanic environment. The use of algae in the process of cleaning domestic and industrial waste is also one of the most recent issues. Compounds, such as nitrogen and phosphorus, can be removed from the environment by using algae as a source of nutrients during the cleaning process (Shelknanloymilan et al. 2012). Algae studies were initially in the fields of physiology and biochemistry, specifically to explain the secrets of photosynthetic cycles. In subsequent studies, determining optimal culture conditions for various species has been the focus of many research studies. Thus, algae can be used in biotechnological applications. Researchers are currently surveying the vast range of microalgal species for novel compounds of economic value, such as food, cosmetics, aquaculture, pharmaceutical industries, treatment of wastewater, anti-tumor and anti-bacterial compounds (M.A. Borowitzka & L.J. Borowitzka 1988; Cohen 1999; Smith et al. 2001; Rasmussen & Morrissey 2007; Hosikian et al. 2010). The aim of this review study is to bring together advanced microalgae preservation methods towards preserving microalgae due to their increasing needs and also, seen as a hope in the industrial world, and their importance on events, such as scientific studies and climate change.

In this study, creating a microalgae culture collections (GAZI-MACC) in Turkey is important for biotechnological applications intended to provide a basis for this kind of work. In addition, the inability to provide the specific strains in Turkey makes the issue even more important. Many studies have been carried out systematically in Central Anatolia. In one of these studies, a new algae species, *Clipeoparvus anatolicus* J.Woodbridge, E.J.Cox & N.Roberts, has been identified in the Cappadocia region (Woodbridge et al. 2010). A similar study on saline soils to identify and produce halophilic algae for systematic purposes was performed by Atıcı et al. (2001). According to Yumuşak et al. (2005) in Turkey, culture collection centers are organizations where previously defined specifications of standard microorganisms, cell lines and other biological products and their biochemical, morphological, physiological and genetic specifications are preserved originally, where the strains are stored for long period of time and are provided to researchers when needed. In Turkey there are six culture collection centers, which are members of the World Federation for Culture Collection (WFCC). The first and oldest one is the Refik Saydam National Type Culture Collection (RSKK), which was founded at 1954. However, in the last decade, many new collections in Turkey have been set up, especially in the university laboratories, such as the GAZI-MACC.

The Central Anatolian Region is within the study area with a surface area of 151,000 km^2 and which occupies 20% of the territory of Turkey (Figure 1). It is believed that such a collection will provide a basis for other studies. The algae collections are important as resources of biological origins such as cells, genes and different living organisms with the relevant matrices and are the essential materials for the enhancement of the study of biotechnology, healthcare, and developments

in the sciences, especially as they affect human beings. Therefore, it becomes the duty of states and industry to do everything possible through appropriate projects to preserve and sustain biological resources in nature and ensure their proper usage. Such biological resources are the source of materials for scientific research studies leading to discoveries and inventions that add to the great strides made in biotechnology and the other bio-industries. Living organisms are resources with millions of genes and molecules available for life science and biotechnology (OECD 2007). It is necessary to identify personnel to be responsible for each stage in the processes, while preparing the Culture Collection's Quality Management System and preparing manuals and definition of quality management system. The basic principle is to identify pure, healthy, and live cultures with good qualities and store them under suitable conditions for the formation of long-period culture preservation. Pure, living cultures of microorganisms without variation or mutation, are of utmost importance for both the scientific world and as well as the economy.

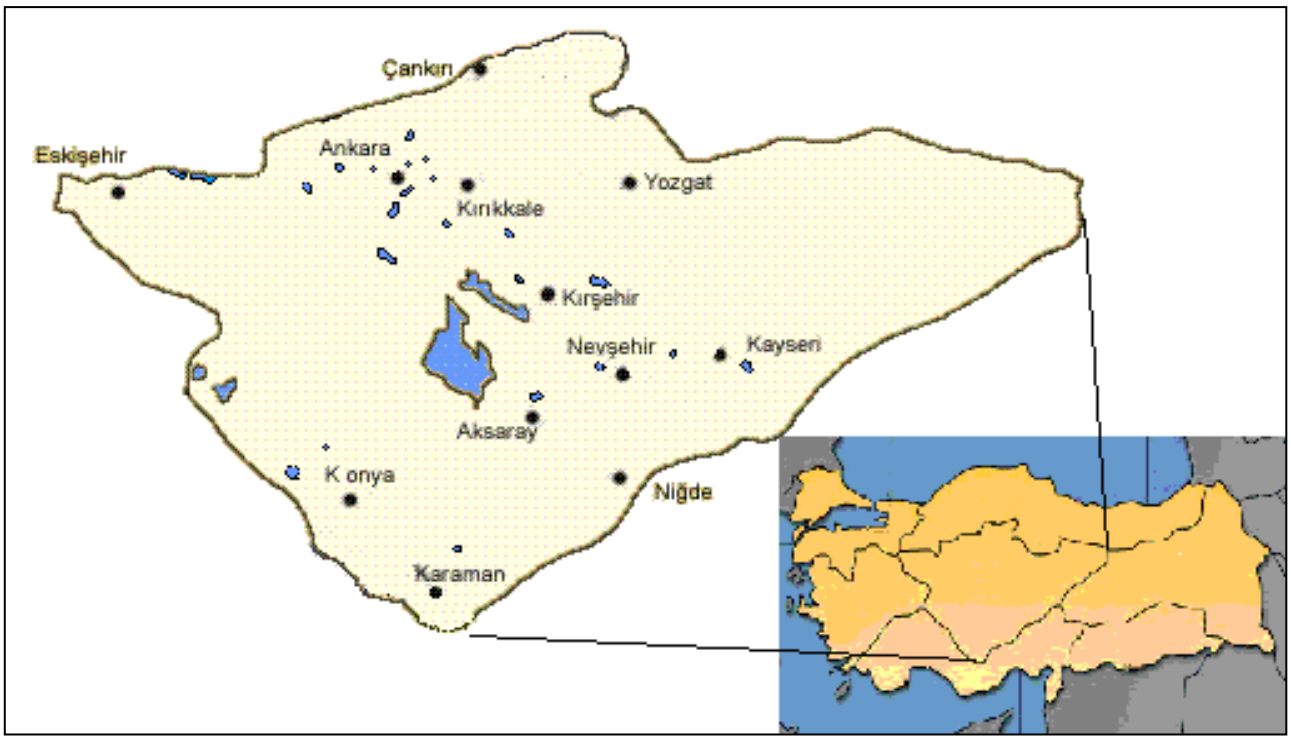

Figure 1. Lake and dam lakes in Central Anatolia.

The biotechnological importance of microalgae in the world, nutrition, and especially protein requirements, are searched through biotechnological methods. Algae constitute an important group among the single-celled organisms used to obtain single-cell proteins or whose usability has been proven by in-vitro studies. It is currently known that algae are sold in Central Africa and Japan (Ciferri 1983).

The optimum cultivation temperature for most microalgae to be produced within the scope of aquaculture is 20°C for environmental conditions and cultural collections (Munir et al. 2015). The sudden elevation and lowering of temperature in an algal culture medium leads to deterioration of the culture and affects the continuity of the existing culture. Photoautotrophic algae require light energy for photosynthesis. Therefore, light is a criterion that is effective with photosynthesis and optimum algal efficiency. Continuous illumination is usually required for achieving rapid growth.

Although the algal growth rate increases due to an increase in light intensity, the photosynthetic capacity decreases after cells reach a certain light intensity.

The intensity of light acting on algal cultures should be adjusted according to the desired growth rate to be obtained in cultures. Specimens obtained from pure culture should be stored at +4°C in slanted agar or suitably lyophilized. A microalgae culture collection belonging to the studied area would be created in this manner.

MATERIAL AND METHOD

Collection of samples

In the study, the lake and reservoir lakes mentioned above were visited 4 times during the study and samples were taken from the designated areas (littoral and pelagic). After the water samples were placed in sterile containers and placed in a laboratory environment, they were transferred to various nutrients prepared under sterile conditions.

Media used and production

In this study, Rippka et al. (1979) and Becker (1994) recommended the microalgae used for the medium. Room temperature, 3000 lux lighting and 2% CO_2 were used for production. BG-11, Allen media modified medium, Bold Wynne medium, Bourrely medium, Beggiota, SOT medium, Bristol modified medium, Zehnder (z-8), Basal medium, *Chlamydomonas* medium, Prat medium and modified Tamiya medium were used (Allen 1984).

Phytoplankton production is based on the artificial formation of autotrophic conditions that will enable rapid development of algae populations. Each species has a different ecological need and it is necessary to prepare the required nutrient medium at the beginning of production. The basic substances required for this are: macro-elements (N, P, C, Ca, Mg, Na), oligo-elements (Fe, Mn), trace elements (Zn, Mo, Cu, Co, B, Si), vitamins (Carotene, Cyanocobalamin, Thiamine) and growth regulators.

In most observations, it has been reported that plant hormones or other growth regulators can be applied to algae. These are auxins, cytokinins and gibberellins. As the development of algae culture depends on the quality of the culture in the test tubes, it is particularly recommended to use pure culture for a stable algae production. The original and purified algae stored in a refrigerator were re-isolated using microbiological methods.

RESULTS AND DISCUSSION

The list of microalgae produced as pure culture under laboratory conditions and taken from the natural environment has been given below (Table 1). When we look at the statistics of culture collection based on the situation in Turkey, it appears to be quite inadequate. The situation is even worse when it comes to services, such as providing samples to private entrepreneurs, etc. This inability has been considered normal, so that even the standards of the Turkish Standards Institute (TSI) are indexed to collections in foreign countries. For example, there are many TSI standards indexed to the American Type Culture Collection. It is also quite difficult for researchers to obtain cultures from these collections. It is necessary to encourage researchers to establish microorganism collections due to this situation. Technological developments in the field of algal biotechnology can be achieved through personal, sectoral, and scientific research. In this research, a total of seventeen

microalgae products were prepared as a pure culture, and these species were added to the GAZI-MACC culture collection.

Table 1. List of the pure microalgae cultures and their phyla (divisions).

	Genus	**Medium**	**Habitats**	**Location**
1	*Anaebena* sp. Phylum: Cyanobacteria	BG-11	Planktonic, Epiphytic	Asertepe Dam Reservoir
2	*Chlamydomonas* sp. Phylum: Chlorophyta		Planktonic, Epiphytic	Kumludere Dam Reservoir
3	*Chlorella vulgaris* Beyerinck Phylum: Chlorophyta	BG-11	Planktonic, Epipelic, Epiphytic	Mogan Lake, Asertepe Dam Reservoir
4	*Chroococcus* sp. Phylum: Cyanobacteria	BG-11	Epiphytic, Epilithic	Mogan Lake, Hirfanlı Dam Reservoir
5	*Cladophora fracta* (O.F.Müller ex Vahl) Kützing Phylum: Chlorophyta		Epipelic, Epiphytic	Mogan Lake
6	*Coelastrum* sp. Phylum: Chlorophyta	BG-11	Planktonic, Epipelic	Seyfe Lake
7	*Cosmarium* sp. Phylum: Chlorophyta	BG-11	Planktonic, Epipelic	İvriz Dam Reservoir
8	*Dactylococcopsis* sp. Phylum: Cyanobacteria	BG-11	Planktonic, Epiphytic, Epilithic	Tuz Lake, Mogan Lake, Sarıyar Dam Reservoir
9	*Dunaliella* sp. Phylum: Chlorophyta	BG-11	Planktonic, Epiphytic	Tuz Lake
10	*Lyngbya* sp. Phylum: Cyanobacteria	BG-11	Epipelic, Epiphytic	Mogan Lake
11	*Microcystis aeruginosa* (Kützing) Kützing Phylum: Cyanobacteria	BG-11	Planktonic, Epipelic, Epiphytic	Asertepe Dam Reservoir
12	*Oocystis lacustris* Chodat Phylum: Chlorophyta	BG-11	Planktonic, Epilithic	Karagöl
13	*Pediastrum boryanum* (Turpin) Meneghini Phylum: Chlorophyta	BG-11	Planktonic, Epipelic, Epiphytic	Mogan Lake
14	*Scenedesmus quadricauda* (Turpin) Brébisson Phylum: Chlorophyta	BG-11	Planktonic, Epiphytic, Epilithic	Susuz Lake, Seyfe Lake Asertepe Dam Reservoir
15	*Selenastrum* sp. Phylum: Chlorophyta	BG-11	Planktonic, Epiphytic	Sarımsaklı Dam Reservoir , Mogan Lake
16	*Spirogyra* sp. Phylum: Chlorophyta	BG-11	Epipelic, Epiphytic	Çavuşçu Lake
17	*Ulothrix zonata* (F.Weber & Mohr) Kützing Phylum: Chlorophyta	BG-11	Planktonic, Epilithic	Eymir Lake

The World Data Center for Microorganisms (WDCM) currently has 469 cultural collections from 62 countries (GCM 2020). Cultural collections and culture samples from the WDCM give us extensive information about the culture collections and cultural examples of the world. In addition to the distribution of cultural collections in the world, it is possible to obtain information about the world support of these cultural collections, the number and diversity of the cultural samples, the education, consulting, identification, storage and distribution services of the organizations that have cultural collections, and the provision of patented deposits.

A comparative study was conducted on the distribution of cultural collections registered at the WDCM, and it was observed that Asia and Europe have the most cultural collections based on continents, followed by America and Africa, respectively. The countries with the most cultural collections in Asia are Thailand, Japan, China, Indonesia, and India, respectively. However, the number of culture samples is not proportional to the distribution of cultural collections and different rates are observed by countries. In the EU countries, France has the highest distribution of the number of microalgae culture collections, followed by England and Germany, respectively. Ireland has the lowest share and from the EU countries, Luxembourg is not included here. England has the highest share of cultures in this regard, followed by the Netherlands, Belgium, Sweden, and France with the same share. Among the EU acceding countries and candidate countries, while the Czech Republic has the greatest share of the distribution of microalgae culture collections, it is followed by Poland, Turkey, and Hungary. The Czech Republic is again in the lead for cultures, followed by Bulgaria, Poland, Hungary, and Romania.

In contrast to the expectations in the Americas, Brazil has a significant margin for cultural collections, followed by the United States, Canada, and Mexico. However, this situation changes for culture samples and the ranking is the United States, Canada, and Brazil. In addition, the United States is the country with the highest number of cultures among the 62 countries registered and has more than twice the number of culture samples, even after Korea.

Australia has more cultural collections and culture examples in the Australia, Papua New Guinea, and New Zealand group. According to these data, it is possible to observe Turkey's shortcomings on this subject. Turkey has a very favorable climate for algae culture. However, microalgae are obtained from cultural collections abroad for many research and technological applications (Derakhshandeh et al. 2020). In 2004, the EGE-MACC was established within the Ege University Science, Technology, Application, and Research Center (EBILTEM) (Conk-Dalay & Cirik 2004). It is known that so far this is the only center in Turkey that has been established on this subject. Algae are cultivated in some state-owned institutions, but there is no formal collection of culture and species.

Strains in serial manners and subcultures, are normally stored in laboratories, but with specific functions and were chosen in large quantities for special purposes. Though sustainability through subcultures may be used for different organisms, the method is in progress, while the strains of organisms involved are faced with the high risks of being contaminated or a total loss as a result of inadequate knowledge. A Biological Research Center (BRC) is a research center that carries out the duties of developing technologies used in storing and preserving the algal cultures. It is generally a center where high technique studies and development are carried out. Therefore, such centers are of great importance for current knowledge and information on taxonomy, characterization, preservation, biosafety, and shipment. Added to the above, they have relevance as mentoring

centers for education and training on issues within the scope. To set up and run a Biological Resource Center (BRC), utmost attention must be paid for credible and trustworthy storage techniques capable of implementation to ensure the desired control for allowing a proper recovering of the strains and other materials originally used in the isolated culture. Different kinds of BRCs exist depending on the types of materials found there and the needs those materials serve (Becker 1994). Whatever the case, these centers, and the materials they hold, are meant to be of good quality and information good enough for use to allow the achievement of the required goals and standards. It is our opinion that the results of this study will contribute and shed light on enhancing future research studies in this field in Turkey.

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