The Characteristics and Importance of Microalgae Culture Collections

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Geliş : 09.11.2016
Kabul : 01.03.2017

Review / Derleme

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E-Dergi ISSN: 1308 – 7517

Abstract

Cultural collections, whether created by government or private organizations, are important to meet the needs of scientific studies, biotechnology and bioindustry. They are also regarded as an important center for biodiversity and the conservation of the natural resources of the countries. Biological Research Center (BRC) serves as depots for live resource materials, supplies, and as sources of relevant information to the public. When culture collections are planned, it is necessary to take into account culture species, quantity and the preservation method. Along with several other methods applied toward preservation, lyophilization and cryopreservation are the most widely used in micro algal collections. The necessary criteria such as memorandum of agreements (MoA), collaboration with different centers, legal and quality control procedures, required to be possesed by a culture collection, have all been defined by the World Federation of Culture Collection (WFCC). This paper therefore, reviews the postions of microalgae in ecosystem, aims the protection of microalgae as biological resources for human life and activities, the establishement of culture collections and provides a general view about the subject.

Keywords: Culture collection, microalgae, lyophilization, cryopreservation

Mikroalg Kültür Koleksiyonlarının Özellikleri ve Önemi

Özet

Kültür Koleksiyonları, ister kamu ister özel sektör tarafından yürütülmüş olsun, bilimsel çalışmalar, biyoteknoloji ve biyo-endüstrinin ihtiyaçlarının karşılanması için önem taşımaktadır. Aynı zamanda, kültür koleksiyonları biyolojik çeşitlilik ve ülkelerin doğal kaynaklarının korunması için bir merkez oluşturmaktadırlar. Biyolojik Kaynak Merkezleri (BRC) canlı kaynaklar için depolama sağlamakta, örnek temin etmekte ve kamu yararına gerekli bilgileri tedarik etmektedirler. Kültür koleksiyonları planlanırken, kültür türleri, miktarı ve koruma yönteminin göz önüne alınması gerekmektedir. Kültür korunmasına yönelik olarak pek çok metot uygulanmakla birlikte, mikroalg kültür koleksiyonlarında en yaygın uygulanan liyofilizasyon ve dondurarak saklama metotlarıdır. World Federation of Culture Collection (WFCC) tarafından, kültür koleksiyonlarının sahip olması gereken tüm kriterler, protokoller, çeşitli merkezlerle işbirliği ile yasal ve kalite kontrol prosedürleri tanımlanmıştır. Bu derleme çalışması mikroalglerin ekosistemdeki yerleri, insan yaşamı ve faaliyetleri için biyolojik kaynaklar olarak korunması ve kültür koleksiyonlarının oluşturulması ile ilgili olarak genel bir bakış sunmaktadır.

Anahtar Kelimeler: Kültür koleksiyonu, mikroagler, liyofilizasyon, kriyoprezervasyon

INTRODUCTION

Microalgae are photosynthetic microorganisms. They can convert solar energy into chemical energy through photosynthesis. Microalgae consist of prokaryotic cyanobacteria and eukaryotic algae. While some are multicellular, microalgae as a group, consist largely

Alıntılama: YALÇIN DUYGU, D., UDOH, A.U., ÖZER, T. & A.ERKAYA, İ. (2017). The characteristics and importance of microalgae culture collections. *Süleyman Demirel Üniversitesi Eğirdir Su Ürünleri Fakültesi Dergisi*, 13(1), 80-87.

of single-celled forms (Thompson, 2002; Hosikian et al., 2010). They are involved in nutrient cycling and energy conduction in aquatic systems. Around 40 % of global photosynthesis is assumed to be performed by microalgae (Falkowsky, 1980; Murdock and Wetzel, 2009) thus, becoming important in the alteration of inorganic nutrients (e.g, C, N, and P) and transformation into organic forms (Murdock and Wetzel, 2009), contributing to approximately 50 % of the total planetary primary productivity (Shelly et al., 2002). They yield more protein per unit dry-weight than the rest of the terrestrial plants. They are also the main source of some organic molecules important in human nutrition and physiology such as long chain polyunsaturated omega three fatty acids (Thompson, 2002). Presently, researchers are surveying a wide range of microalgal species for important compounds with economic value as food, cosmetics, aquaculture, pharmaceutical industries, treatment of waste water, anti-tumor and anti-bacterial compounds (Borowitzka and Borowitzka, 1988; Cohen, 1999; Smith et al., 2001; Thompson, 2002; Rasmussen et al., 2007; Hosikian et al., 2010). The aim of this review, is to bring advanced microalgal storing methods together towards preserving microalgae because of its increasing needs and also, seen as a hope in the industrial world, and its importance in scientific studies.

The Importance of Culture Collections

Resources of biological materials such as cells, genes and other living organisms with relevant matrixes, are the essential raw materials for the advancement of biotechnology, human health, research and development in the life science. It is therefore, the duty of states and industry to initiate appopriate projects to preserve and sustain biological resources in natural forms and ensure their proper usage. Live resource materials are the sources of study samples for scientific research leading to discoveries and inventions which contribute to the great achievements made in biotechnology and other bio-industries. Biological organisms are resource materials, with millions of genes and molecules available for life science and biotechnology (OECD, 2007).

The basic principle of cultural protection is to maintain healthy and livelihoods without losing the qualities of pure cultures and long-term preservation in appropriate conditions. As we take into account of the scientific and economic world, the culture collections are very important due to the fact that microorganism cultures preserved as pure, viable, kept unmutated and undamaged.

Cultural collections present their cultural materials to academic, public, private and commercial activities for the development of industrial processes, as reference strains in academic studies, for taxonomic studies and biological assays. Cultural collections are for the conservation of biodiversity to preserve national resources of a country (Stacey and Day, 2007). Different countries and institutions have established or are setting up community-supported culture collection centers for microorganisms. In this context, culture collections purpose of rendering services to countries and regions are to enforce their own research activities (WFCC, 2010). BRCs in several countries have played many roles. They preserve and provide biological resources for scentific, industrial, agricultural, environmental and medical R&D applications and also, for the conservation of biodiversity. An increasing number of test methods rely on the use of certified, stable and validated biological materials. A small but rising number of BRCs are seeking and achieving certification for the supply of such biological reference material (OECD, 2007).

From the past till present, the earth has been experiencing great extinctions in the loss of varieties of species due to one factor or the other. However, the current extinction crisis differs in and it is the direct result of human activities. As a result, it is believed that a great number of algal species would have been totally extincted or hugely reduced mostly in freshwaters due to discharges of domestic, agricultural and industrial wastes (Lugo, 1988; Maxted et al., 1997).

To counter the above or reduce its occurence, strategic efforts are being made, either to restore or preserve biodiversities, in or ex situ. In-situ preservation of algae is to keep the variations in genetic materials at where it happens, wild or through conventional cultivation systems. To achieve the above, it is pertinent to set up standard natural reserves or parks across the globe. For ex-situ conservation, algal strains are preserved as live collections (e.g. culture collections) or as spores, cysts, DNA, etc, and under special unnatural conditions (Kirsop and DaSilva, 1988; Smith, 2001; Watanabe, 2005).

Characteristics of Culture Collections

The World Federation of Culture Collection (WFCC) had indicated that it is necessary to decide on the aim of each collection suitable with its policy, based on its identity, species of cultures and the quantity to be preserved. In addition to this, preparation of quality control and records keeping, collection catalogues, demand for the cultures, preparation of procedures to be followed and basic logistics for the movement of materials both, locally and internationally among partnership organizations, have been defined (UKNCC, 1998; Smith, 2001; Yumuşak et al., 2005). While preparing the Culture Collection's Quality Management System, preparation of Mannuals and the definition of quality managment system, it is necessary to identify personels to be responsible for each stage in the processes. Quality managers are in charge of controlling and overseeing of all the documents related to the quality standards of the collection works. In the application of the collection quality policies, generally three levels of documentations are normally employed. These are: Quality Manual, Laboratory Procedures Manual or Standard Operating Procedures (SOPs) and laboratory records. Quality Manuals deal mainly with policy administration and management. Laboratory Procedures Manual focuses on procedures and technical functions. Series of laboratory records are kept by the collection centers, including laboratory books. Microorganisms to be deposited at the center must fullfill the acquisition criteria of the collection documentation showing isolation data especially, the country of origin and identity or characterisation information (UKNCC, 1998; WFCC, 2010). The biological materials must be kept under environmental parameters that ensure the stability of its properties as documented (OECD, 2007).

In order to make delivery to the customers, attend to orders, customer undertaking, supply, information provided about the organisms supplied, packaging, invoicing, and refunds, constitute the important information that should be defined as to how they would be enforced and records kept (UKNCC, 1998; WFCC, 2010). BRCs must give at least, minimum information to the user, e.g. biological material identifier, accession and batch numbers, estimate of shelf-life, storage conditions, storage instructions and if appropriate, conditions of growth, instructions for opening ampoules or vials, safety data sheet and material transfer agreement (OECD, 2007).

Members of staff should be trained on duties specific to the job and offered regular training services. Periodic audits are performed by the management toward ensuring that collection policies and procedures, as set out in the manual, are strictly applied.

Measurement instruments and equipment must be calibrated accordingly to provide traceability and reproducibility (UKNCC, 1998; WFCC, 2010).

Accurate identification and numenclature of species at Collection Centers are required while attending to orders by clients, otherwise, this may lead to wrong application during research. Hard copies or electronic catalogues of the strains ready for distribution, should be produced and revised periodically. Risk evaluation must be done before the materials are brought into the collection center and specific procedures applied (WFCC, 2010). There are three fundamental features available to be observed during the collection of biological materials to ensure the values of stored materials: (1) purity (freedom from contaminant organisms); (2) authenticity (correct identity of each strain), and (3) stability, including correct functional qualities (Stacey and Day, 2007). The viability, purity, identity, stability, developmental needs, and directives on maintenance and/or storage of the organisms should be determined and records kept. These data are saved along with information on preservation and growth (Smith, 2001; WFCC, 2010).

Preservation of Cultures

With the advancement in science and technology, different methods for culture preservations have been successfully applied, bearing in mind that there is no single method for all microorganisms. For different groups of microorganisms, a unique procedure and varieties of a similar procedure are recommended (Topal, 1989; Smith and Onions, 1983; Halkman and Dogan, 2000). Regarding each culture strain, a suitable preservation method (s) must be applied by the culture collection center based on its own experience or in agreement with the depositor (OECD, 2007).

Microorganisms often require special preservation methods for optimal viability, recovery of the preserved culture, and avoidance of contaminants in the culture, purity, authenticity, genome integrity and storage (OECD, 2007; WFCC, 2010). Freeze-drying (lyophilization/L-drying) or storage in ultra low temperature in liquid nitrogen or mechanical freezers of temperature (-140°C) or lower (cryopreservation), is the commonly used approach for sustainable preservation of microbial cultures for long periods. These are the best methods allowing high quality, long-term storage, recovery and use of strains, minimising risks of gene change. Where freezing is only available in certain instances, duplicates should be preserved in separate refrigerators with different electrical supplies (UKNCC, 1998; OECD, 2007; WFCC, 2010).

Compared with other groups of microorganisms, relatively little research has been done on the improvement of long-term preservation methods for microalgae (Day et al., 2000). Lyophilization and cryoprservation are the most commonly used methods for preservation. Lyophilization, in other words, dry-freezing, in culture collections, or industry, is widely used today. In lyophilization, the material is simply frozen, and through sublimation, made to dry by vaccum-sucking the water vapour out (Halkman and Dogan, 2000). Together with its general principles, lyophilization is based on three basic phases: freezing, pre-drying (sublimasion) and intermediate drying (desorption). With this technique, the solution is initially cooled between -40° and -50°C after which, it is vaccum-sucked and warmed gradually allowing the ice to vaporize while the water separates from the solution. Lyophilization method has succesfully been applied in preserving yeast and sporulating fungi as well as bacteria. The advantages of the method include protection from contamination or infestation during storage, sustainable viability,

and the facilitation of strain distribution (Berny and Hennebert, 1991). Despite some positive results obtained with microalgae using this method, others have been negative.

Halkman and Dogan (2000) had reported that during lyophilization, viability and activities are affected by several factors which include type of species, age, reproduction medium, pre-process cell density, reaction of preservative with the medium, atmosphere of culture after the processes (vaccum, inert gases, etc), moisture retained in culture, storage conditions (temp, period, light) compounds of solvents used in watering, and temperature (Halkman and Dogan, 2000). Corbett and Parker (1976) indicated that with some species of *Cyanobacteria*, lyophilization method was successful, while with others, it failed. After the process of lyophilization, many of the *Cyanobacteria* that remianed alive were filamentous organisms, which had bred heterocyst or akinet (spors) - special cells showing possible resistance against freezing and drying (Corbett and Parker, 1976). McGrath et al. (1978), out of 106 algal species, had successfully preserved 39 through lyophilization method, with *Chlorella* and *Scenedesmus* being majority and *Chamydomonas* as minority.

Cryopreservation is a method of preservation with liquid nitrogen at vapor phase (-140°C) or liquid nitrogen at liquid phase (-196°C). Liquid phase (-196°C) today, is the most effective known preservation method without loss in culture viability and activeness for many years (according to type and species up to 30-40 years) (Halkman and Dogan, 2000; Baust, 2002). Dimethyl sulphoxide (Me_2SO) is the most frequently used cryoprotectant (CPA) for cryopreserving algal species, although methanol (MeOH) is also reported to be an effective CPA for some algae. Glycerol has been used successfully as a CPA for animal cells and a few eukaryotic algal cells, but some researchers found glycerol to be toxic to *Chlorella* and *Chlamydomonas* (Johnson and Dutcher, 1993).

Cryopreservation has been successfully employed to maintain algae (Day, 1998). Culturable *Cyanobacteria* and soil microalgae can be cryopreserved with relatively high viability. Also, different freshwater and marine eukaryotic algae can be successfully cryopreserved, but typically with lower post-thaw viability levels (Day, 2007). Day and Brand (2005) had successfully applied cryopreservation method in preserving *Cyanobacteria*, marine diatoms and morphologically simple single cells of green algae (Day and Brand, 2005). The viability of marine algae (Cox et al., 2009; Tanniou et al., 2012), diatom (Pilar and Sa´nchez-Saavedra, 2006; Buhmann et al., 2013), eakaryotic algae (Myron and Parker, 1992; Park, 2006) isolates were investigated by different researchers upon freezing in liquid nitrogen. The protocols developed and tested, further enhance application of cryopreservation. Findings imply that cryopreservation could be an easy and time-saving sustainable preservation method for microalgae.

According to a guideline published by the OECD in 2007, L-drying cryopreservation in or above liquid medium in ultra low temperature (below -140°C), and freeze-drying serial transfer (if long term preservation is not possible) are appropriate for *Cyanobacteria*; and for microalgae, sterile liquid medium, sterile semi-solid medium (agar, alginate beads) and cryopreservation below (-140°C) methods, were recommended (OECD, 2007).

There are microalgae culture collection centres in many countries around the world with large numbers of strains being examined in these collections. Some of these are: 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), and Pasteur Culture Collection (PCC).

CONCLUSION

A large number of culture strains with unique functions have been selected for specific applications, and such strains generally are maintained in laboratories through serial subculture. If maintenance by subculture can be applied to a wide variety of microalgae, this technique requires a lot of human power and strains can be lost due to human error and can be contaminated. An extinction through out the microalgae species may come up with a wide range of activities of people and some natural phenomena. Reference strains, whose characteristics are well known during scientific researches, are also needed. Researchers may need to provide some strains that are not available in their own country. When all these factors are taken into consideration, the need for Biological Resource Centers is emerging. A BRC must have a reliable protection technology and quality control management in order to protect the properties of isolated and conserved cultures characteristics.

A BRC is a research center that carries out the duties of developing technologies used in storing and preserving algal cultures. It is generally a center where such high-technique studies and developments are done. Therefore, these centers are very important in terms of current knowledge and information on taxonomy, charactization, preservation, biosafety and shipment. In addition, it is the center of guidance for education and training on issues within the scope. Different kinds of BRCs exist depending on the types of materials they habour, and the needs those materials serve. Nevertheless, the centers and their materials, are meant to be of good quality and information, good enough for use, to allow the achievment of the required goals and standards. With this review study, the importance of microalgae; why microalgae culture collections are necessary and what basic qualifications they have to contain has been briefly summarized and a general outlook has been tried to be brought over this issue.

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