REPUBLIC OF TURKEY
ISTANBUL MEDENIYET UNIVERSITY
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IMU-ASYA Culture Collection: Molecular Barcoding and Cryopreservation of Microalgae Isolated During IVth National Antarctic Science Expedition

MASTER OF SCIENCE THESIS

ASİYE SİNACİ

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SUPERVISOR
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DECLARATION

I hereby declare that all information in this dissertation has been obtained and presented in accordance with academic rules and ethical conduct. I also declare that, as required by these rules and conducts, I have fully cited and referenced all material and results that are not original to this work.

Asiye SİNACİ

Signature

Asiye Sinaci, a M.Sc. student of Istanbul Medeniyet University, successfully defended the thesis/dissertation entitled “IMU-ASYA Culture Collection: Molecular Barcoding and Cryopreservation of Microalgae Isolated During IVth National Antarctic Science Expedition”, which she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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Dedicated to my lovely family

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LIST OF SYMBOLS

A: Absorbance
cm: Centimeter
°C: Degree Celsius
Ω: Doubling time
g: Gram
g: g-force or rcf
μ: Growth Rate
km: Kilometer
L: Liter
M: Meter
μg: Microgram
μL: Microliter
μm: Micrometer
mg: Milligram
mL: Milliliter
min: Minute
M: Molarity
nm: Nanometer
✇: Number of Generations
NA: Not available
%: Percentage
rcf: The relative centrifugal force
rpm: Revolutions per minute
w/w: Weight by weight

LIST OF ABBREVIATIONS

ATP: Adenosine Triphosphate

BLAST: Basic Local Alignment Search Tool

BG-11: Blue-Green Medium

BBM: Bold-Basal Medium

DNA: Deoxyribonucleic acid

DMSO: Dimethyl Sulfoxide

mRNA: Messenger RNA

NCBI: National Center for Biotechnology Information

PCR: Polymerase Chain Reaction

RNA: Ribonucleic acid

rDNA: Ribosomal deoxyribonucleic acid

SSU: Small Subunit

TAE: Tris-Acetate-EDTA

TAP: Tris-Acetate-Phosphate Medium

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ABSTRACT

IMU-ASYA Culture Collection: Molecular Barcoding and Cryopreservation of Microalgae Isolated During IVth National Antarctic Science Expedition

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Master Thesis, Molecular Biology and Genetics Department, Molecular Biology and Genetics Program

Supervisor: Prof. Dr. Turgay ÇAKMAK

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Culture collections are of special importance for scientific studies as they serve as safe depositories of biological materials, significantly contributing to acquiring taxonomic, physiological, genetic, and morphological information. As a consequence of increasing scientific curiosity in the investigation of algae at the end of the nineteenth century, several algae culture collections were established. There are 44,596 eukaryotic algae - 17,819 diatoms - documented in AlgaeBase as of July 2022. A vast majority of them have been deposited in microalgae culture collections. This study contributes to microalgae biodiversity information by providing a genomic and morphological evaluation of the eukaryotic microalgae isolated from Horseshoe Island and Dismal Island in Marguerite Bay of the Antarctic Peninsula during the IVth National Antarctic Science Expedition.

In order to sample microalgae, sediment, stone, ice, snow, and water samples were collected on-site. For isolation, the samples were introduced to selective microalgae growth media in liquid and agar forms on the same day of field sampling. Moreover, samples were also transported to the laboratory for further isolation. The axenic cultures were obtained mainly by plate-streaking and serial dilution and were analyzed morphologically and genomically. Genomic DNA was extracted for genomic identification, 18S rDNA coding fragments were amplified, and then sequence reads were obtained by Sanger sequencing. Lastly, the strains were submitted to the NCBI, accession numbers were obtained, the phylogenetic trees were produced, and all strains were represented on a web page (www.imuasya.org).

All isolates in the IMU-ASYA collection are deposited as cryopreserved as well as serial sub-culturing. The collection contains 62 strains from 26 taxa, of which 13 from *Trebouxiophyceae*, 3 from *Ulvophyceae*, 4 from *Chlorophyceae*, 2 from

Klebsormidiophyceae, 1 from *Xanthophyceae*, and 3 from *Bacillariophyceae*. This is the first Antarctic microalgae culture collection in Turkey. It is a unique microalgae culture collection that supports the biotechnological application and provides information better to understand the nature and use of psychrotolerant microalgae.

Keywords: Antarctica, cryopreservation, culture collection, microalgae

ÖZET

IMU-ASYA Kültür Koleksiyonu: IV. Ulusal Antarktika Bilim Seferi Sırasında İzole Edilen Mikroalglerin Moleküler Barkodlanması ve Kriyoprezervasyonu

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Danışman: Prof. Dr. Turgay ÇAKMAK

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Kültür koleksiyonları, biyolojik materyallerin güvenli depoları olarak hizmet ettikleri ve taksonomik, fizyolojik, genetik ve morfolojik bilgilerin elde edilmesine önemli ölçüde katkıda bulundukları için bilimsel çalışmalarda özel bir öneme sahiptir. 19. yüzyılın sonlarında alg araştırmalarında artan bilimsel merakın sonucu olara çeşitli alg kültür koleksiyonları oluşturulmuştur. Bugüne kadar, Temmuz 2022 itibariyle AlgaeBase'de belgelenen 44.596 ökaryotik alg vardır ki bunların 17.819'u diatomdur. Bu mikroalglerin büyük bir çoğunluğu mikroalg kültür koleksiyonlarında depolanmıştır. Bu çalışma, IV. Ulusal Antarktika Bilim Seferi sırasında Antarktika Yarımadası'nın Marguerite Körfezi'nde yer alan Horseshoe Adası ve Dismal Adası'ndan izole edilen ökaryotik mikroalglerin genomik ve morfolojik bir değerlendirmesini sağlayarak mikroalg biyoçeşitliliği bilgisine katkıda bulunmaktadır.

Mikroalg örneklemesi için sahada tortu, taş, buz, kar ve su örnekleri toplanmıştır. İzolasyon için, aynı gün içinde toplanan örnekler sıvı ve agar formlarında seçici mikroalg yetiştirme ortamına ekilmiştir. Ayrıca örnekler daha sonra izolasyon için laboratuvara taşınmıştır. Aksenik kültürler, esas olarak çizgi ekim yöntemi ve seri seyreltme yaklaşımlarıyla elde edilmiştir. Elde edilen aksenik kültürler morfolojik ve genomik olarak analiz edilmiştir. Genomik tanımlama için, genomik DNA ekstrakte edilmiş ve 18S rDNA kodlama fragmanları amplifiye edilerek Sanger Dizileme ile dizi okumaları elde edilmiştir. Son olarak suşlar NCBI'ye gönderilmiş, erişim numaraları elde edilerek filogenetik ağaçları üretilen tüm suşlar IMU-ASYA web sayfasında (www.imuasya.org) tanıtılmıştır.

IMU-ASYA koleksiyonundaki tüm izolatlar, seri alt kültürlemenin yanı sıra kriyoprezerve edilmiş olarak da depolanmaktadır. Koleksiyonunda toplam 62 suş ve

26 takson bulunmaktadır; 13'ü *Trebouxiophyceae,* 3'ü *Ulvophyceae*, 4'ü *Chlorophyceae,* 2'si *Klebsormidiophyceae,* 1'i *Xanthophyceae* ve 3'ü *Bacillariophyceae* dir. Bu koleksiyon, Türkiye'deki ilk Antartik mikroalg kültür koleksiyonu olma özelliği taşımaktadır. IMU-ASYA, psikrotolerant mikroalglerin doğasını ve potansiyel kullanım alanlarını daha iyi anlamak ve biyoteknolojik uygulamaları desteklemek için bilgi sağlayan benzersiz bir mikroalg kültür koleksiyonu olma özelliği taşımaktadır.

Anahtar kelimeler: Antarktika, kültür koleksiyonu, kriyoprezervasyon, mikroalg

1. INTRODUCTION

1.1. IMPORTANCE OF MICROALGAE CULTURE COLLECTION

Culture collections protect genetic material, serve as repositories for biodiversity in natural environments, and provide research and development resources. They are vital for building biodiversity knowledge because they serve as safe depositories of biological materials, significantly contributing to acquiring taxonomic, physiological, genetic, and morphological information. Prof. Frantisek Král established the first bacterial culture collection at the German University of Prague in 1890. Since then, many other cultural collections have been established.

As a consequence of increasing scientific curiosity in the investigation of algae at the end of the nineteenth century, multiple specified culture collections were established. The first collection of laboratory-cultured algae was created by J. Horejsi (1910) at Charles University in Prague, and he successfully purified symbiotic cyanobacteria from *Cycas revoluta* and V. Uhlir isolated cyanobacteria from the lichen *Collema*. Samples were cultured in test tubes or Petri dishes using a mineral nutrient solution mixed with soil extract. V. Uhlir's collection was lost after his death. In the 1920s, Ernst Georg Pringsheim, a Professor of Plant Physiology, established an Algae Culture Center at Charles University in Prague. The first cultures were isolated by Pringsheim and his associate V. Czurda (1897-1945), and 49 isolates maintained in the culture collection were published. Due to political unrest, Prof. Pringsheim left Prague in 1938 and traveled to England with some species to establish a new Culture Collection at Cambridge University, the UK, named the Culture Collection of Algae and Protozoa (CCAP) (www.ccap.ac.uk, 2022).

When E.G. Pringsheim relocated to Germany in 1953, cultures from this collection were used to establish the Sammlung von Algenkulturen in Göttingen (SAG) (sagdb.uni-goettingen.de, 2022). The SAG collection is an extensive biological

center of living algae; it is one of the world's three largest algal service culture collections. The SAG microalgae collection plays an essential role in building the infrastructure for scientific research by maintaining and providing algal resources like other microalgae culture collections. Algal culture collections function as a resource and service infrastructure and storage of expert knowledge on identifying, isolating, and culturing algae (Friedl & Lorenz, 2012).

R.C. Starr, Pringsheim's collaborator at Cambridge, used nearly 400 strains of green algae cultures to create the Indiana University Culture Collection (IUCC), also provided by Pringsheim. This living algae collection was expanded, diversified, and later moved to its current location and renamed the Culture Collection of Algae at the University of Texas at Austin (UTEX) in the United States in 1976 (Jaenicke, 1998). UTEX's primary resource is its extensive collection of live algae. More than 3,000 different algae species representing over 500 genera are available (utex.org, 2022). The collection preserves a wide range of freshwater and soil algae. All strains in the collection have been obtained in isolation from natural sources. About half of the UTEX strains are axenic, and all cultures distributed to the public are unialgal. UTEX aims to encourage, support, and enable algae use for research and education. The cultures in the collection are used for research, teaching, water quality assessment, biotechnology development, and various other purposes. UTEX does not impose any restrictions on the use of purchased cultures and assumes no liability for cultures sent from the facility.

During World War II (WWII), V. Czurda maintained cultures left in Prague by E.G., Pringsheim, and cultures from S. Prat. When the Czech Charles University was reconstituted at the end of WWII, S. Prat returned to the Institute of Plant Physiology with his partners, R. Retovsky (1904-1964) and M. Baslerova (1905-1979), who succeeded in preserving the collection. In 1979, most of the Prague collection was relocated to Trebon and formed part of what is now known as the Culture Collection of Autotrophic Organisms (CCALA). New isolates are step by step added to the CCALA collection. The collection's primary purpose is to collect a variety of essential clonal cultures of algae and cyanobacteria. The CCALA collection has microalgae from polar, snow, and thermal springs.

The Culture Collection of Cryophilic Algae at Potsdam (Germany) (CCCryo) was founded in 1999 by Thomas Leya and the supervisor of his doctoral thesis, G.R. Fuhr. The CCCryo is located in the Bioanalytical and Bioprocesses Branch (IZI-BB) of the Fraunhofer Institute for Cell Therapy and Immunology in Potsdam near Berlin. The CCCryo specializes in cryophilic freshwater and permafrost microalgae from arctic and alpine conditions. The CCCryo provides an extremophile bioresource for public and industrial research (Leya, 2020). Strains or species can also be searched through the Global Catalog of Microorganisms.

Microbial Culture Collection at the National Institute for Environmental Studies in Japan (MCC-NIES) was established in 1983. The researchers isolated most MCC-NIES species directly, but some were taken from exchanges with other collections. About 80% of the MCC-NIES strains were initially sourced from Japan, giving the collection a high level of authenticity (Kawachi & Noël, 2014).

WFCC is a Multidisciplinary Commission of the International Union of Biological Sciences (IUBS) and a Federation within the International Union of Microbiological Societies (IUMS). The WFCC is concerned with collecting, validating, maintaining, and distributing microorganism cultures and cultured cells. It aims to encourage and support the establishment of cultural collections and related services and establish a network of contacts and information between the collections and their users.

There are twelve WDCM culture collection centers in Turkey. The first and oldest is the Refik Saydam National Type Culture Collection, established in 1954. Many new collections in Turkey have been recently established, especially in the university laboratories such as IMU-ASYA, IMBIYOTAB, MAKU-MACC, EGE-MACC, and GAZI-MACC. IMU-ASYA stands for the only psychrotolerant microalgae culture collection in Turkey. It is a unique microalgae culture collection that supports the biotechnological application and provides information better to understand the nature and use of psychrotolerant microalgae.

1.2. MICROALGAE

Algae are living organisms like all plants that obtain their food from sunlight through photosynthesis. Algae can be found in the sea, oceans, rivers, lakes, and ponds. Unlike plants, they lack roots, stems, and leaves but have cellulose cell walls. Algae cells are classified into two main categories as macroalgae and microalgae. Macroalgae are multicellular organisms ranging from millimeters to meters, whereas microalgae are small and unicellular organisms with sizes from 0.2 to 100 μm. The reasons why microalgae are preferred as a biological source:

(a) Microalgae have high photosynthesis efficiency and are adaptable to different light and temperature conditions (Nascimento et al., 2013).

(b) Microalgae can grow in water with varying nutrient levels and adapt to growth conditions, and nutrient uptake ability can change with this adaptation mechanism.

Microalgae are single-celled, photosynthetic, microscopic organisms found in freshwater and seawater (Barbera et al., 2016; Torres et al., 2017). They are classified as either eukaryotic or prokaryotic microorganisms. Cyanobacteria (Cyanophyceae) can be examples of prokaryotic microorganisms, while green algae (Chlorophyta) and diatoms (Bacillariophyta) are examples of eukaryotic microalgae. It is estimated that around 272,500 eukaryotic algae are diversified on different earth biomes. Of these microalgae, 200,000 are diatoms, and 72,500 are represented by other eukaryotic algae (Guiry, 2012). However, the fact remains that only a total of 44,596 eukaryotic algae - 17,819 of these are diatoms - have been documented in AlgaeBase of July 2022. Major microalgae phyla and class distribution retrieved from the AlgaeBase website are given below (www.algaebase.org/browse/taxonomy, July-2022).

Empire: Eukaryota (51.261 species)
- **Kingdom:** Plantae (20.028 species)
  - **Subkingdom:** Biliphyta (7.494 species)
    - **Phylum/Division:** Rhodophyta (7.491 species)
      - **Class:** Bangiophyceae (186 species)
      - **Class:** Compsopogonophyceae (73 species)
      - **Class:** Cyanidiophyceae (6 species)
      - **Class:** Florideophyceae (7.090 species)
      - **Class:** Porphyridiophyceae (10 species)
      - **Class:** Rhodellophyceae (7 species)
      - **Class:** Rhodophyta classis incertae sedis (69 species)
      - **Class:** Stylonematophyceae (50 species)
  - **Subkingdom:** Glaucoplantae (26 species)
    - **Phylum/Division:** Glaucophyta (26 species)
      - **Class:** Glaucophyceae (26 species)
  - **Subkingdom:** Viridiplantae (12.408 species)
    - **Phylum/Division:** Chlorophyta (7.185 species)
      - **Class:** Chlorodendrophyceae (65 species)
      - **Class:** Chlorophyceae (3.702 species)
      - **Class:** Chlorophyta classis incertae sedis (20 species)
      - **Class:** Chloropicophyceae (8 species)
      - **Class:** Chuariophyceae (3 species)
      - **Class:** Mamiellophyceae (25 species)
      - **Class:** Nephroselmidophyceae (28 species)
      - **Class:** Pedinophyceae (24 species)
      - **Class:** Pyramimonadophyceae (141 species)
      - **Class:** Trebouxiophyceae (919 species)
      - **Class:** Ulvophyceae (2.249 species)
    - **Phylum/Division:** Charophyta (5.213 species)
      - **Class:** Charophyceae (892 species)
      - **Class:** Chlorokybophyceae (5 species)
      - **Class:** Coleochaetophyceae (36 species)
      - **Class:** Klebsormidiophyceae (48 species)
      - **Class:** Mesostigmatophyceae (1 species)
      - **Class:** Zygnematophyceae (4.231 species)
    - **Phylum/Division:** Prasinodermatophyta (10 species)
      - **Class:** Palmophyllophyceae (8 species)
      - **Class:** Prasinodermatophyceae (2 species)
- **Kingdom:** Chromista (27.939 species)
    - **Phylum:** Bacillariopyta (17.819 species)
      - **Class:** Bacillariophyceae (14.096 species)
      - **Class:** Bacillariophyta classis incertae sedis (485 species)
      - **Class:** Coscinodiscophyceae (1.482 species)
      - **Class:** Mediophyceae (1.756 species)
    - **Phylum:** Cryptophyta (224 species)
      - **Class:** Cryptophyceae (224 species)
    - **Phylum:** Haptophyta (1.509 species)
      - **Class:** Coccolithophyceae (1.416 species)
      - **Class:** Haptophyta incertae sedis (79 species)
      - **Class:** Pavlovophyceae (13 species)
    - **Phylum:** Ochropyta (4.486 species)
      - **Class:** Chrysophyceae (1.195 species)
      - **Class:** Phaeophyceae (2.100 species)
      - ***Class: Xanthophyceae (739 species)***

Members of Chlorophyta are photoautotrophic algae that can be unicellular or multicellular and vary significantly in shape and size. The majority of the species live in freshwater. Some species have adapted to live in harsh environments such as deserts, arctic, hypersaline, acidic, and alkaline habitats. The chlorophyll is the main pigment (especially chl *a* and chl *b*). Many species have flagellated motile swimming cells. Some of the most biotechnologically important microalgae which belong to the Chlorophyta include *Chlorella vulgaris, Haematococcus pluvialis, Dunaliella salina* are used in commercial areas for different purposes, such as nutritional supplements and animal feeding (Koyande et al., 2019).

Bacillariophyta, known as diatoms, are mostly unicellular but may also be colonial or filamentous. The cell wall (frustule) is silica and consists of two valves. Most diatoms are photosynthetic, but some species lack chlorophyll and live heterotrophically among decaying marine algae.

Microalgae have a short generation time and multiply exponentially in favorable environmental conditions. As a result of their evolutionary adaptation to a wide range of habitats and extreme environments, microalgae have an abundance of biological and genetic diversity, potentially producing a variety of bioactive compounds. Some microalgae species can grow well in brackish water, wastewater, or seawater, and these distinct growth conditions can affect the content of microalgae.

1.3. ANTARCTICA

Antarctica, the fifth largest continent globally with a surface area of 14 million square kilometers, is located in the Southern Hemisphere. The continent has one of the hardest habitats for life on Earth. Extremely low temperatures, limited liquid water resources, low atmospheric humidity, long dark and light cycles, and high radiation periods make the continent unsuitable for living populations.

Antarctica acts as a natural laboratory concept for different climatic studies, geophysics, biology, and other scientific areas and has abundant natural resources.

Scientists predict that the continent hosting various studies will have abundant mineral reserves.

Temperatures as low as –89.2°C and wind speeds of 248 kilometers per hour have been reported, as temperature decreases by up to 65 °F (36 ° C) in 12 minutes. It is the most challenging area on Earth where continuous logistical support has been tried. During the winter, sea ice covers 18 million square kilometers and shrinks to 2-3 million square kilometers during the summer. The climatic system is balanced via sea ice accumulation.

The climate of modern Antarctica is not proper for the growth of vegetation. Freezing temperatures, poor soil quality, a lack of moisture, and a lack of sunlight affect plant growth. Therefore, the diversity of plant life is shallow and has a minimal distribution in Antarctica. The continent's flora is dominated by bryophytes (about 100 species of mosses and 25 species of liverworts), which only two flowering plants found in the Antarctic Peninsula: *Deschampsia antarctica* (Antarctic hair grass) and *Colobanthus quitensis* (Antarctic pearlwort) (Chwedorzewska et al., 2015). The growth season occurs in the summer and lasts only a few weeks. The accumulation of algae is significant because it creates the base of the food chain and serves as a house and breeding ground for various species.

1.4.TECHNIQUES FOR PRODUCING AXENIC MICROALGAE CULTURES

Axenicity, also called sterility or purity of culture, is a required microbial condition of microalgae cultures and means that the culture is not contaminated or co-cultivated with any other species (Andersen & Kawachi, 2005). Physiological, biochemical, or molecular biological experiments are performed to analyze microalgae and should always be run under axenic conditions to avoid immeasurable effects that affect the outcome of the experiments. Contaminations of cultures are the main problem in most cases and are difficult to avoid during long-term experiments involving many sequential cell transfers. However, contamination can reduce microalgal cell concentration, inhibit biomass growth rates, and induce cell stress (H.

Wang et al., 2013). Scientists have developed many different methods to purify and isolate microalgae cultures. Some of these techniques will be explained following.

1.4.1. Streak Plate Method

Streaking is a technique for isolating a species from a mixture of microorganisms to analyze and identify selected species. In this method, a sterile inoculation loop takes a sample, and the chosen colony is seeded onto a new agar plate with a specific pattern.

Single microalgae species may now be differentiated in the colonies based on morphological (size/shape/color) distinctions, and the latter is chosen and spread until no other microorganisms can be found in consecutive agar plates.

One colony must be selected and placed in a drop of sterile culture medium on a glass slide from the agar plate to ensure that the desired microorganism has been isolated and is pure. If the sample is axenic, the same colony is taken one more time and streaked on an agar slant for stock culture. The technique assumes that each colony on the agar surface results from a single dividing cell. While this may seem minor, it is not always the case, particularly with some microalgae whose growth depends on symbiotic relationships, and separating these species is impossible.

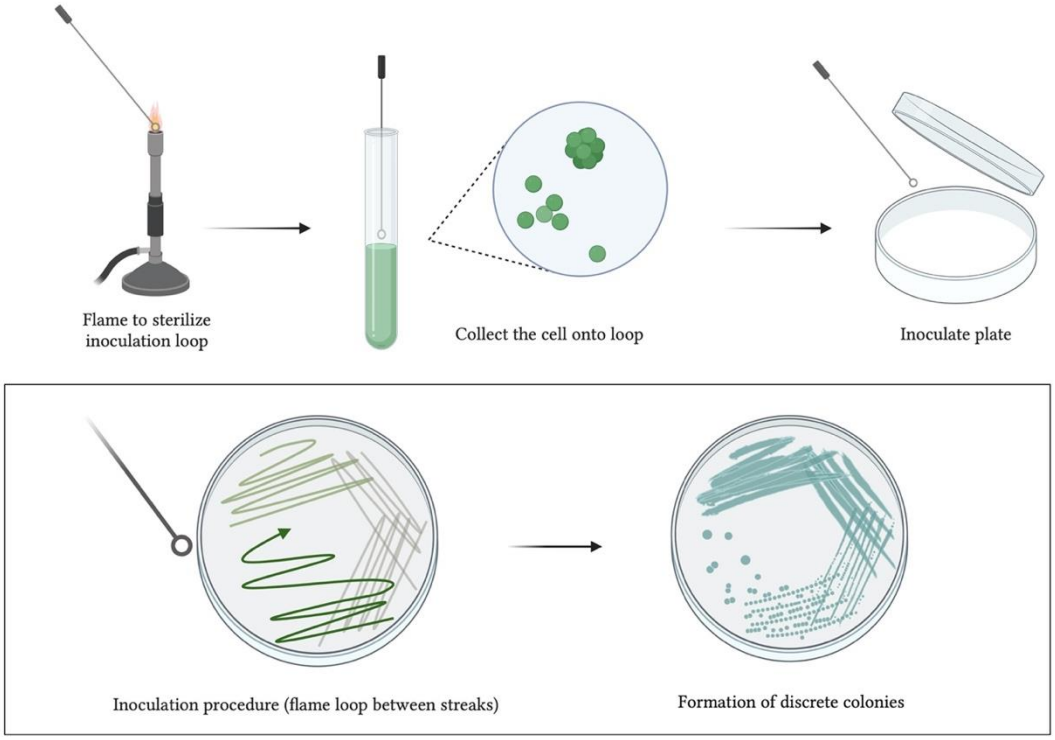


Figure 1.1 Streak Plate Method illustration

1.4.2. Dilution in Liquid Media

A serial dilution protocol is a method for reducing the cell concentration in a liquid growth media. It starts with test tubes filled with a specific amount of liquid medium (i.e., 9 mL). From the enriched microalgae sample, 1 mL is taken and diluted in the first test tube (10^{-1}) by inverting gently. Next, 1 mL is taken from the diluted culture solution using a pipette and transferred into the new test tube (10^{-2}). The test tube was mixed gently by inverting 3 to 5 times. This is repeated until all tubes have been used for dilution; in the result, if ten test tubes were used, the last one will represent a 10^{-10} dilution. Due to this technique, an axenic culture should grow in the highest diluted tubes.

The test tubes are placed in a shaking incubator with algal inoculum and incubated for 7 to 20 days at 15 °C under 16-hour light and 8-hour darkness. The light intensity, photoperiod, and incubation time are determined by the microalgae isolated species. To determine the purity of culture, a small amount of culture medium is taken from different dilution tubes, and samples are examined using a light

microscope every week. Therefore, the number of microalgae species in culture media can be determined. The highest purity is expected from the most diluted culture media at the end of this procedure. The culture media that contain more than one microalgae species need extra dilution steps to obtain a culture containing only one type of microalgae species.

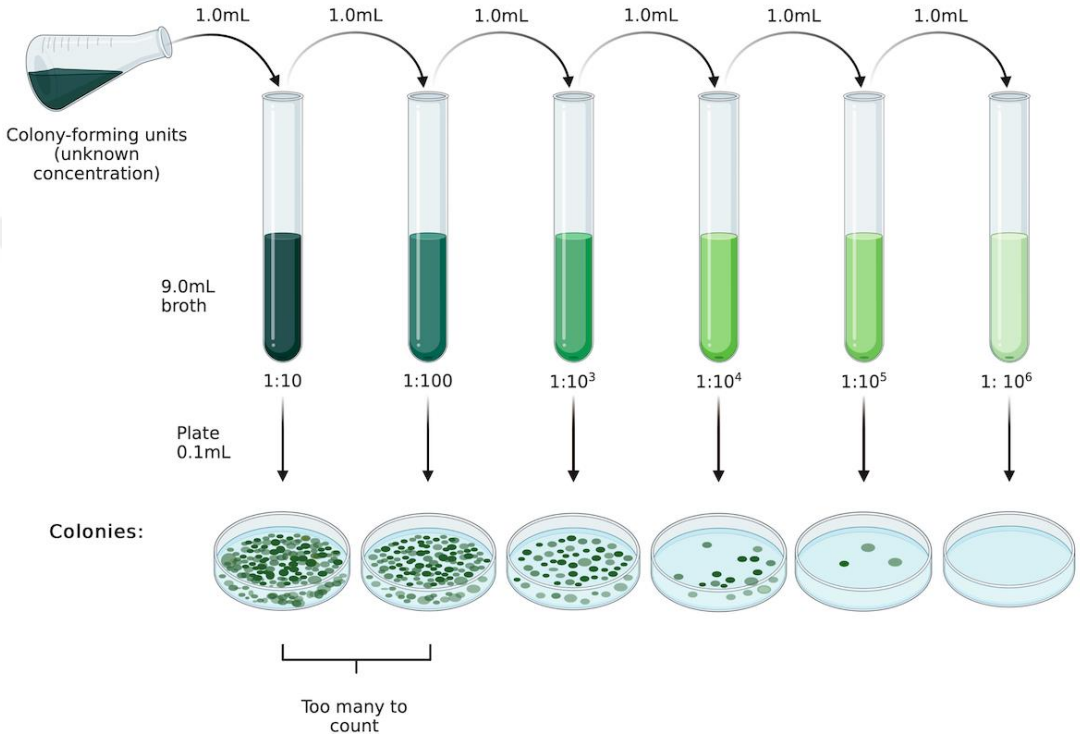


Figure 1.2 Serial Dilution Method illustration

1.5. MICROALGAE NUTRITIONAL REQUIREMENTS

Microalgae have different nutrient requirements depending on their species. However, the essential nutrients for algal growth and metabolism are nitrogen, phosphorus, and carbon (Juneja et al., 2013). Notably, silicate is also a macronutrient desired by some marine microalgae species (diatoms). The presence of macronutrients during cultivation significantly impacts the algal growth rate and oil content (Lardon et al., 2009; Solovchenko et al., 2008).

Proteins and nucleic acids both require nitrogen to be synthesized. The other vital nutrient is phosphate, which is found in the structure of ATP. Moreover, phosphate is a critical component in the backbone of DNA and RNA structures, which are the essential macromolecules for all living organisms. Phosphate is found in the content of phospholipids, so its depletion can affect the organism's metabolism. Nitrogen and phosphorus deficiency, for example, affect lipid metabolism and enhance lipid storage instead of producing membrane lipid. The other required element is carbon, which plays an essential role in photosynthesis, affecting cell growth and reproduction processes. Therefore, decreased carbon fixation rate can affect the growth of the cell.

Photosynthesis in algae requires an inorganic carbon supply. The different forms of carbon are used under other conditions. While CO_2, carbonate, or bicarbonate forms can be used in autotrophic growth conditions, glucose or acetate forms are used in heterotrophic growth conditions.

Specialized studies have been conducted to determine the nutrient requirements of microalgae more accurately. There are numerous recipes for microalgae growing media in the laboratory. Many of these are modifications of previously published methods, while others are derived from water analysis in natural habitat and ecological considerations (Vonshak, 1993). The following are the primary considerations in developing a nutrient recipe for algal cultivation:

- Carbon source (carbon dioxide or bicarbonate).
- Nitrogen source (nitrate).
- Trace elements.
- Vitamins.
- Hydrogen potential (pH).
- Salinity (is determined by the natural habitat of the microalgae).

Based on this general guide, many distinct culture media have been developed, evaluated, and established. Some are very general and support the growth of many microalgae species, while others show species-specific effects for some microalgae. The Culture Collection of Algae and Protozoa (CCAP), The Culture Collection of

Cryophilic Algae (CCCryo), and the Culture Collection of Algae at the University of Texas at Austin (UTEX) provide a comprehensive list of recipes.

1.6.CONDITIONS FOR GROWTH AND MAINTENANCE OF CULTURES

Microalgae culture is affected by nutrient availability (N, P, C, etc.) and other environmental and cultural conditions such as temperature, pH, salinity, inorganic carbon, oxygen, light intensity, and CO_2 (Mata et al., 2010). Stirring and mixing, harvest time interval, and dilution rate are significant factors in culture success. The biomass and content of microalgae show changes in environmental conditions and culture parameters.

1.6.1. Light

One of the most significant factors in microalgae growth is light intensity. Light duration and power considerably impact microalgae photosynthesis and affect the biochemical content of microalgae and biomass yield (Krzemińska et al., 2014). Growth rate and biomass productivity can be determined by arranging the light in outdoor or indoor algal culture systems (Huesemann et al., 2013). Light intensities show variation within the culture and decrease with culture depth; this aspect should be considered when modeling the bioreactor or open pond system. The amount of light required by different algae species for maximum growth and biomass accumulation varies.

1.6.2. Temperature

The temperature has a significant impact on microalgae growth by influencing photosynthesis. The optimal temperature for exponential growth varies by species, and deviating more or less from this optimal point can impact growth and activity (Béchet et al., 2017).

1.6.3. pH

The pH of the media is critical for microalgae cell growth and biomass production. Previous research indicates that the optimal pH for marine algae is 7.9-8.3 and for freshwater microalgae, 6.0-8.0 (Pandey et al., 2010; Ying et al., 2014). The optimal pH range varies greatly depending on the natural habitat of microalgae (Prokop et al., 2015). Khalil et al. (2010) discovered that *Chlorella ellipsoidea* could grow in pH ranges ranging from 4 to 10. Bartley et al. (2014) investigated how pH affected the growth and lipid accumulation in *Nannochloropsis salina* and discovered that growth rates were highest at pH 8.0-9.0.

Most algae species are affected by pH and show different growth profiles, and few can withstand a pH range as wide as C. *vulgaris. C. vulgaris* can grow in a wide pH range; however, the highest growth rates and biomass productivities have been reported at pH 9–10 (Daliry et al., 2017). The salinity of the culture media can affect the pH rise, which harms algae cells' viability.

1.6.4. Salinity

Cultivating microalgae at optimal salinity may affect the biomass composition of certain algae species. Renaud et al. (1991) discovered that fatty acid's gross chemical and composition in *Isochrysis sp*, *Nannochloropsis oculata*, and *Nitzschia sp* could be distinguished under different salinities. Furthermore, experiments on marine diatoms (*Amphora sp., Navicula sp., and Cymbella sp*.) and cyanobacteria (*Oscillatoria sp*.) at various salinities revealed that diatom growth was significantly higher at 35 ppt than at lower salinities. (Rao et al., 2007) also observed that salinity influenced the development and biochemical composition of *Botryococcus braunii*, including hydrocarbon, carbohydrate, fatty acid, and carotenoids. However, species and strains may impact salinity stresses. Overall, more observation should be conducted to ensure that specific species' optimum salinity can be identified for optimal microalgae biomass production.

1.6.5. Aeration and Mixing

Mixing and aeration provide equal nutrients, air, and CO_2 distribution in microalgae culture. They give light penetration within the cell culture and inhibit the cell's aggregation. If all other requirements are supplied but the lack of mixing, biomass productivity is affected and significantly reduced. On the other hand, microalgae cultures must be constantly shaken to keep all cells suspended and exposed to light. In a photobioreactor, a proper mixing system allows nutrient dispersion and light penetration into the culture and sufficient gaseous exchange.

1.7. TECHNIQUES IN MICROALGAE CULTURE PRESERVATION

A primary function of culture collections is the preservation of organisms without change in their morphological, physiological, biochemical, and genetic properties. A reliable and straightforward technique for long-term preservation is essential in an algal culture collection. When the axenic culture has been established, the next step is to preserve it for an extended period.

The environmental changes affect the morphology and physiology of the microalgae cells. Microalgae species can be preserved with a periodic transfer, freeze-drying, and cryopreservation techniques. The method of choice is primarily determined by the laboratory's capabilities, the type of culture, and the expense of materials and workers. The preservation techniques show differences according to the desired species because different microalgae strains can be affected by distinct stress factors. For instance, some strains can be preserved via freeze-dried and cryopreserved, while other species cannot be preserved. To minimize losses, it is recommended to have at least two preservation methods.

1.7.1. Periodic Transfer/ Sub-culturing

The serial subculture method (periodic transfer to fresh media) has been mainly used to preserve most cyanobacteria (blue-green algae) and eukaryotic microalgae. The strain's growth characteristics largely determine the frequency of transfer. Aseptic

serial sub-culturing involves transferring the cell culture sample to the late log phase or stationary phase into the sterilized, fresh culture media. This preservation method provides morphologically and physiologically coherent single axenic cell culture. This technique has several drawbacks, such as time-consuming, costly, possible contamination risks, and risk of genetic instability due to mutation and selection. Despite these technique limitations, it provides advantages to preserving microalgae cultures that resist other preservation methods like cryopreservation. This approach is insufficient in conserving extensive culture collection because this technique cannot guarantee the long-term preservation and preservation of cell culture as stable and healthy. It also necessitates considerable effort. Other issues to be concerned about include the possibility of axenic culture contamination and handling errors creating a potential risk for culture. Another limitation of this technique is the time and energy because the continuous sub-culturing procedure is time-consuming and tedious for workers. These limitations can cause the loss of some species.

1.7.2. Freeze-drying

This method removes water from frozen cell culture through a sublimation process under decreased pressure. The cell's water content is vaporized by applying reduced pressure and accumulates in a condenser. Microalgae that have been freeze-dried retain their original shape and texture. Some researchers have used this technique to conserve the cyanobacterial strains, showing that this technique could be an effective way to preserve. However, this method is not an effective storage method for many microalgae, with lower viability (1% of the original population) and a further decrease in viability with prolonged storage (Arguelles et al., 2020; J. G. Day, 2007).

1.7.3. Lyophilization (L-Drying) Technique

Lyophilization (liquid drying) reduces some limitations of other preservation techniques. For instance, the risk of contamination and handling errors can be decreased by this technique even if it is applied in a continuous routine. This technique also has some benefits compared with other preservation techniques, such

as less storage size, lower cost, and fewer workers. It is also the most effective method for transportation of cultures.

In the preservation process with the lyophilization method, microalgae are preserved by keeping above the freezing temperature, and moisture is removed under vacuum pressure (Gana et al., 2014). Moreover, the lyophilization method is seen as effective due to its longevity and ease of application. Due to the advantages aspects of lyophilization, it is preferred to preserve several microalgae.

1.7.4. Cryopreservation Technique

Cryopreservation in liquid nitrogen is an option for achieving genome stability over long periods. It reduces the risk of contamination with other organisms while saving time and space for organism maintenance. Cryopreservation provides the storage of a species at a low temperature to survive after defrosting. Many microalgae can be cryopreserved, but some show decreased viability. Several cyanobacteria and eukaryotic microalgae species have been successfully cryopreserved using cryoprotective agents (CPAs) such as methanol (MeOH), dimethylsulfoxide (DMSO), or glycerol. The cryopreservation technique decreases species' cultivation cost, purity, and stability and reduces the contamination risk due to handling. The procedure and cryoprotectant agents (CPAs) used species-specific and showed differences between distinct strains. Cryopreservation frequently entails placing 1.0 ml of the culture at the desired temperature, either -20°C, -80 °C, or -170 °C (vapor phase of liquid nitrogen), using the selected CPA. For some strains, a two-step protocol may be required. In the first step, CPA addition is needed to the culture, and later solution should be cooled to a specified subzero temperature to assist the dehydration and rapidly cooled to the final storage temperature.

To examine cell viability, the cryotubes are typically incubated in a 40 °C water bath for 5 minutes before being moved into 50 ml of a suitable growth medium, and later cells are incubated under appropriate conditions. In general, strains with specific cell characteristics such as unicellularity, sphericity, lack of spines, and lack of vacuoles are observed after the thawing process. Specialized equipment, consumables, and training are the primary needs of cryopreservation to decrease drawbacks. The

improper thawing and realization under uncontrolled conditions, even if only for a short period, can lead to the death of cells.

After cryopreservation, the viability of the cells should be analyzed by measuring their metabolic activity and reproduction capacity of cryopreserved cells. The most practical approaches are vital staining with fluorescein diacetate or cell regrowth. Typically, a minimum cell viability level should be nearly 30%. Furthermore, no significant differences in genotype or phenotype should exist between control and post-thaw cultures. The cryopreservation method should be improved considering the species because it is insufficient for some species, which may need some practical studies.

This study focuses on the identification and cryopreservation of psychrotolerant microalgae isolated during the 4th Turkish Antarctic Expedition (08 February-08 March 2020) from water sources as well as snow, ice, soil crust, and mosses on Dismal Island (68°05′S-68°51′W) and Horseshoe Island (67°51′S-67°12′W) in Antarctica. The main subject of this thesis is to systematically organize the Antarctic strains that have been isolated and conserved in our laboratory and establish a microalgae culture collection for biotechnological studies.

Thereby, the specific goals of the thesis can be outlined as follows:

- Purification of the algal samples obtained from several locations of Dismal Island (68°05′S-68°51′W) and Horseshoe Island (67°51′S-67°12′W) in Antarctica
- Obtaining axenic culture of eukaryotic microalgae using specified methods
- Characterization of the isolates both morphologically and genomically based on 18s rDNA amplifications
- Uncovering the phylogenetic relationship of the Antarctic isolates

2. MATERIALS AND METHODS

2.1. SAMPLE COLLECTIONS

2.1.1. Sampling Area

In order to sample microalgae, sediment, stone, ice, snow, and water samples were collected on Dismal Island (68°05′S-68°51′W) and Horseshoe Island (67°51′S-67°12′W) in Antarctica.

Dismal Island is the largest of the Faure Islands, measuring 1.9 kilometers and 60 meters in height. It is located in Marguerite Bay, off the west coast of Graham Land. The French Antarctic Expedition led by Jean-Baptiste Charcot discovered and mapped the Faure Islands in 1909.

Horseshoe Island is an island 6.5 miles long and 3 miles wide, occupying most of the entrance to Square Bay on the west coast of Graham Land in Antarctica. It was discovered and named by the British Graham Land Expedition under John Rymill, which mapped the area by land and air in 1936-37. Its name refers to the crescent-shaped orientation of its 600- to 900-meter peaks, which give the Island a similar shape. Station Y is located at the northwestern end of the Island, also known as Horseshoe Base, an inactive but relatively unaltered and fully equipped British research station of the late 1950s. As part of the 3rd Turkish National Antarctic Science Expedition (TAE 3) in 2019, a temporary Turkish scientific research base has been established on Horseshoe Island.

Most stations indicated on the map could be sampled at least twice from the same station. Transportation was not possible on the south side of the Island.

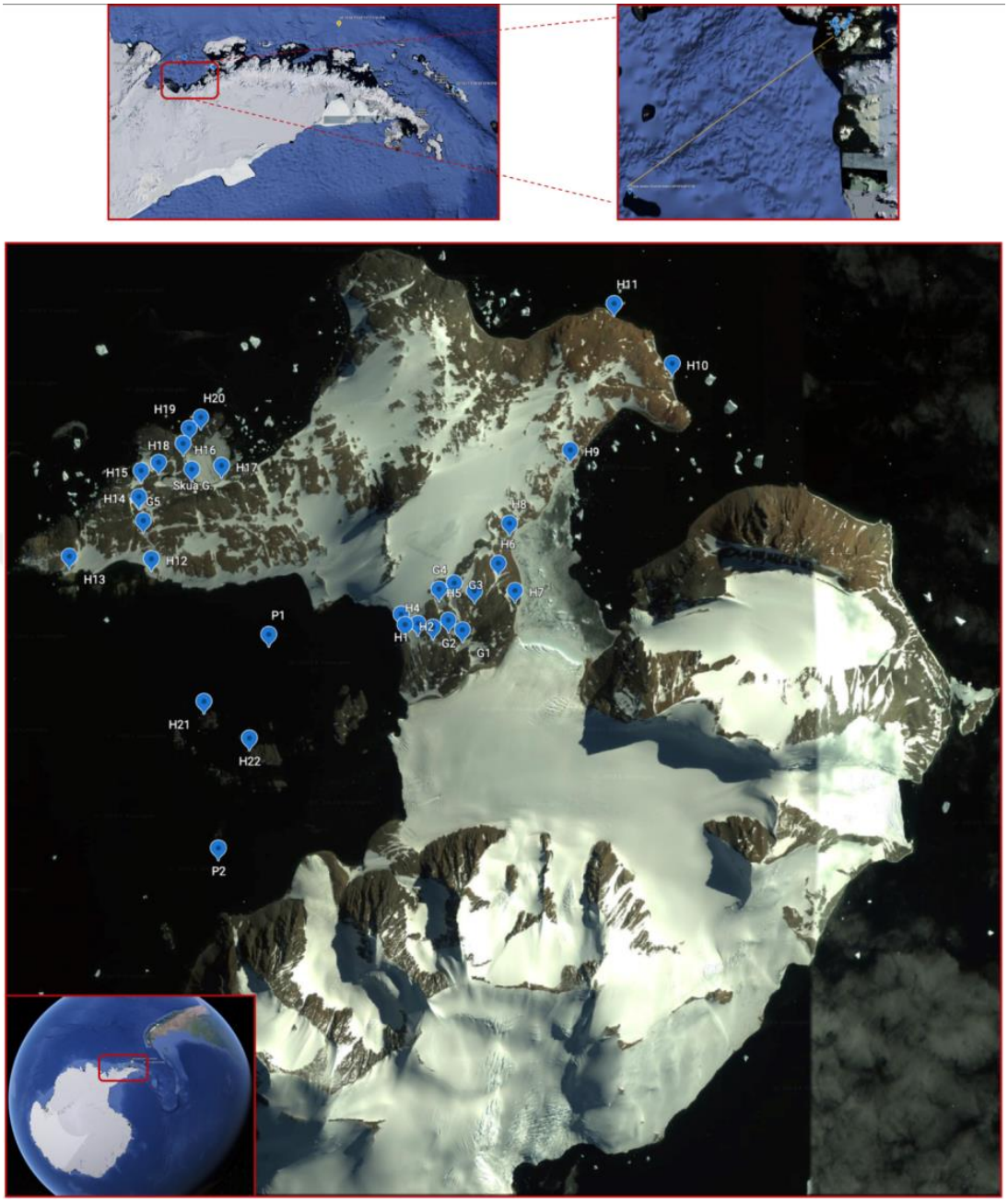


Figure 2.1 Location of Dismal and Horseshoe Island

2.1.2. Physico-chemical characters of the lakes

Physicochemical parameters of the ponds (L1, L2, L3, L4, and L5) and Skua Lake on Horseshoe island were measured. In addition, seawater samples obtained from the shore of Dismal island and Horseshoe island were analyzed. Temperature, pH, conductivity, total dissolved solids, dissolved oxygen (DO) concentration, and

salinity measurements for all water samples were recorded on-site using a multiparameter reader (YSI proplus).

The NO_3^-, NO_2^-, Cl^-, and $S_2O_4^-$ concentrations that the water samples may contain were measured by Dionex ICS-5000+ IC ion chromatography (Thermo Fisher Scientific, USA) using an AS9-HC column (chromatography conditions; flow rate = 1 mL/min, injection volume = 25 µL, column temperature = 30 ± 0.02 °C). The kit determined the amount of ammonium in the water samples (Hatch, LCK303).

2.1.3. Sampling

Microalgae were sampled from water sources as well as snow, ice, soil crust, and mosses. Overall experimental workflow for microalgae sampling, identification, and library construction is shown below;

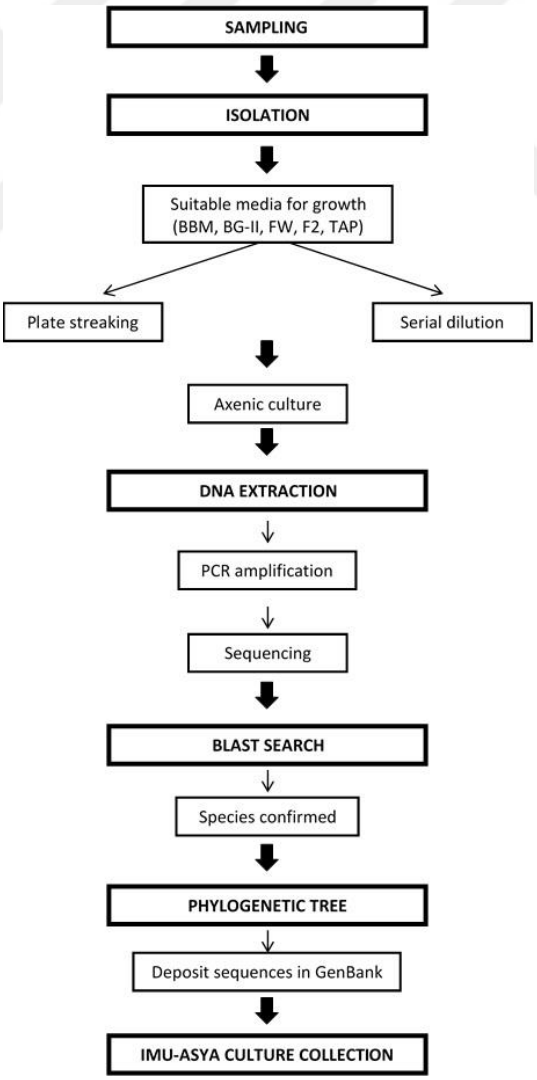


Figure 2.2 Workflow of microalgae strain isolation

2.1.4. Isolation

The collection process of species was made using 50 mL falcon tubes. Later, collected samples were cultivated into five different culture media prepared before the expedition to Antarctica. These media were determined concerning the literature on microalgae growth, and BBM, BG-11, F2, FW, and TAP mediums were selected as suitable growth media. The biochemical compositions of the media are presented in Tables 2.1-2.6.

Then, collected samples were brought to the laboratory, and the most suitable medium for the growth of each species was determined by comparing the growth situation in five different mediums. The macroscopic screening was made according to the culture in the Erlenmeyer flasks for the medium determination. The medium of each species shows differences due to the content of culture mediums. In pursuit of the growth medium selection, the situation of species was examined with microscopic screening, and axenic and mixed cultures were detected. While axenic cultures were cultivated for further characterization and analysis, the mixed cultures were seeded with a serial dilution and continual sub-culturing in agar plates to obtain axenic culture forms.

Axenic microalgae were collected from agar plates and cultivated onto new agar. Subcultures were done every two months into new agar media. All agar plates were stored at 15 °C, 8:16 h light/dark photoperiod under 50 µmol photon $s^{-1}m^2$ light intensity. Meanwhile, individual colonies were cultured in liquid media for further analysis. Cultivation was performed in 100 mL Erlenmeyer flasks with 50 mL working volume. Samples were grown in liquid media at 15 °C in an incubation-shaker cabinet (CERTOMAT BS-T, Sartorius) with continuous light (50 µmol photon $s^{-1}m^2$ light intensity). Identification of the purified microalgae was achieved both by morphological and genomic analysis.

Table 2.1 Biochemical compositions of TAP Medium

TAP Medium (Tris-Acetate-Phosphate Medium)				
Stock Solution (SL)	**Volume**	**Component**	**Concentration in SL**	**Conc. in final Medium**
Tris base	2.42 g	$H_2NC(CH_2OH)_3$ Tris(hydroxymethyl)-aminomethan		$2.00 . 10^{-2}$ M
TAP-salts (Beijerinck salts)	25 mL	NH_4Cl	15 g . L^{-1}	$7.00 . 10^{-3}$ M
		$MgSO_4.7H_2O$	4 g . L^{-1}	$8.30 . 10^{-4}$ M
		$CaCl_2 . 2H_2O$	2 g . L^{-1}	$4.50 . 10^{-4}$ M
Phosphate solution	1 mL	K_2HPO_4	28.8 g . 100 mL^{-1}	$1.65 . 10^{-3}$ M
		KH_2PO_4	14.4 g . 100 mL^{-1}	$1.05 . 10^{-3}$ M
Trace elements solution	1mL	$Na_2EDTA.2H_2O$	5.00 g . 100 mL^{-1}	$1.34 . 10^{-4}$ M
		$ZnSO_4.7H_2O$	2.20 g . 100 mL^{-1}	$1.36 . 10^{-4}$ M
		H_3BO_3	1.14 g . 100 mL^{-1}	$1.84 . 10^{-4}$ M
		$MnCl_2.4H_2O$	0.50 g . 100 mL^{-1}	$4.00 . 10^{-5}$ M
		$FeSO_4.7H_2O$	0.50 g . 100 mL^{-1}	$3.29 . 10^{-5}$ M
		$CoCl_2.6H_2O$	0.16 g . 100 mL^{-1}	$1.23 . 10^{-5}$ M
		$CuSO_4.5H_2O$	0.16 g . 100 mL^{-1}	$1.00 . 10^{-5}$ M
		$(NH_4)_6MoO_3$	0.11 g . 100 mL^{-1}	$4.44 . 10^{-6}$ M
Acetic acid, conc.	1 mL	CH_3COOH		

- **For 1 L final culture medium, add the prepared stock solutions to 850 mL distilled water.**
- **Adjust medium to final pH of 6.0 and autoclave at 121°C for 20 min.**

Table 2.2 Biochemical compositions of BG-11 Medium

BG-11 Medium				
Stock Solution (SL)	**Volume**	**Component**	**Concentration in SL**	**Conc. in final Medium**
SL 1	60 mL	$NaNO_3$	2.50 g . 100 mL^{-1}	$1.76 . 10^{-2}$ M
SL 2	10 mL	$MgSO_4.7H_2O$	0.75 g . 100 mL^{-1}	$3.04 . 10^{-4}$ M
SL 3	20 mL	Na_2CO_3	0.10 g . 100 mL^{-1}	$1.89 . 10^{-4}$ M
SL 4	5.3 mL	K_2HPO_4	0.75 g . 100 mL^{-1}	$2.28 . 10^{-4}$ M
SL 5	14 mL	$CaCl_2.2H_2O$	0.25 g . 100 mL^{-1}	$2.38 . 10^{-4}$ M
SL 6	6 mL	Citric acid	0.10 g . 100 mL^{-1}	$3.12 . 10^{-5}$ M
SL 7	10 mL	Ferric ammonium citrate	0.06 g . 100 mL^{-1}	$2.26 . 10^{-5}$ M
SL 8	10 mL	$Na_2EDTA.2H_2O$	0.01 g . 100 mL^{-1}	$2.69 . 10^{-6}$ M
SL 9	1 mL	H_3BO_3	61.0 mg . L^{-1}	$9.87 . 10^{-7}$ M
(trace elements solutions)		$MnSO_4.H_2O$	169.0 mg . L^{-1}	$1.00 . 10^{-6}$ M
		$ZnSO_4.7H_2O$	287.0 mg . L^{-1}	$9.98 . 10^{-7}$ M
		$CuSO_4.5H_2O$	2.5 mg . L^{-1}	$1.00 . 10^{-8}$ M
		$(NH_4)_6Mo_7O_{24}.4H_2O$	12.5 mg . L^{-1}	$1.01 . 10^{-8}$ M
SL 10	1 mL	Vit. B_1 (Thiamine HCl)	0.100 g . 100 mL^{-1}	$2.97 . 10^{-6}$ M
(vitamin mix)		Vit. H (Biotin)	0.025 mg . 100 mL^{-1}	$1.02 . 10^{-9}$ M
		Vit. B_{12} (Cyanocobalamin)	0.015 mg . 100 mL^{-1}	$1.11 . 10^{-10}$ M

- **For 1 L final culture medium, add the prepared stock solutions to 850 mL distilled water.**
- **Adjust medium to final pH of 8.4 and autoclave at 121°C for 20 min.**

Table 2.3 Biochemical compositions of FW Medium

fwDiatom Medium (freshwater medium for diatoms)				
Stock Solution (SL)	**Volume**	**Component**	**Concentration in SL**	**Conc. in final Medium**
SL 1	20 mL	$Ca(NO_3)_2.4H_2O$	0.2 g . 100 mL^{-1}	1.70 . 10^{-4} M
SL 2	10 mL	K_2HPO_4	0.1 g . 100 mL^{-1}	5.74 . 10^{-5} M
SL 3	25 mL	$MgSO_4.7H_2O$	0.1 g . 100 mL^{-1}	1.02 . 10^{-4} M
SL 4	20 mL	Na_2CO_3	0.1 g . 100 mL^{-1}	1.89 . 10^{-4} M
SL 5	50 mL	$Na_2SiO_3.5H_2O$	0.1 g . 100 mL^{-1}	2.36 . 10^{-4} M
SL 6	10 mL	Ferric-citrate	0.1 g . 100 mL^{-1}	4.08 . 10^{-5} M
SL 7	10 mL	Citric acid	0.1 g . 100 mL^{-1}	5.20 . 10^{-5} M
SL 8	5 mL	Micronutrient solutions	see below	
SL 9 (soil extract)	30 mL	garden soil		
SL 10 (vitamin mix)	1 mL	Vit. B_1 (Thiamine HCl)	0.100 g . 100 mL^{-1}	2.97 . 10^{-6} M
		Vit. H (Biotin)	0.025 mg. 100 mL^{-1}	1.02 . 10^{-9} M
		Vit. B_{12} (Cyanocobalamin)	0.015 mg. 100 mL^{-1}	1.11 . 10^{-10} M

- **For 1 L final culture medium, add the prepared stock solutions to 850 mL distilled water.**
- **Adjust medium to final pH of 6.5 and autoclave at 121°C for 20 min.**

Table 2.4 Micronutrient Solution of FW Medium

SL 8 Micronutrient Solution				
Solution	**Volume**	**Component**	**Concentration in SL**	**Conc. in final Medium**
Solution I	1 mL	$ZnSO_4.7H_2O$	0.1 g . 100 mL^{-1}	1.74 . 10^{-8} M
	2 mL	$MnSO_4.4H_2O$	0.1 g . 100 mL^{-1}	4.48 . 10^{-8} M
	5 mL	H_3BO_3	0.2 g . 100 mL^{-1}	8.09 . 10^{-7} M
	5 mL	$Co(NO_3)_2.6H_2O$	0.02 g . 100 mL^{-1}	1.72 . 10^{-8} M
	5 mL	$Na_2MoO_4.2H_2O$	0.02 g . 100 mL^{-1}	2.07 . 10^{-8} M
	1 mL	$CuSO_4.5H_2O$	0.0005 g . 100 mL^{-1}	1.00 . 10^{-10} M
	0.4 g	Na_2EDTA		-
	881mL	distilled water		
Solution II	0.7 g	$FeSO_4.7H_2O$		1.26 . 10^{-5} M
	0.4 g	Na_2EDTA		-
	100 mL	distilled water		

- **Prepare and autoclave solutions I and II separately, let both cool down, and then pool.**

Table 2.5 Biochemical compositions of BMM Medium

BBM Medium (Bold's Basal Medium + soil extract + vitamins)				
Stock Solution (SL)	**Volume**	**Component**	**Concentration in SL**	**Conc. in final Medium**
SL 1	10 mL	$NaNO_3$	2.50 g . 100 mL^{-1}	2.94 . 10^{-3} M
SL 2	10 mL	$MgSO_4.7H_2O$	0.75 g . 100 mL^{-1}	3.04 . 10^{-4} M
SL 3	10 mL	NaCl	0.25 g . 100 mL^{-1}	4.28 . 10^{-4} M
SL 4	10 mL	K_2HPO_4	0.75 g . 100 mL^{-1}	4.31 . 10^{-4} M
SL 5	10 mL	KH_2PO_4	1.75 g . 100 mL^{-1}	1.29 . 10^{-3} M
SL 6	10 mL	$CaCl_2$	0.25 g . 100 mL^{-1}	1.70 . 10^{-4} M
SL 7	1 mL	$ZnSO_4.7H_2O$	8.82 g . L^{-1}	3.07 . 10^{-5} M
(trace elements solutions)		$MnCl_2.4H_2O$	1.44 g . L^{-1}	7.28 . 10^{-6} M
		MoO_3	0.71 g . L^{-1}	4.93 . 10^{-6} M
		$CuSO_4.5H_2O$	1.57 g . L^{-1}	6.29 . 10^{-6} M
		$Co(NO_3)_2.6H_2O$	0.49 g . L^{-1}	1.68 . 10^{-6} M
SL 8	1 mL	H_3BO_3	1.14 g . 100 mL^{-1}	1.85 . 10^{-4} M
SL 9	1 mL	$Na_2EDTA.2H_2O$	5.00 g . 100 mL^{-1}	1.71 . 10^{-4} M
		KOH	3.10 g . 100 mL^{-1}	5.53 . 10^{-4} M
SL 10	1 mL	$FeSO_4.7H_2O$	4.98 g . 1 L^{-1}	1.79 . 10^{-5} M
SL 11 (soil extract)	50 mL	garden soil		
SL 12	1 mL	Vit. B_1 (Thiamine HCl)	0.100 g . 100 mL^{-1}	2.97 . 10^{-6} M
(vitamin mix)		Vit. H (Biotin)	0.025 mg. 100 mL^{-1}	1.02 . 10^{-9} M
		Vit. B_{12} (Cyanocobalamin)	0.015 mg . 100 mL^{-1}	1.11 . 10^{-10} M

- **For 3N-BBM, add a triple quantity of $NaNO_3$.**
- **For 1 L final culture medium, add the prepared stock solutions to 850 mL distilled water.**
- **Adjust medium to final pH of 5.5/6.5 and autoclave at 121°C for 20 min.**

Table 2.6 Biochemical compositions of F/2 Medium

F/2 Medium			
Stock Solution (SL)	**Volume**	**Component**	**Concentration in SL**
SL 1	1 mL	$NaNO_3$	7.50 g . 100 mL^{-1}
SL 2	1 mL	$NaH_2PO_4.2H_2O$	0.565 g . 100 mL^{-1}
SL 3	1 mL	$Na_2EDTA.2H_2O$	0.416 g . 100 mL^{-1}
(trace elements)		$FeCl_3.6H_2O$	0.315 g . 100 mL^{-1}
		$CuSO_4.5H_2O$	0.001 g . 100 mL^{-1}
		$ZnSO_4.7H_2O$	0.002 g . 100 mL^{-1}
		$CoCl_2.6H_2O$	0.001 g . 100 mL^{-1}
		$MnCl_2.4H_2O$	0.018 g . 100 mL^{-1}
		$Na_2MoO_4.2H_2O$	0.0006 g . 100 mL^{-1}
SL 4	1 mL	$Na_2SiO_3.5H_2O$	0.005 g . 100 mL^{-1}
SL 5	1 mL	Vit. B_1 (Thiamine HCl)	0.1000 g. $1L^{-1}$
(vitamin mix)		Vit. H (Biotin)	0.0005 g. $1L^{-1}$
		Vit. B_{12} (Cyanocobalamin)	0.0005 g. $1L^{-1}$

- **For 1 L final culture medium, add the prepared stock solutions to filtered natural seawater.**
- **Adjust medium to final pH of 8.0 and autoclave at 121°C for 20 min.**

2.2. MOLECULAR ANALYSIS

2.2.1. DNA extraction

Two different methods were applied for DNA extraction: (1) QIAGEN DNeasy Plant Mini Kit and (2) modified CTAB procedure (modified from (Aljanabi & Martinez, 1997; Winnepenninckx, 1993).

Approximately 100 mg of fresh microalgae biomass was frozen in liquid nitrogen and stored at -80˚C for use. The manufacturer's instructions were followed when the QIAGEN DNeasy Plant Mini Kit procedure was applied. The modified CTAB method was applied as follows;

- 200 µl of CTAB extraction buffer (pre-warmed to 60 °C) was added to 100 mg of microalgal pellet and was transferred to a pre-labeled 2 ml screw-capped microcentrifuge tube containing an average of 70 mg glass beads (autoclaved).
- Each sample was homogenized for 1 min using a cell homogenizer.
- After homogenization, 200 µl of extraction buffer was added to the cells. 40 µl SDS (10%) and 8 µl Proteinase K (20mg/ml) were added, vortexed for 5 seconds, and the cells were incubated for 1 hour at 60 °C with a thermoshaker set to low speed (100 rpm) during incubation.
- The samples were cooled to room temperature after incubation (which takes 2 min), gently the microcentrifuge tube was inverted 3 times, 400 µl phenol: chloroform: isoamyl alcohol (25:24:1) were added, and the tube was mixed by inverting for 2 min (use a 20 rpm rotator).
- The tubes were centrifuged for 10 min at 14000 g (there should be 3 layers: the bottom layer, an organic phase with green pigment; the protein boundary layer, which should be white, or the cells will not be lysed; and the upper aqueous phase containing DNA.).
- The upper aqueous phase was transferred to a new pre-labeled microcentrifuge tube. Adding Phenol: chloroform: isoamyl alcohol (25:24:1) steps (second wash) were repeated.

- 400 µl of Phenol: chloroform: isoamyl alcohol (25:24:1) were added, and the tube was mixed for 2 minutes by inverting (use a 20 rpm rotator).
- Then, the tube was centrifuged at 14000 g for 10 minutes. The upper aqueous phase was transferred to a new pre-labeled microfuge tube (If the samples were seen visibly contaminated, this cleaning step was repeated one more time).
- An equal volume of cold isopropanol was added. The tube was mixed by inverting it for 3 minutes (use a rotator at 20 rpm) and was incubated for at least 1 hour (it can be 2 hours or more, the DNA must be visible after incubation) at -20 °C (If the DNA was not visible after 1 hour, the samples could be stored overnight at -20 °C).
- The sample was spun at 14000 g for 10 minutes at 4 °C, and carefully the supernatant was removed. 750 µl of ice-cold EtOH (70%) was added and rotated for 2 minutes (use a rotator at 20 rpm).
- The tube was centrifuged at 14000 g for 1 min at room temperature to form the washed pellet.
- The supernatant was discarded, and the DNA was allowed to dry by leaving the tube open in the sterile cabinet for 10 minutes.
- Then, the DNA was dissolved in 100 µl of nuclease-free ddH_2O. A nanodrop spectrophotometer was used for spectrophotometric quantification at Abs 260/280.
- The solution's serial dilution (1, 1:1, 1:4, and 1:8 DNA: ddH2O) was performed to determine the optimal dilution ratio for quantification.
- After quantification, a 50 µl aliquot was made, and the extract was stored at 4 °C for immediate use or at -20 °C for long-term storage.
- The presence and purity of DNA were confirmed, and PCR amplification was performed.

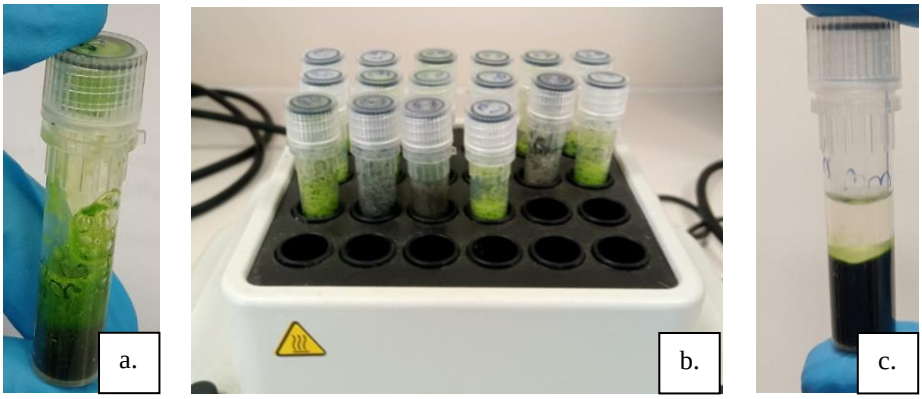


Figure 2.3 DNA extraction steps

a. Mixture of cells in CTAB extraction buffer after homogenization, b. Incubation of the cells for 1 hour at 60 °C, c. Separation of aqueous and organic phases after centrifugation.

2.2.2. PCR amplification

PCR amplification was performed with different primers (in Table 2.7) by using 100 ng/mL of genomic DNA.

Table 2.7 Primers used for PCR amplification

Primers	**Sequence 5'- 3'**	**Reference**
EAF3	TCGACAATCTGGTTGATCCTGCCAG	(Marin et al., 2003)
ITS055R	CTCCTTGGTCCGTGTTTCAAGACGGG	(Marin et al., 2003)
Euk-F	AACCTGGTTGATCCTGCCAGT	(Sands et al., 2008)
Euk-R	TGATCCTTCTGCAGGTTCACC	(Sands et al., 2008)
D512F	ATTCCAGCTCCAATAGCG	(Marić Pfannkuchen et al., 2018)
D978R	GACTACGATGGTATCTAATC	(Marić Pfannkuchen et al., 2018)

The total volume of PCR mix was 50 µl and consisted of 25 µl master mix [EcoTaq 2x PCR Master Mix], 100 ng/mL template DNA, 0,5 µl of each primer (forward and reverse) (10µM), and up to 50 µl water. The PCR reaction was performed in a thermal cycler (T100 Thermal Cycler, BIORAD), and the PCR condition details are as stated in Table 2.8.

Table 2.8 Thermal cycling parameters

Stage	Temperature	Time	Cycles
Initial Denaturation	95 ºC	2 min	1 cycle
Denaturation	95 ºC	1 min	30 cycles
Annealing	55 ºC	1 min	
Extension	68 ºC	3 min	
Final extension	72 ºC	5 min	1 cycle

Then, the PCR products were run on a 1.0% agarose gel using 1X Tris-Acetate-EDTA (TAE) buffer (4.85 g Tris base, 1.14 mL glacial acetic acid, 2 mL 0.5 M EDTA, pH 8.0) containing 10 mg/mL ethidium bromide (EtBr). Agarose gel electrophoresis was performed at 80 volts for 100 minutes, and the gels were photographed using a UV gel documentation system (Azure Gel Imaging Systems, VWR). Samples were stored at -20 °C for use. After the DNA isolation procedure was completed, the PCR products were sent to an institution for Sanger sequencing analysis. The primers used for the sequencing are seen in Table 2.9.

Table 2.9 Primers used for Sequencing

Primers	Sequence 5'- 3'	Reference
E528F	TGCCAGCAGCYGCGGTAATTCCAGC	(Marin et al., 2003)
BR	TTGATCCTTCTGCAGGTTCACCTAC	(Marin et al., 2003)
26SR	TTGGGCTGCATTCCCAAACAAC	(Yakimovich et al., 2021)
1422F	CAGGTCTGTGATGCCCTTAG	(Remias et al., 2010)
SSU	CTGCGGAAGGATCATTGATTC	(Piercey-Normore & DePriest, 2001)
LSU	AGTTCAGCGGGTGGTCTTG	(Piercey-Normore & DePriest, 2001)
D512F	ATTCCAGCTCCAATAGCG	(Zimmermann et al., 2011)
D978R	GACTACGATGGTATCTAATC	(Zimmermann et al., 2011)

2.2.3. Phylogenetic Tree Analysis

The sequence data obtained from the 18S rDNA region of cultured microalgae were submitted to the NCBI system, and accession numbers were obtained. The sequence results were used for BLAST analysis (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Matching sequence sequences of the closest species were taken from the system, combined in FASTA format, and aligned with the Clustal W algorithm.

The evolutionary history was inferred using the Maximum Likelihood method and the Kimura 2-parameter model (Kimura, 1980). The bootstrap consensus tree was inferred from 100 replicates taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (100 replicates) are shown next to the branches (Felsenstein, 1985). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with a superior log-likelihood value. This analysis involved 99 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. There were a total of 805 positions in the final dataset. Evolutionary analyses were conducted in UGENE v38.1 (Okonechnikov et al., 2012). Lastly, a graphical viewer (FigTree v1.4.4) was employed to organize and make up the last version of the phylogenetic tree.

2.3. CRYOPRESERVATION

Cryopreservation of microalgae was achieved as follows;

- Dimethyl sulfoxide (DMSO, 10%) was used as a cryoprotectant.
- Axenic microalgae were grown in a 50 mL 3N-BBM medium of 100 mL Erlenmeyer flask at 15 °C in an incubation-shaker cabinet (CERTOMAT BS-T, Sartorius) under continuous light (50 µmol photon $s^{-1}m^2$) until cells reached to the linear growth phase.
- The cells were centrifuged and resuspended in fresh 3N-BBM media, and the cell concentration was counted with a Neubauer hemocytometer before being adjusted to 10^6cells/mL.
- An equal volume of pre-cooled cryoprotective solution (0.8 ml) and algal culture (0.8 ml) was added to 2 ml of pre-labeled cryotubes.
- The cryotubes were covered with aluminum foil to protect them from light and were incubated for 15 minutes in a rocker-shaker device.
- The cryotubes were placed into the freezing container and were stored at -80 °C for 1 h.
- Then cryotubes were put in liquid nitrogen (-196 °C) for cryopreservation. The overall experimental scheme is shown below;

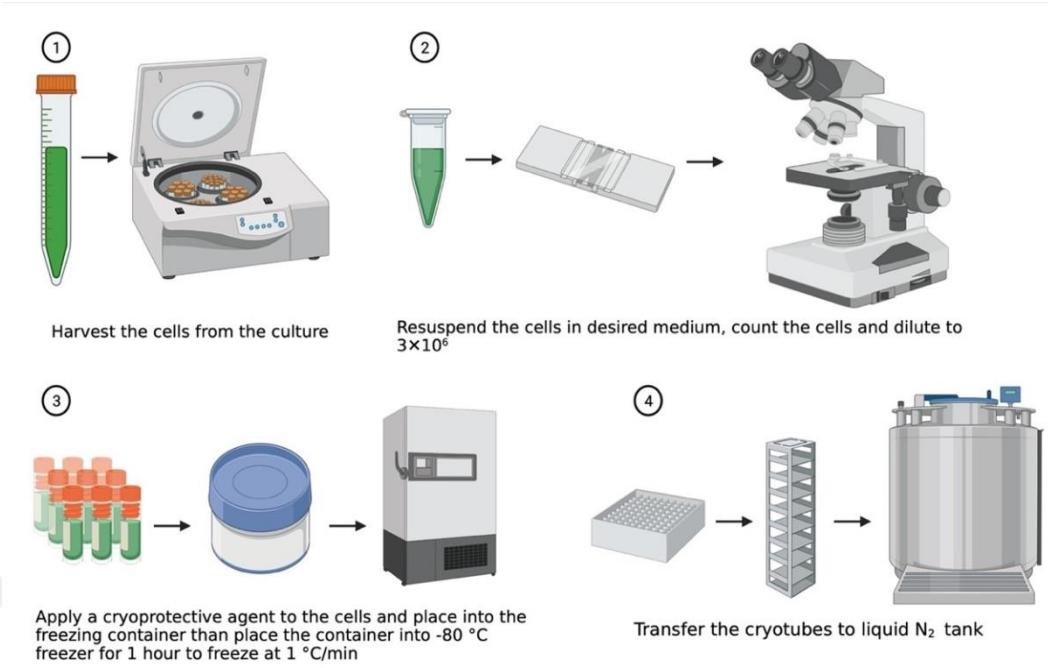


Figure 2.4 Workflow for the cryopreservation of microalgae

In order to test recovery of microalgae;

- The cryo-vials were defrosted in a water bath at 40 °C for 15 min (applied in the dark).
- Then the sample was centrifuged at 3000 g for 3 minutes at 15 °C, and the supernatant was removed.
- The pellet was resuspended in 1mL of fresh 3N-BBM medium and transferred to 24 wells plates with 2 mL 3N-BBM medium for each well.
- Lastly, growth was followed by measuring the absorbance values of microalgae solution at 680nm wavelength. Moreover, a change in the morphology of the cryopreserved microalgae was checked by microscopic analysis (Figure 2.5). The specific growth rate and generations were calculated as formulated below;

  Doubling time: ln(2) / μ

  Specific growth rate (μ) = (lnN2-lnN1) / (t2-t1)

  Number of generations (ng) =logN2-logN1/log2

  * t stands for time

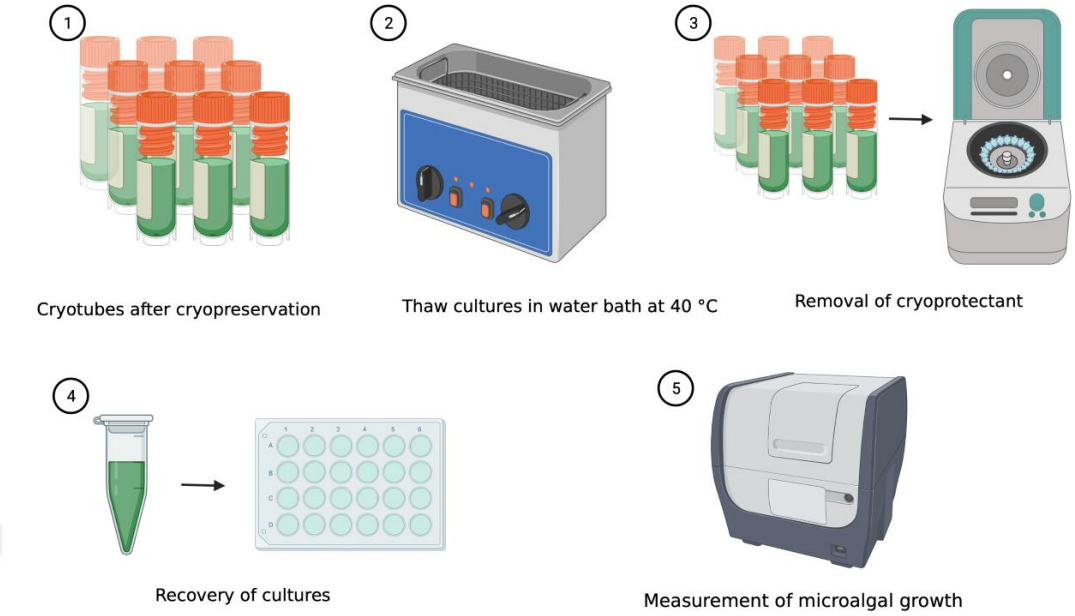


Figure 2.5 Recovery of microalgae after cryopreservation process

3. RESULTS and DISCUSSION

3.1. SAMPLING

Istanbul Medeniyet University Culture Collection of Antarctic Microalgae Research (IMU-ASYA) (https://www.imuasya.org) was established to present the purified strains isolated during the 4th Turkish Antarctic Expedition. Most microalgae (*Chloromonas, Chlamydomonas, Coccomyxa, Stichococcus, Micractinium, Chlorella, Klebsormidium, Xanthonema* genera) that were dominant on Dismal and Horseshoe Island were purified and cultured.

During the sampling, seawater and lake water samples were also collected. Physicochemical properties of seawater sampled from Horseshoe and Dismal Island were measured as close to each other. Seawater temperature was measured between 1.6-1.9°C, salinity levels at 30%, and a slightly basic pH value. Skua Lake and all other ponds on Horseshoe Island are fed by melted snow water. Skua Lake and other ponds have been described as oligotrophic, with slightly basic pH and extremely low salinity. Table 3.1 shows the recorded results of temperature, pH, conductivity, total dissolved solids, dissolved oxygen (DO) concentration, and salinity measurements for all collected water samples.

Table 3.1 Physical and chemical properties of the water sample

Sampling area	Temperature (°C)	Dissolved oxygen (mg/L)	Conductivity (uS/cm)	Total dissolved solids (mg/L)	Salinity (ppt)	pH
Horseshoe seawater	1,6	64,8	24689	29085	29,26	7,85
Dismal seawater	1,9	67,1	25774	29898	31,14	7,74
Skua Lake	1,8	58	85,6	89,05	0,07	7,84
H. Lake 1	1,2	66,7	68,92	65,2	0	8,02
H. Lake 2	1,8	70,7	80,5	89,05	0,07	8,11
H. Lake 3	1,4	98,1	95	92,3	0,07	7,7
H. Lake 4	1,9	72,8	89	85,4	0,06	8,2
H. Lake 5	1,8	81,4	78,2	60,4	0,02	7,9

Nitrite ions were not detected in the samples. The concentrations of ammonium ions in seawater samples were low, and the contents of nitrate, sulfate, and chlorine were found to reflect the salty nature of the ocean water. On the other hand, in other ponds analyzed with Skua Lake, ammonium, nitrate, and phosphate ions were either absent or traced, and chlorine and sulfate ion concentrations were deficient.

Table 3.2 Ion concentrations of water samples

Sampling area	NH_4^+(mg/L)	Cl^-(mg/L)	NO_3^-(mg/L)	SO_4^-(mg/L)
Horseshoe seawater	0,6	20931	0,68	2780
Dismal seawater	0,8	22426	1,29	3017
Skua Lake	0,04	110,5	0,12	5,17
H. Lake 1	0	20,4	0	1,6
H. Lake 2	0	80,1	0	10,7
H. Lake 3	0	92,4	0,07	6,8
H. Lake 4	0	31,5	0,03	4,52
H. Lake 5	0	40,8	0	3,8

Boron (B), Manganese (Mn), Iron (Fe), Cobalt (Co), Copper (Cu), Zinc (Zn), and Molybdenum (Mo) elements can be found in Skua Lake and other ponds on Horseshoe Island and can be used as micronutrients by microalgae. The ICP-MS device was used for the analysis of lake waters to determine the concentrations of some metal ions such as Chromium (Cr), Arsenic (As), Cadmium (Cd), and Lead

(Pb), which are toxic to life even in low amounts (Perkin Elmer Nexion 2000). For this purpose, elemental analysis was carried out in ICP-MS tubes by adding concentrated nitric acid solution with a final concentration of 2% into the water samples passed through a 0.2um filter. Standard solutions containing elements in concentrations of 0, 20, 50, and 100 ppm/ppb (prepared at ppm level for macroelements and ppb level for microelements) were designed from each element, and quantitative identification was carried out with the help of the standard solutions. While Zn, As, Cd, and Pb elements were not found in the analyzed water samples, B, Mn, and Fe ions were detected in almost all samples. On the other hand, Cr, Co, and Cu metal ions were determined at deficient levels.

Table 3.3 Concentrations of heavy metals (ppm) in water samples

	11B	**52Cr**	**55Mn**	**57Fe**	**59Co**	**65Cu**	**66Zn**	**75As**	**95Mo**	**111Cd**	**208Pb**
Skua Lake	1,24	0,04	0,6	2,4	---	0,11	---	---	0,02	---	---
H. Lake 1	0,21	0,08	0,4	1,8	0,02	0,08	--	--	0,03	--	--
H. Lake 2	3,2	0,05	---	0,2	0,01	---	---	--	---	---	---
H. Lake 3	1,4	---	0,2	1,5	---	0,04	---	--	---	--	--
H. Lake 4	---	---	0,2	3,2	0,01	---	---	--	---	---	---
H. Lake 5	0,8	0,02	0,3	2,5	---	---	---	--	---	--	--

3.2. IDENTIFICATION

As a result of the samplings, 56 taxa were identified; 10 belong to Chlorophyceae, 5 to Klebsormidiophyceae, 12 to Trebouxiophyceae, 4 to Zygnematophyceae, 5 to Xanthophyceae, 5 to Cyanophyceae, and 15 to Bacillariophyceae. The distribution of species by stations is given in Table 3.4. Thus, 38 taxa were identified on Horseshoe Island, and 18 taxa were identified on Dismal Island.

Table 3.4 Systematic presentation of microalgae species identified in the sampling area. S-snow, R-rock surface, SE-sediment, SL-Skua Lake, HL-Horseshoe Lakes M-mosses, SW-seawater

	Dismal Island				Horseshoe Island					
	S	**R**	**SE**	**SL**	**HL**	**S**	**R**	**SE**	**M**	**SW**
Phylum: Chlorophyta										
Class: Chlorophyceae										
Coccomyxa sp. Schmidle, 1901	*	*				*	*			
Gloeocystis sp. Nägeli, 1849					*					
Heterotetracystis akinetos Cox & Deason 1968								*		
Monoraphidium sp. Komárková-Legnerová, 1969						*				
Pseudococcomyxa simplex (Mainx) Fott 1981: 5								*		
Scotiellopsis oocystiformis (Lund) Puncochárová & Kalina 1981		*								
Chloromonas nivalis (Chodat) Hoham & Mullet 1978	*					*				
Chloromonas sp.	*		*		*	*	*	*		
Chlamydomonas nivalis (F.A.Bauer) Wille 1903	*					*				
Chlamydomonas sp.	*		*		*	*	*			
Class: Klebsormidiophyceae										
Klebsormidium dissectum (F.Gay) H.Ettl & Gärtner 1995: 601		*								*
Klebsormidium flaccidum (Kützing) P.C.Silva, K.R.Mattox & W.H.Blackwell 1972						*	*			*
Klebsormidium klebsii G.M.Smith 1933			*							
Klebsormidium subtile (Kützing) Mikhailyuk 2015								*		
Raphidonema sp. Lagerheim, 1892										*
Class: Trebouxiophyceae										
Chlorella sp.					*	*				
Choricystis minor (Skuja) Fott 1976		*								
Koliella antarctica C.Andreoli 1998							*			*
Stichococcus bacillaris Nägeli 1849	*					*	*			
S. chlorelloides Grintzesco & Péterfi 1932						*				
S. minutus Grintzesco & Péterfi 1932						*				
Stichococcus sp.							*			
Micractinium simplicissimum H.Chae, H.-G. Choi & J.H.Kim 2019						*			*	
Micractinium sp.	*	*	*			*	*	*	*	

Prasiolopsis sp.						*	
Prasiola sp.						*	
Keratococcus bicaudatus (A.Braun ex Rabenhorst) J.B.Petersen 1928							*
Phylum: Charophyta							
Class: Zygnematophyceae							
Zygnema sp. C.Agardh, 1817					*		
Zygnema sp. 2					*		
Cylindrocystis brebissonii (Ralfs) De Bary 1858		*					
Cosmarium sp. Corda ex Ralfs, 1848						*	
Phylum: Orchophyta							
Class: Xanthophyceae							
Chlorella simplex (Artari) Migula 1907							
Tribonema vulgare Pascher 1925	*						*
Tribonema sp.					*		
Xanthonema exile (Klebs) P.C.Silva 1979					*		*
Xanthonema debile (Vischer) P.C.Silva 1979					*		*
Xanthonema sp.					*		
Phylum: Bacillariophyta							
Class: Bacillariophyceae							
Achnanthes sp.							*
Achnanthes brevipes C. Agardth 1824				*			
Fragillaria sp.			*				
Amphora sp.							*
Phaeodactylum tricornutum							*
Navicula pyllepta							*
Navicula tripunctata							*
Pinnularia sp.			*				
Gamphonema sp.			*				
Gomphonema abbreviatum C. Agardh			*				
Coscinodiscus argus Ehrenberg 1839							*
Chaetoceros tetrachaeta Ehrenberg							*
Nitzschia sp.			*	*			
Humidophilia sp.				*			
Plannothidium delicatulum (Kützing)				*			
Phylum: Cyanobacteria							
Class: Cyanophyceae							
Leptolyngbya foveolarum (Gomont) Anagnostidis & Komárek 1988	*						
Leptolyngbya sp.					*		
Plectolyngbya hodgsonii Taton, Wilmotte, Marda, Elster & Komárek 2011							*
Merismopedia sp. Meyen, 1839						*	
Calothrix sp.						*	
Phormidium sp. Kützing ex Gomont, 1892						*	

Microalgae morphology can take on quite different morphological appearances in response to environmental changes and even in different stages of life cycles. Thereby, the identification of microalgae was done by the Sanger sequencing method as well as morphological analysis. For this purpose, DNA extraction of cultured microalgae was performed, and DNA samples were purified and amplified using the primers specified in the method section. Agarose gel images of the amplicons are presented in Figure 3.1.

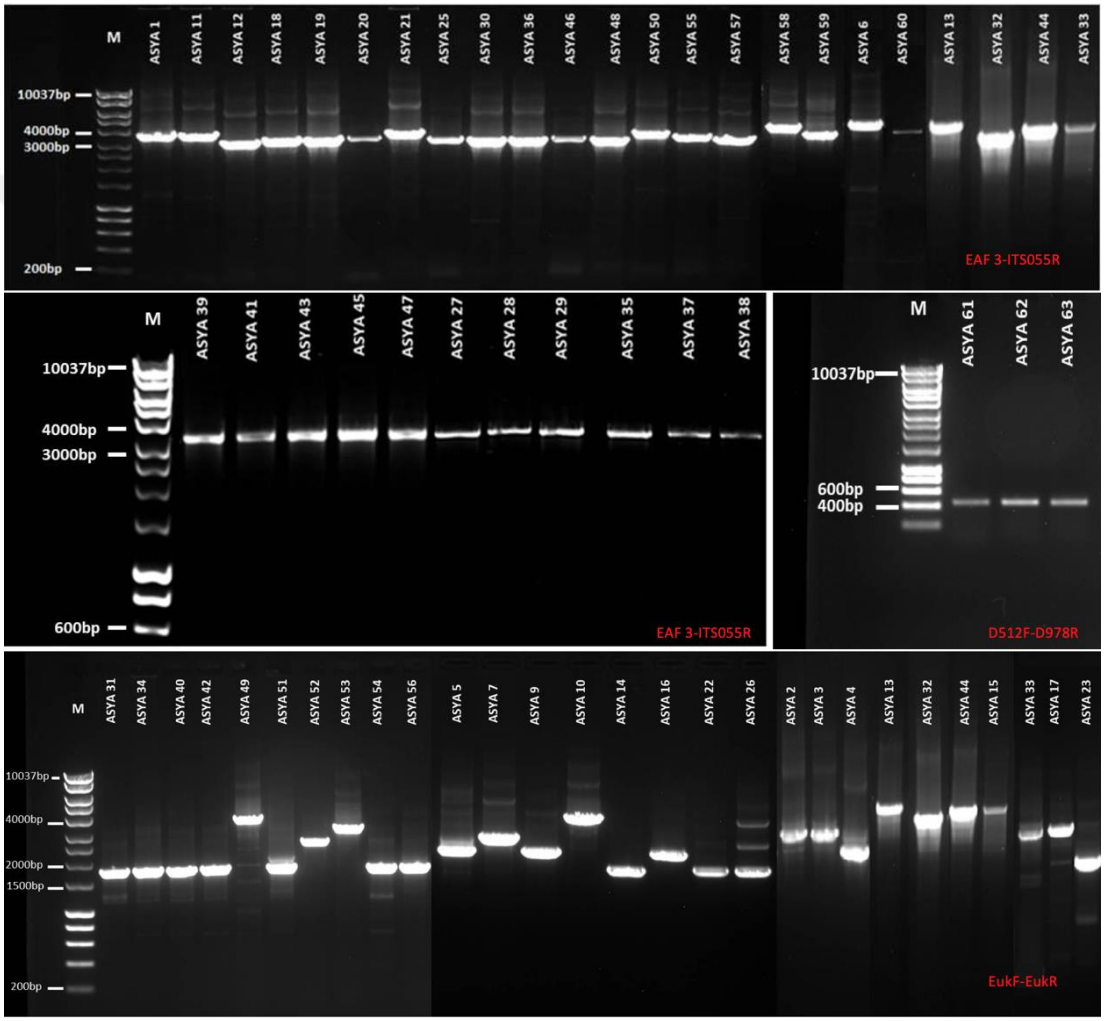


Figure 3.1 Gel electrophores image of the PCR amplicons. The primer pairs are denoted as red.

3.3. PHYLOGENETIC ANALYSIS OF THE ISOLATES

Sanger sequence analysis data obtained from the 18S rDNA region of microalgae were submitted to the NCBI system, and accession numbers were provided. The recorded sequence results were analyzed by NCBI/BLAST program (https://blast.ncbi.nlm.nih.gov). The matching sequence sequences of the closest species were retrieved from the system, combined in FASTA format, and aligned with the Clustal W algorithm using the UGENE program. The phylogenetic tree was constructed using the Maximum Likelihood method and 100 Bootstrap repetitions. FigTree (v1.4.4) program was also used to organize the phylogenetic trees produced. The phylograms are presented below.

There are three distinct diatom species isolated, cultured, and identified. All three diatoms are marine diatoms Figure 3.2.

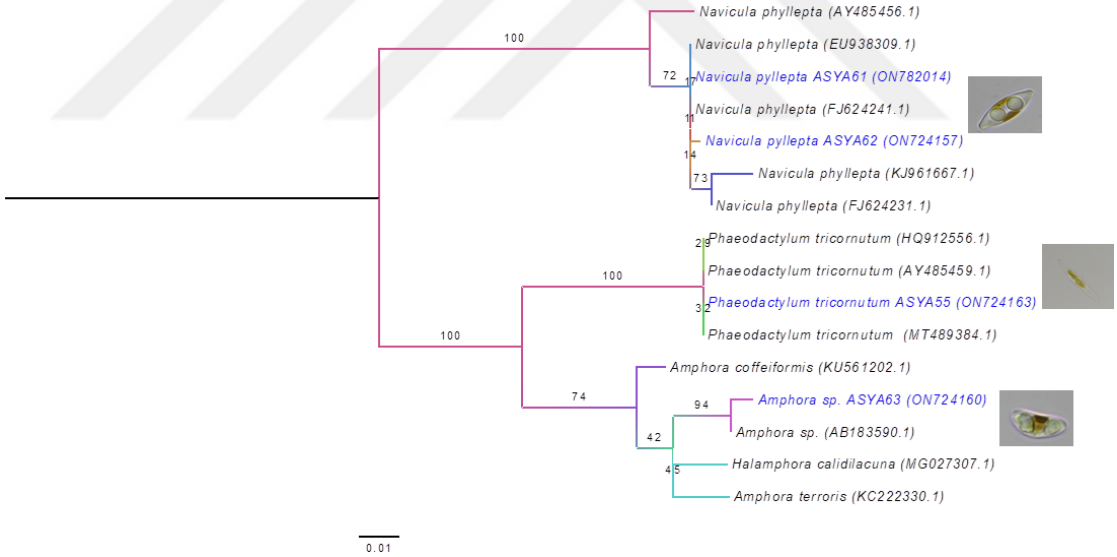


Figure 3.2 Phylogenetic analysis of the Antarctic diatom isolates

Navicula phyllepta is a widely distributed benthic diatom, and close relatives of this diatom differentiate in their salinity tolerance (Vanelslander et al., 2009). The strains *N. phyllepta* ASYA61 and *N. phyllepta* ASYA62 were isolated from planktonic samples on the coast of Horseshoe Island. *N. phyllepta* strains are known to have broad adaptability to changes in the salinity level, and the strain distribution may depend on strain-specific ecophysiological traits other than the salinity level itself.

The phylogenetic analysis showed that the *N. phyllepta* strains isolated in this study were most close to strains given NCBI accession numbers FJ624241.1, and EU938309.1, which were isolated from the North Sea - Westerschelde estuary of the Netherlands (Vanelslander et al., 2009).

Phaeodactylum tricornutum is the only species in the genus Phaeodactylum. This marine diatom can form several morphological differences, e.g., fusiform, oval, and some more intermediate forms. This is not usual for most diatoms as the Silica content of the cell wall in *P.tricornutum* is incomparably lesser than the other diatoms (Martino et al., 2007). The marine diatom *P. tricornutum* is widely studied for its biotechnological properties. This strain has been isolated from mainly brackish and seawater worldwide. Some Antarctic *P. tricornutum* strains have also been reported from King George Island (Cabrita et al., 2017). The strain *P. tricornutum* ASYA55 was isolated from planktonic samples on the coast of Horseshoe Island.

The *Amphora* strains are widely distributed in marine systems with over 1000 identified species. Few of them are also reported from freshwater systems. The Southern Ocean is no example, and several *Amphora* and *Halamphora* strains have been reported (P. Wang et al., 2014a). The strain *Amphora* sp. ASYA63 was isolated from planktonic samples on the coast of Dismal island. Phylogenetically close *Amphora* strains *Amphora sp.* (AB183590.1) and *Amphora terroris* (KC222330.1) are isolates from Pacific ocean planktonic and stone samples (P. Wang et al., 2014b).

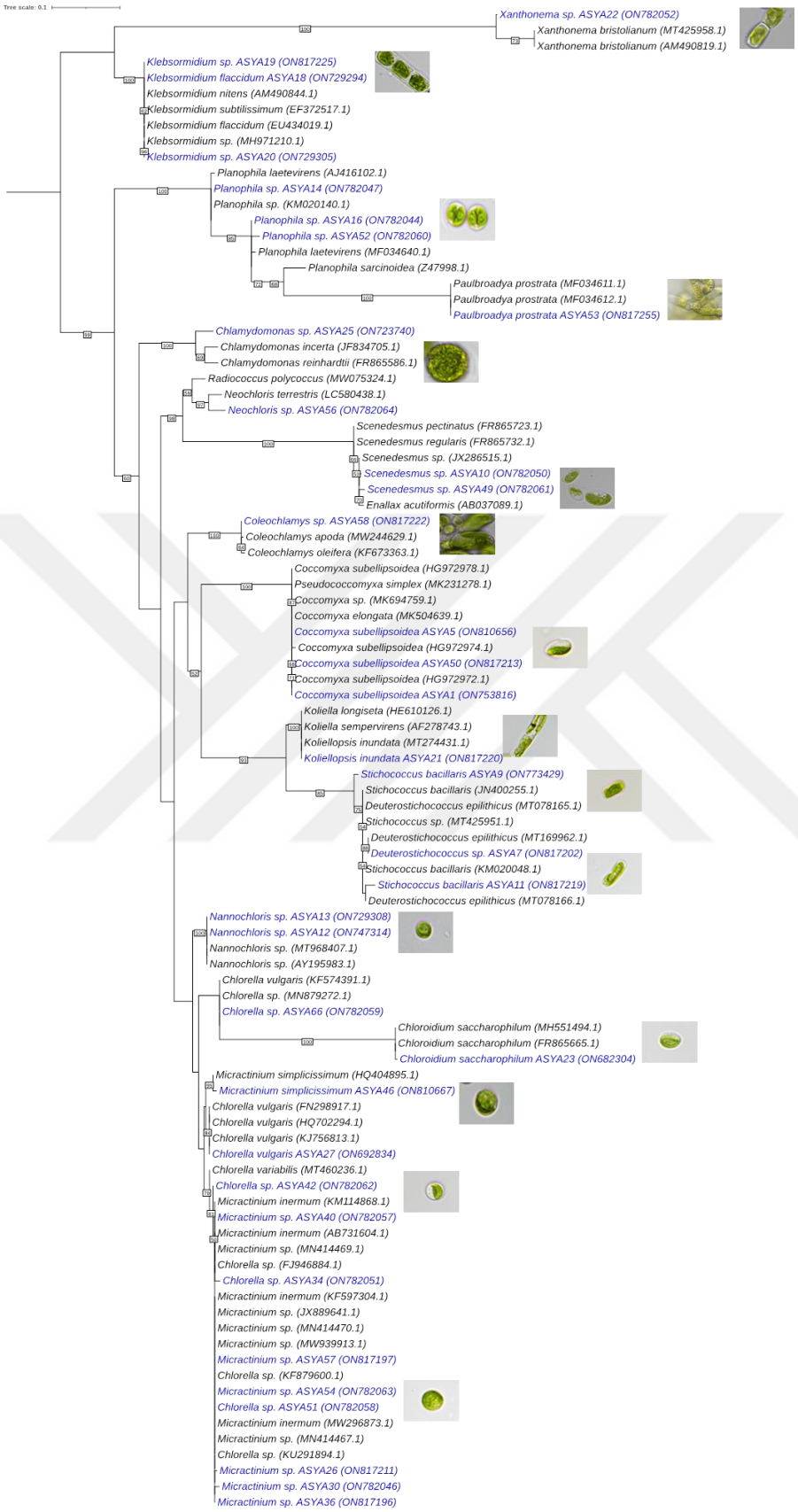


Figure 3.3 Phylogenetic analysis of the Antarctic microalgae isolates

The microalgal diversity of Antarctica's terrestrial and freshwater areas has been extensively studied. The class of Xanthophyceae consists primarily of freshwater organisms, though a significant number can also be found in moist soil (Andersen, 2004). Several Xanthophyceae have colonized alpine and polar environments (Rybalka et al., 2009). *Xanthonema* has been isolated from water-flushed soils near glaciers, stone surfaces, and moss in Antarctica. The strain *Xanthonema* sp. ASYA22 was isolated from the rock cavity on Horseshoe Island. Phylogenetically close Xanthonema strains *Xanthonema bristollanum* (AM490819.1) and *Xanthonema bristollanum* (MT425958.1) were isolates from Belanske Tatry, Slovakia (from snow) and Russia (arable chernozem), respectively (Maistro et al., 2009a). Some Antarctic *Xanthonema* strains have also been reported from King George Island (Rybalka, 2015).

Microalgae species belonging to the *Klebsormidium* genus are among the most common aeroterrestrial groups distributed in many different habitats worldwide (Rindi et al., 2008). The phylogenetic analysis showed that the *K. flaccidum* strains isolated in this study were most close to strains given NCBI accession numbers MK201754.1 and AM490844.1, which were isolated from the Crimea, Leninsky District, Vicinities of Kazantip Nature Reserve, Ukraine (from biological soil crust) and WI, Madison, Barlow, USA respectively (Mikhailyuk et al., 2015; Sluiman et al., 2008). Some Antarctic *K. flaccidum* strains have also been reported from King George Island (from green sand near the bird nests) (Elster et al., 2008). The strain *K. flaccidum* ASYA18 was isolated from rock surface-lakeside on the coast of Horseshoe Island.

Planophila is a ulvophyte that has been isolated from terrestrial or freshwater habitats. (Friedl & O'KELLY, 2002). The strains *Planophila* sp. ASYA14 and *Planophila* sp. ASYA16 were isolated from the rock surface on the coast of Horseshoe Island. The phylogenetic analysis showed that the *Planophila* sp. strains isolated in this study were most close to strains given NCBI accession numbers AJ416102.1 and MF034642.1, isolated from soil and freshwater samples- in Italy and Switzerland (Darienko & Proeschold, 2017; Friedl & O'KELLY, 2002). Some Antarctic *Planophila* sp. strains have also been reported from King George Island (Câmara et al., 2021).

Paulbroadya has been found to form symbiotic relationships with several other freshwater and marine lichens in the Verrucariaceae family. (Gasulla et al., 2019; Thüs et al., 2011). Although the majority of the taxa in the family are marine, there are also freshwater and aerophytic taxa (Škaloud et al., 2018). The strain *Paulbroadya prostrata* ASYA53 is isolated from the snow surface by the sea on Horseshoe Island. Phylogenetically close Paulbroadya strains *Paulbroadya prostrata* (MF034611.1) and *Paulbroadya prostrata* (MF034612.1) were isolates from soil and rocks samples (green epilithic crusts on rocks) on Ross Island, Cape Royds, Antarctica (Darienko & Proeschold, 2017).

Chlamydomonas sp. is a unicellular freshwater green alga used as a model organism for physiological, molecular, biochemical, and genetic research (Rasala & Mayfield, 2011). *Chlamydomonas* can be found in stagnant water, damp soil, freshwater, seawater, and polar regions (Hoham et al., 2002). *Chlamydomonas* sp. is a valuable model system for studying various scientific fields, including responses to environmental conditions (de Carpentier et al., 2019). The strain *Chlamydomonas* sp. ASYA25 was isolated from planktonic samples of Skua Lake on Horseshoe Island. The phylogenetic analysis showed that the *Chlamydomonas* sp. strain isolated in this study was most close to strains given NCBI accession numbers FR865586.1 and JF834705.1, which were isolated from the freshwater- muddy pool in Edgewood Park, Connecticut, the USA, and Umian Lake of Shillong, Meghalaya, India respectively (Melero-Jiménez et al., 2022; Ratha et al., 2012). Some Antarctic *Chlamydomonas* sp. strains have also been reported (Zhang et al., 2021).

The strain *Neochloris* sp. ASYA56 was isolated from the sediment on Horseshoe Island. Phylogenetically close Neochloris strains *Neochloris terrestris* (LC580438.1) and *Radiococcus polycoccus* (MW075324.1) are isolates from freshwater samples in the United Kingdom (Fučíková et al., 2014). Some Antarctic *Neochloris* sp. strains have also been reported from Antarctica (Cameron, 1972).

Scenedesmus sp. is one of the most common freshwater algae genera, but their identification is difficult due to the highly diverse morphologies found within species. *Scenedesmus* sp. ASYA10 and *Scenedesmus* sp. ASYA49 were isolated from a natural freshwater pond on Horseshoe Island. The phylogenetic analysis

showed that the *Scenedesmus* sp. strains isolated in this study were most close to strains given NCBI accession numbers jX286515.1 and AB037089.1, which were isolated from the surface of wet rocks at the Zhongshan Station of Antarctica (Chen, Gong, et al., 2012).

Coleochlamys can be found in both freshwater and terrestrial environments. The strain *Coleochlamys* sp. ASYA58 was isolated from the rock surface on Horseshoe Island. Phylogenetically close Coleochlamys strains *Coleochlamys oleifera* (KF673363.1) and *Coleochlamys apoda* (MW244629.1) were isolates from freshwater and snow samples in the South Orkney Islands, Signy Island, Antarctica, and High Tatra Mts, Slovakia (Barcytė et al., 2021; Broady, 1979).

Coccomyxa species can be found in terrestrial green biofilms, soil algae associated with mosses, and planktonic in limnic ecosystems (Darienko et al., 2015). However, no record of the life of *Coccomyxa* in the marine environment has been published. *Coccomyxa* has a worldwide distribution, can form biofilms, is dominant in specific ecosystems, and exhibits excellent adaptability in habitat and lifestyle. Representatives of this genus are distinguished by their small size and irregular elliptical to spherical cell shape. The strains *Coccomyxa* sp. ASYA1 and *Coccomyxa* sp. ASYA5 were isolated from the snow surface on Horseshoe Island. The phylogenetic analysis showed that the *Coccomyxa* sp. strains isolated in this study were most close to strains given NCBI accession numbers HG972974.1 and HG972972.1, which were isolated from the freshwater samples, Vestfold Hills, Princess Elizabeth Land, Antarctica (Darienko et al., 2015).

Koliella is a small genus of green algae with 35 species that thrive in freshwater, in the surface layers of alpine glaciers and snow (Guiry & Guiry, 2019). The strain *Koliellopsis* sp. ASYA21 was isolated from the snow surface of Dismal island. Phylogenetically close Koliellopsis strains *Koliellopsis inundata* (MT274431.1) and *Koliella sempervirens* (AF278743.1) were isolated from freshwater.

More than 100 species names are given to *Stichococcus* strains isolated worldwide; however, information regarding their morphology and ecology has yet to be completed (Guiry & Guiry, 2019). An authentic strain of this species has not been reported so far. A *Stichococcus* strain can be found in any terrestrial habitat, mainly

soil samples. The first type species *S. bacillaris* was described by Nageli from Switzerland. Stichococcus strains' morphology differs from barrel shape to ovoid, from a single cell to multicellular (Proeschold & Darienko, 2020). The Stichococcus strains isolated in this study were close relative to *S. Bacillaris* (JN400255.1), which was also isolated from Antarctica (Zhongshan station, from the surface of rocks) (Chen, He, et al., 2012).

The group of *Nannochloris*-like algae is difficult to identify in general and requires taxonomic revision, including strains from ecologically diverse environments (Sommer et al., 2020). *Nannochloris* sp. can be found in brackish and marine systems. The strains *Nannochloris* sp. ASYA12 and *Nannochloris* sp. ASYA13 were isolated from the rock surface on the coast of Horseshoe Island. The phylogenetic analysis showed that the *Nannochloris* sp. strains isolated in this study were most close to strains given NCBI accession numbers AY195983.1 and MT968407.1, which were isolated from light biocrust on sandy in Germany (Sommer et al., 2020). Some Antarctic *Nannochloris* sp. strains have also been reported from freshwater samples (Vishnivetskaya, 2009).

Chloroidium species live in many habitats, from streams to oceans, and they can also be found on the land, including in deserts (Nelson et al., 2017). *Chloroidium saccharophilum* ASYA23 was isolated from the stone surface on Horseshoe Island. The phylogenetic analysis showed that the *C. saccharophilum* strain isolated in this study was most close to strains given NCBI accession numbers MH551494.1 and FR865665.1, isolates from freshwater- in Italy (Darienko et al., 2010). Some Antarctic *C. saccharophilum* strains have also been reported from Queen Maud Land, East Antarctica (Faluaburu et al., 2019).

Chlorella-like microalgae are challenging to identify due to their morphology and high plasticity. The genus *Micractinium* was described by G. Fresenius (Fresenius, 1858). This genus currently contains 20 microalgae species (Guiry & Guiry, 2019). *Micractinium* members are found in many habitats, including freshwater and brackish water reservoirs, hot springs, and polar regions (Krivina et al., 2022). The strain *Micractinium* sp. ASYA26 was isolated from the stone surface of Horseshoe Island. The phylogenetic analysis showed that the *Micractinium* sp. strains isolated in

this study were most close to strains given NCBI accession numbers JX889641.1 and MN414470.1, which are isolated from freshwater- Sendai, Miyagi, Japan, and King George Island, South Shetland Islands, Antarctica, respectively (H. Chae et al., 2019; Hoshina & Fujiwara, 2013). *Micractinium simplicissimum* ASYA46 was isolated from the snow on Horseshoe Island. Phylogenetically close *Micractinium simplicissimum* (HQ404895.1) was isolated from King George Island, South Shetland Islands, Antarctica (from the snow surface) (Leya, 2020). Some other Antarctic *M. simplicissimum* strains have also been reported from King Sejong Station, Antarctica (H.-J. Chae et al., 2021).

Chlorella was described by Dutch microbiologist Martinus Willem Beijerinck in 1890 (Beijerinck, 1890). *Chlorella* is a cosmopolitan genus of green algae (Chlorophyta) with small globular cells. It includes strains with high-temperature tolerance, as some strains can grow at temperatures ranging from 15 to 40 °C. Chlorella strains grow autotrophically in an inorganic medium as well as mixotrophically and heterotrophically (e.g., with the addition of acetic acid and glucose). *Chlorella* sp. members can be found in various habitats, including fresh and brackish waters, hot springs, and even rock surfaces in Antarctica (Hu et al., 2008). In comparison to aquatic and terrestrial environments, little is known about the presence and biology of *Chlorella*-like organisms in brackish and marine environments (Darienko et al., 2019). The strain *Chlorella vulgaris* ASYA27 was isolated from the stone surface in the running on Horseshoe Island. Phylogenetically close Chlorella strains *Chlorella vulgaris* (KJ756813.1) and *Chlorella vulgaris* (HQ702294.1) were isolates from marine samples on Loch Linnhe, Argyll, Scotland, UK, and freshwater samples on Jinhae Bay, South Korea (Darienko et al., 2019; Lee & Hur, 2012). Some Antarctic *Chlorella* sp. strains have also been reported from the snow and water samples (Apostolova et al., 2014; Rivas et al., 2016).

The unrooted phylograms show the evolutionary distances of the strains regardless of time. Figure 3.4 shows an unrooted phylogram of diatom isolates. *Navicula phyllepta* can live in different habitats with a wide range of salinity levels. This strain differentiates from two other diatom isolates distinctly. On the other hand, diatom *P.tricornutum* possesses a unique feature in its cell wall where the Silicium level is way less than the other diatoms, giving flexibility to this diatom in different environments.

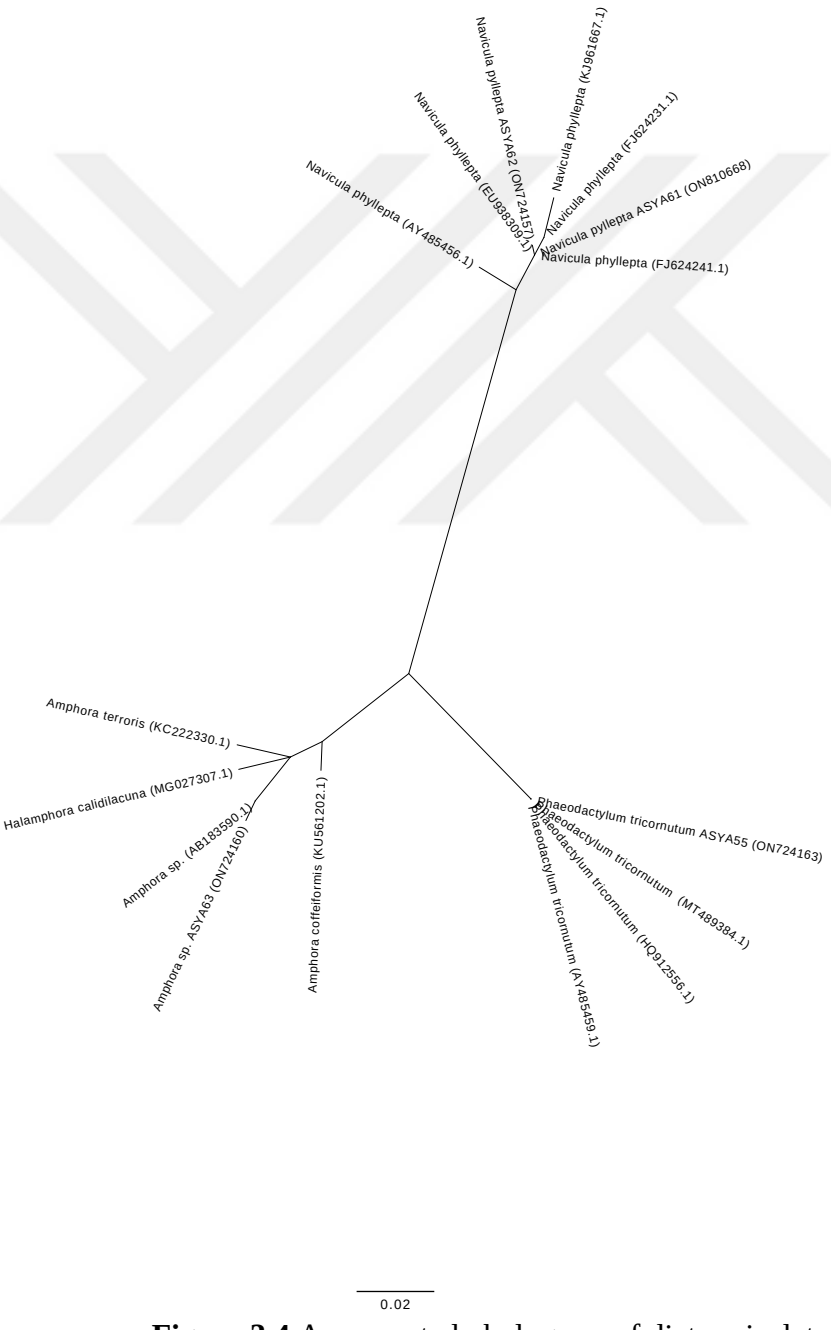


Figure 3.4 An unrooted phylogram of diatom isolates

Figure 3.5 shows an unrooted phylogram for the other Antarctic isolates. Being included in the Trebouxiophyceae clade, *Chloroidium saccharophilum* seems distinctly differentiated from other *Chlorella*, *Nannochloris*, and *Micractinium* strains. On the other hand, *Coleochlamys*, *Coccomyxa*, *Koliellopsis*, and *Stichococcus* form a group in the Trebouxiophyceae clade that share more similar features with *Chlorophyceae* than the others. Lastly, possessing a cutin-like layer covering the cell wall, *Klebsormidium* strains are separated from most others at the phylum level (Charophyta), sharing more similar features with Xanthonema, which is also grouped in another phylum (Ochrophyta).

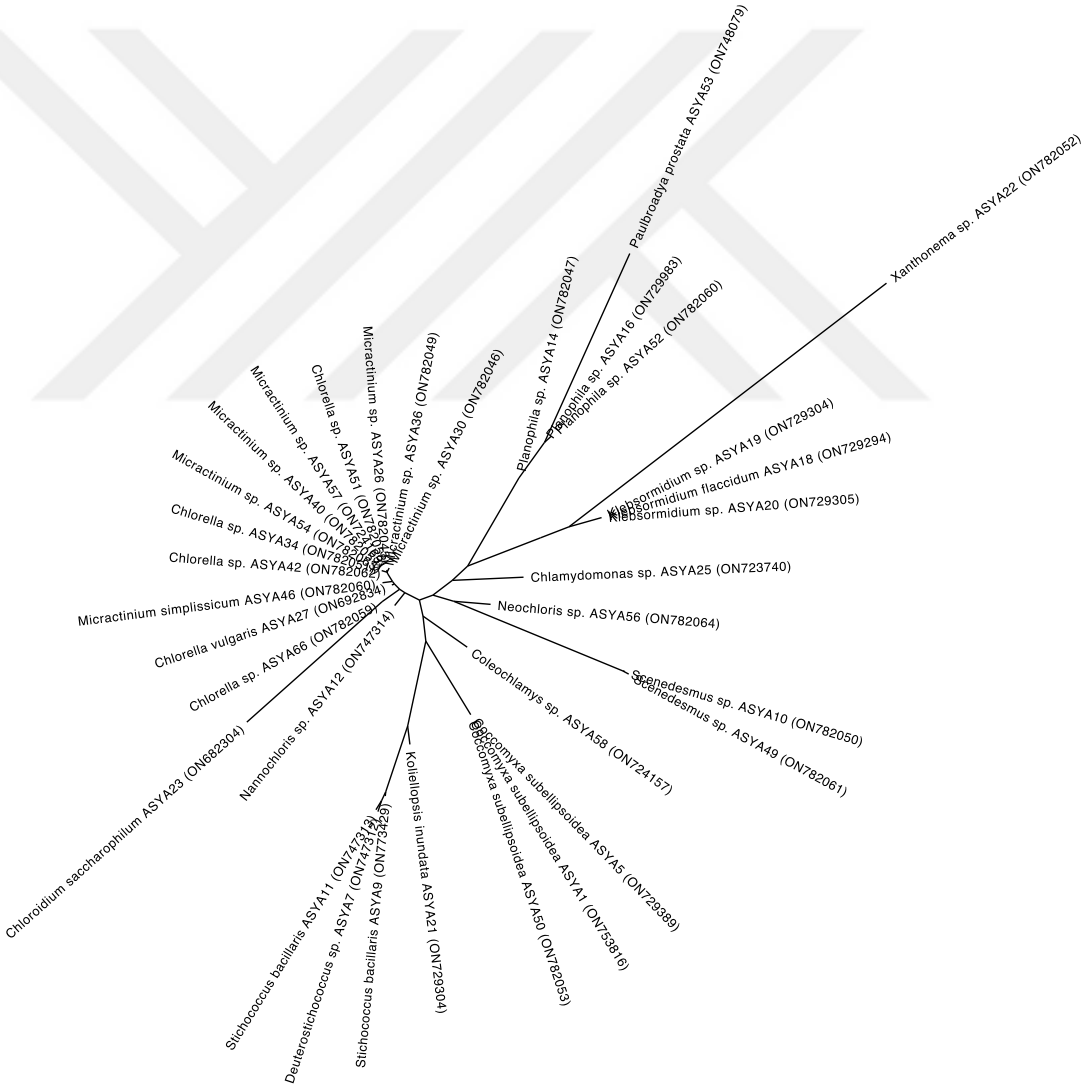
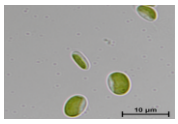
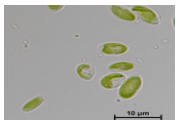
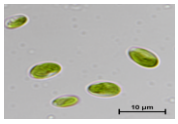


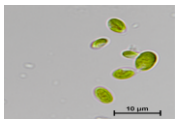
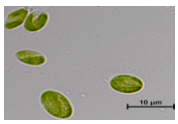
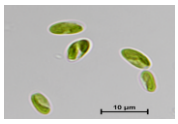
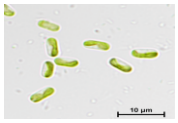
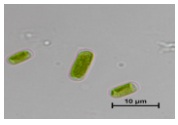
Figure 3.5 An unrooted phylogram for the other Antarctic isolates

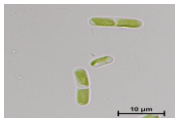
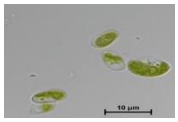
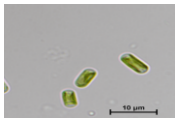
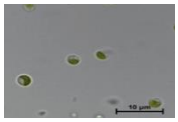
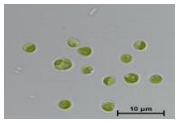
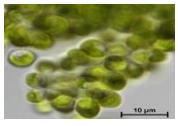
3.4. MORPHOLOGY AND ECOPHYSIOLOGY OF THE ISOLATES

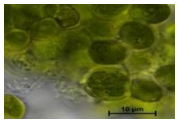
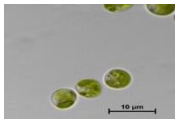
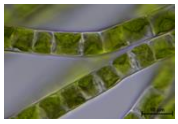
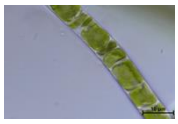
The isolates' morphological characteristics and habitat image are given below, along with the microscopic images and NCBI accession numbers attributed to the strains.

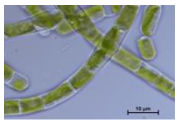
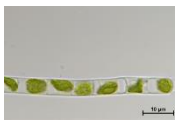
Table 3.5 Morphological characteristics of IMU-ASYA strains

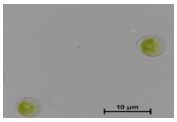
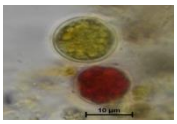
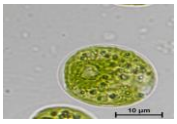
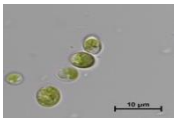
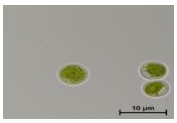
Strain	Characteristics	NCBI accession #	Habitat / Coordinates	Microscope image
Coccomyxa subellipsoidea ASYA1	Class: Trebouxiophyceae *Coccomyxa* cells are small, unicellular, and non-motile with a single parietal chloroplast. The cells are ovoid or ellipsoidal in shape. Under standard conditions, the average size of mature vegetative cells is 3-4×5-7 µm. Chloroplasts are large and located close to the cell wall. Pyrenoid is absent in the cells.	ON753816	 67°49'52"S 67°14'17"W	
Coccomyxa subellipsoidea ASYA2	Class: Trebouxiophyceae *Coccomyxa* cells are small, unicellular, and non-motile with a single parietal chloroplast. The cells are ovoid or ellipsoidal in shape. Under standard conditions, the average size of mature vegetative cells is 3-6×5-10 µm. Chloroplasts are large and located close to the cell wall. Pyrenoid is absent in the cells.	ON729389	 67°49'51.9"S 67°14'17.5"W	
Coccomyxa subellipsoidea ASYA3	Class: Trebouxiophyceae *Coccomyxa* cells are small, unicellular, and non-motile with a single parietal chloroplast. The cells are ovoid or ellipsoidal in shape. Under standard conditions, the average size of mature vegetative cells is 4-5 × 5-8 µm. Chloroplasts are large and located close to the cell wall. Pyrenoid is absent in the cells.	ON747312	 67°81'28.5"S 67°22'39.1"W	

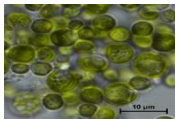
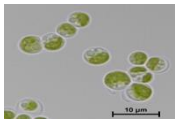
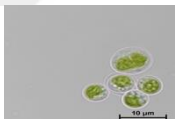
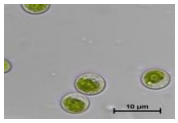
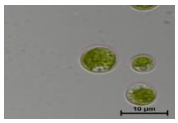
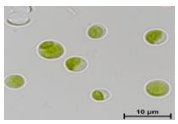
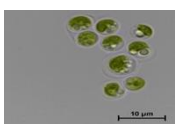
Coccomyxa subellipsoidea ASYA4	Class: Trebouxiophyceae *Coccomyxa* cells are small, unicellular, and non-motile with a single parietal chloroplast. The cells are ovoid or ellipsoidal in shape. Under standard conditions, the average size of mature vegetative cells is 2-5×4-7 µm. Chloroplasts are large and located close to the cell wall. Pyrenoid is absent in the cells.	ON747313	 67°49'33.7"S 67°12'25.6"W	
Coccomyxa subellipsoidea ASYA5	Class: Trebouxiophyceae *Coccomyxa* cells are small, unicellular, and non-motile with a single parietal chloroplast. The cells are ovoid or ellipsoidal in shape. Under standard conditions, the average size of mature vegetative cells is 3-8 × 6-10 µm. Chloroplasts are large and located close to the cell wall. Pyrenoid is absent in the cells.	ON810656	 68°05'3.1"S 68°50'59.2"W	
Coccomyxa subellipsoidea ASYA6	Class: Trebouxiophyceae *Coccomyxa* cells are small, unicellular, and non-motile with a single parietal chloroplast. The cells are ovoid or ellipsoidal in shape. Under standard conditions, the average size of mature vegetative cells is 3-4 × 6-8 µm. Chloroplasts are large and located close to the cell wall. Pyrenoid is absent in the cells.	ON747314	 67°49'52"S 67°14'17"W	
Deuterostichococcus sp. ASYA7	Class: Trebouxiophyceae *Deuterostichococcus* cells are usually unicellular. In marine conditions, unbranched filaments can form out of 2-4 cells, which are easy to fragment. In a freshwater medium, cells are cylindrical with rounded ends. The cells are cylindrical with a 2-3 length/width ratio. The chloroplast is single, large, and parietal. Chloroplasts are located in the cell edge, without pyrenoid.	ON817202	 67°49'33.7"S 67°12'25.6"W	
Stichococcus sp. ASYA8	Class: Trebouxiophyceae *Stichococcus* cells are usually unicellular. In marine conditions, unbranched filaments can form out of 2-4 cells, which are easy to fragment. In a freshwater medium, cells are cylindrical with rounded ends. The cells are cylindrical with a 2-3 length/width ratio. The chloroplast is single, large, and parietal. Chloroplasts are located in the cell edge, without pyrenoid.	ON729983	 67°49'52"S 67°14'17"W	

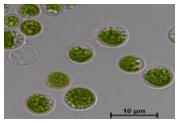
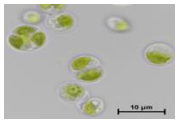
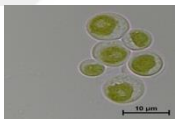
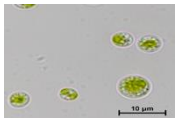
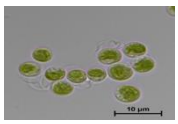
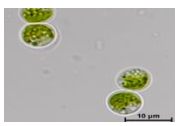
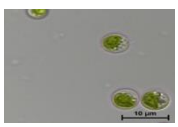
Stichococcus bacillaris ASYA9	Class: Trebouxiophyceae Stichococcus cells are usually unicellular. In marine conditions, unbranched filaments can form out of 2-4 cells, which are easy to fragment. In a freshwater medium, cells are cylindrical with rounded ends. The cells are cylindrical with a 2-3 length/width ratio. The chloroplast is single, large, and parietal. Chloroplasts are located in the cell edge, without pyrenoid. Cells are with polar vacuoles.	ON773429	 67°48'30.7"S 67°10'58.3"W	
Scenedesmus sp. ASYA10	Class: Chlorophyceae *Scenedesmus* cells are unicellular, ellipsoidal and cell size is around 3-6 µm × 5-10 µm. Chloroplast is single, parietal with a pyrenoid. The vacuole is present in some cells.	ON782050	 67°83'21"S 67°22'25.9"W	
Stichococcus bacillaris ASYA11	Class: Trebouxiophyceae *Stichococcus* cells are usually unicellular. In marine conditions, unbranched filaments can form out of 2-4 cells, which are easy to fragment. In a freshwater medium, cells are cylindrical with rounded ends. The cells are cylindrical with a 2-3 length/width ratio. The chloroplast is single, large, and parietal. Chloroplasts are located in the cell edge, without pyrenoid. Cells are with polar vacuoles.	ON817219	 67°50'29.7"S 67°16'44.1"W	
Nannochloris sp. ASYA12	Class: Trebouxiophyceae *Nannochloris* cells are single or in pairs, spherical to cylindrical in shape, and small (the cell size is around 2-5 µm). Chloroplast is single, parietal/cup-shaped without pyrenoid.	ON810657	 67°82'68.5"S 67°22'49.7"W	
Nannochloris sp. ASYA13	Class: Trebouxiophyceae *Nannochloris* cells are single or in pairs, spherical to cylindrical in shape, and small (the cell size is around 2-5 µm). Chloroplast is single, parietal/cup-shaped without pyrenoid.	ON729308	 67°48'06.3"S 67°09'52.9"W	
Planophila sp. ASYA14	Class: Ulvophyceae *Planophila* cells are ellipsoidal or spherical. The cells are clustered. Chloroplast is parietal with an ellipsoidal pyrenoid.	ON782047	 67°49'33.7"S 67°12'32.4"W	

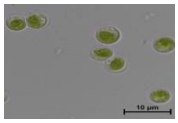
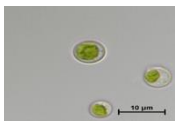
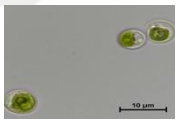
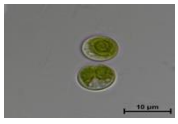
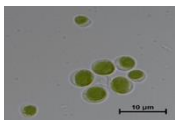
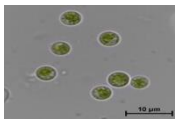
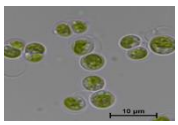
Planophila sp. ASYA15	Class: Ulvophyceae *Planophila* cells are ellipsoidal or spherical. Cells are clustered. Chloroplast is parietal/cup-shaped with an ovoid pyrenoid.	NA	 67°49'25.2"S 67°12'32.4"W	
Planophila sp. ASYA16	Class: Ulvophyceae *Planophila* cells are spherical or ellipsoidal, and cell size is around 4-7×5-9 µm. Chloroplast is large, parietal/cup-shaped with an ovoid pyrenoid.	ON782044	 67°49'25.2"S 67°12'32.4"W	
Klebsormidium sp. ASYA17	Class: Klebsormidiophyceae The filaments are long, tend to disintegrate, and approximately possed a 7.3 µm width. The length-width ratio of the cells is (6.03min) 9.1 (11.21max) x (6.11min) 7.3 (8.56max) µm. The cells are cylindrical-shaped, and the cell wall is moderately thickened. H-fragments are rarely present, and chloroplast covers half to 2/3 of the cell's inner surface with a smooth margin. Pyrenoid is large and surrounded by several layers of starch grains *Klebsormidium sp* cells in liquid media form a superficial hydro-repellent layer, whereas on agar form undulating colonies (Mikhailyuk et al., 2015).	ON729304	 67°49'36.6"S 67°13'38.7"W	
Klebsormidium flaccidum ASYA18	Class: Klebsormidiophyceae The filaments are long, tend to disintegrate, and approximately possed a 6.4 µm width. The length-width ratio of the cells is (4.6min) 12.3 (17.5max) x (7.2min) 8.5 (10.2max) µm. The cells are cylindrical-shaped, and the cell wall is moderately thickened. H-fragments are rarely present, and chloroplast covers half to 2/3 of the cell's inner surface with a smooth margin. Pyrenoid is large and surrounded by several layers of starch grains. *K. flaccidum* cells in liquid media form a superficial hydro-repellent layer, whereas on agar forming undulating colonies (Mikhailyuk et al., 2015).	ON729294	 67°49'52"S 67°14'17"W	

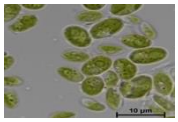
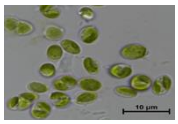
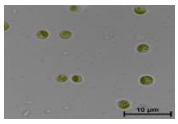
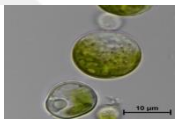
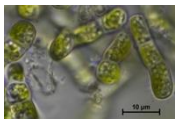
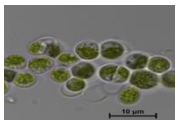
Klebsormidium sp. ASYA19	Class: Klebsormidiophyceae The filaments are long and robust, approximately possed 6.4 µm in width. The length-width ratio of the cells is (6.2min) 9.0 (11.8max) x (7min) 6.4 (7.0max) µm. The cells sometimes grow in rope-like aggregates, and they are cylindrical or barrel-shaped. The cell wall is moderately thickened, and H-fragments are prominently present. The chloroplasts are dissected in four or more lobes with a median incision at the edge covering half to ¾ of the cell interior surface. Pyrenoid is large and surrounded by several layers of starch grains. *Klebsormidium* sp. cells in liquid media form submerged tufts, whereas on agar, forming rough undulating colonies (Mikhailyuk et al., 2015).	ON817225	 67°49'57.6"S 67°14'16.4"W	
Klebsormidium sp. ASYA20	Class: Klebsormidiophyceae The filaments are long and robust, approximately possed 6.1 µm in width. The length-width ratio of the cells is (5min) 8.4 (11.8max) x (5min) 6.1 (7.4max) µm. The cells sometimes grow in rope-like aggregates, and they are cylindrical or barrel-shaped. The cell wall is moderately thickened, and H-fragments are prominently present. The chloroplasts are dissected in four or more lobes with a median incision at the edge covering half to ¾ of the cell interior surface. Pyrenoid is large and surrounded by several layers of starch grains. *Klebsormidium* sp. cells in liquid media form submerged tufts, whereas on agar, forming rough undulating colonies (Mikhailyuk et al., 2015).	ON729305	 67°49'57.6"S 67°14'16.4"W	
Koliellopsis inundata ASYA21	Class: Trebouxiophyceae The filaments are long with some fragmentation tendency and approximately 5.3µm in width. The length-width ratio of the cells is (4.1min) 6.6 (11.15max) x (4.3min) 5.3 (6.6max) µm. The cells are rounded or gradually tapering-shaped, and the cell wall is moderately thickened; likewise, H-fragments are prominent. The chloroplasts are bilobed laminate formation without pyrenoid. *K. inundata* cells reproduce with their biflagellate zoospores and filament dissociation (Lokhorst et al., 2004).	ON817220	 67°49'51.9"S 67°14'17.5"W	

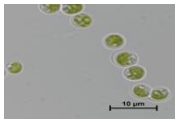
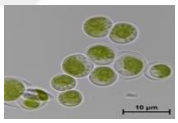
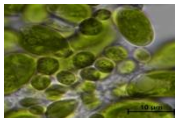
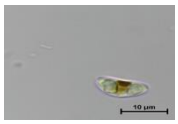
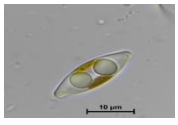
Xanthonema sp. ASYA22	Class: Xanthophyceae *Xanthonema* cells exist as single or few-celled filaments and tend to dissociate easily. The length-width ratio of the cells is (4.5min) 6.5 (8.4max) x (6.0min) 6.8 (7.7max) µm. The cells are barrel-shaped, and the cell wall is moderately thickened; however, H-pieces are not distinctly present. Chloroplasts surround the entire cell wall and exist in 3-to 4 pieces (Maistro et al., 2009b).	ON782052	 67°49'48.7"S 67°14'18.6"W	
Chloroidium saccharophilum ASYA23	Class: Trebouxiophyceae Cells have an ellipsoid shape. Cell size is around 4-10× 5-12 µm. Chloroplast is single, parietal with an ovoid pyrenoid.	ON682304	 67°49'23.6"S 67°18'20.9"W	
Chloromonas sp. ASYA24	Class: Chlorophyceae The shape of unicells is spherical to spindle-shaped. The lack of pyrenoids distinguishes them from *Chlamydomonas* sp. (Guiry & Guiry, 2019). The color of cells, from green to red, depends on the relative concentration of chlorophyll and carotenoids, especially astaxanthin (red).	NA	 68º05'33.2"S 68º50'51.3"W	
Chlamydomonas sp. ASYA25	Class: Chlorophyceae *Chlamydomonas* cells are spherical or broadly ellipsoidal in shape. The size of cells is around 9-15 µm. The cells have 2 apical contractile vacuoles. Chloroplasts are cup-shaped. Pyrenoid is medium, round-slightly ellipsoid in basal position. The cell has an elliptic eyespot in the anterior part. The number of zoospores is 2 or 4.	ON723740	 67°49'36.6"S 67°13'38.7"W	
Micractinium sp. ASYA26	Class: Trebouxiophyceae *Micractinium* sp. cells are ellipsoidal in shape and cell size around 3-6 × 5-11 µm. Chloroplast is single, parietal, large with ellipsoid to ovoid pyrenoid.	ON817211	 67°83'021"S 67°22'25.9"W	
Chlorella vulgaris ASYA27	Class: Trebouxiophyceae *Chlorella vulgaris* cells are spherical, and cell size is around 4-8 µm. Chloroplast is a single parietal with a pyrenoid.	ON692834	 67°48'09.8"S 67°18'16.2"W	

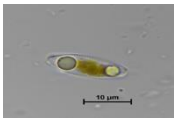
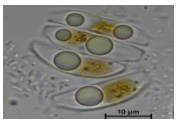
Chlorella sp. ASYA28	Class: Trebouxiophyceae *Chlorella* sp. cells are ellipsoidal or spherical. Cells are clustered. Chloroplast parietal/cup-shaped with an ellipsoidal pyrenoid.	NA	 67°50'15.3"S 67°17'23.7"W	
Chlorella sp. ASYA29	Class: Trebouxiophyceae *Chlorella* sp. cells are spherical with a 3-8 µm diameter. Chloroplast is a single parietal with a pyrenoid. Cells have vacuoles.	NA	 67°49'51.9"S 67°14'17.5"W	
Micractinium sp. ASYA30	Class: Trebouxiophyceae Cells are spherical, and cell size is around 2-6 µm. Chloroplast is single, parietal with an ovoid pyrenoid.	ON782046	 68°05'33.2"S 68°51'10.2"W	
Chlorella sp. ASYA31	Class: Trebouxiophyceae Cells are spherical, and cell size is around 4-10 µm. Chloroplast is large, parietal, with an oval pyrenoid.	ON782045	 68°05'32.4"S 68°51'05.6"W	
Chlorella sp. ASYA32	Class: Trebouxiophyceae Cells are spherical, and cell size is around 4-9 µm. Chloroplast is single, large, parietal with a pyrenoid.	ON833476	 67°49'36.6"S 67°13'38.7"W	
Chlorella sp. ASYA33	Class: Trebouxiophyceae The cells are spherical in shape, and cell size is around 4-6 µm. Chloroplast is single, parietal/cup-shaped, with an ellipsoidal pyrenoid.	ON833475	 67°49'36.6"S 67°13'38.7"W	
Chlorella sp. ASYA34	Class: Trebouxiophyceae The cells are spherical in shape, and cell size is around 4-8 µm. Chloroplast is single, parietal/cup-shaped, with an ellipsoidal pyrenoid.	ON782051	 68°05'38.1"S 68°51'08.5"W	

Chlorella sp. ASYA35	Class: Trebouxiophyceae The cells are spherical in shape, and cell size is around 4-8 µm. Chloroplast is single, parietal/cup-shaped, with an ellipsoidal pyrenoid.	ON833474	 68°05'31.3"S 68°50'48.6"W	
Micractinium sp. ASYA36	Class: Trebouxiophyceae The cells are spherical in shape, and cell size is around 5-8 µm. Chloroplast is single, parietal/cup-shaped, with an ellipsoidal pyrenoid.	ON817196	 67°48'09.8"S 67°18'16.2"W	
Chlorella sp. ASYA37	Class: Trebouxiophyceae Cells are spherical in shape, and cell size is around 4-9 µm. Chloroplast is single, parietal with an ovoid pyrenoid.	ON782049	 67°48'14.6"S 67°18'16.1"W	
Micractinium sp. ASYA38	Class: Trebouxiophyceae The cells are spherical in shape, and cell size is around 5-7 µm. Chloroplast is single, parietal/cup-shaped, with an ellipsoidal pyrenoid.	NA	 68°05'38.5"S 68°50'59.1"W	
Micractinium sp. ASYA39	Class: Trebouxiophyceae The cells are spherical in shape, and cell size is around 4-7 µm. Chloroplast is single, parietal/cup-shaped, with an ellipsoidal pyrenoid.	ON782053	 67°49'36.6"S 67°13'38.7"W	
Micractinium sp. ASYA40	Class: Trebouxiophyceae The cells are spherical in shape, and cell size is around 4-7 µm. Chloroplast is single, parietal/cup-shaped, with an ellipsoidal pyrenoid.	ON782057	 67°49'48.4"S 67°14'20.1"W	
Micractinium sp. ASYA41	Class: Trebouxiophyceae Cells are spherical, and cell size is around 4-8 µm. Chloroplast is single, parietal with a pyrenoid.	NA	 68°05'31.3"S 68°50'48.6"W	

Chlorella sp. ASYA42	Class: Trebouxiophyceae Cells are spherical, and cell size is around 4-7 µm. Chloroplast is single, parietal with a broadly ellipsoidal to spherical pyrenoid.	ON782062	 67°48'06.3"S 67°09'52.9"W	
Micractinium sp. ASYA43	Class: Trebouxiophyceae Cells are spherical, and cell size is around 4-9 µm. Chloroplast is parietal with a pyrenoid.	NA	 67°49'36.6"S 67°13'38.7"W	
Micractinium sp. ASYA44	Class: Trebouxiophyceae Cells are spherical, and cell size is around 4-9 µm. Chloroplast is parietal with an ovoid pyrenoid.	NA	 67°48'32.7"S 67°17'53.2"W	
Chlorella sp. ASYA45	Class: Trebouxiophyceae Cells are spherical, and cell size is around 4-9 µm. Chloroplast is large, parietal with a pyrenoid.	NA	 67°49'48.4"S 67°14'20.1"W	
Micractinium simplicissimum ASYA46	Class: Trebouxiophyceae Cells are spherical, and cell size is around 3-7 µm. Chloroplast is large, parietal with a large pyrenoid.	ON810667	 67°51'35.4"S 67°19'0.4"W	
Chlorella sp. ASYA47	Class: Trebouxiophyceae Cells are spherical in shape, and cell size is around 4-8 µm. Chloroplast is single, large, and parietal with a spherical pyrenoid.	ON748079	 67°48'06.3"S 67°09'52.9"W	
Micractinium sp. ASYA48	Class: Trebouxiophyceae Cells are small and spherical in shape. Cell size is around 3-8 µm. Chloroplast is single, large, and parietal with an ovoid pyrenoid.	ON817212	 67°51'35.4"S 67°19'0.4"W	

Scenedesmus sp. ASYA49	Class: Chlorophyceae Single young cells are ellipsoidal, becoming more spherical with age. For immature cells, cell size is around 2-7× 4-12 µm. For mature cells, cell size is around 4-11× 5-12 µm. Chloroplast is parietal with an ovoid pyrenoid. The vacuole is present in some cells.	ON782061	 67°83'021"S 67°22'25.9"W	
Coccomyxa subellipsoidea ASYA50	Class: Trebouxiophyceae *Coccomyxa* cells are small, unicellular, and non-motile with a single parietal chloroplast. The cells are ovoid or ellipsoidal. Under standard conditions, the average size of mature vegetative cells is 2-5× 5-9 µm. Chloroplasts are large and located close to the cell wall. Pyrenoid is absent in the cells.	ON817213	 67°82'68.5"S 67°22'39.1"W	
Chlorella sp. ASYA51	Class: Trebouxiophyceae Cells are spherical in shape, and cell size is around 2-5 µm. Chloroplast is large, parietal with/without a pyrenoid.	ON782058	 67°48'32.7"S 67°17'53.2"W	
Planophila sp. ASYA52	Class: Ulvophyceae The cells are broadly spherical. Cell size is around 4-15 µm. Chloroplast is single, parietal/cup-shaped with a pyrenoid. The vacuole is present.	ON782060	 67°48'32.7"S 67°17'53.2"W	
Paulbroadya prostrata ASYA53	Class: Ulvophyceae *Paulbroadya* is formed prostrate and erect filamentous systems. The prostrate system comprises rounded cells that are often grouped to form cell packages. The erect system is densely branched, either uni- or bilaterally. Unicellular cells have a parietal chloroplast and one pyrenoid. Starch grains surround the pyrenoid.	ON817255	 67°48'32.7"S 67°17'53.2"W	
Micractinium sp. ASYA54	Class: Trebouxiophyceae Cells are ellipsoidal to spherical. Cell size is around 3-7 µm. Chloroplast is single, parietal, large, with an ellipsoidal pyrenoid.	ON782063	 67°51'35.4"S 67°19'0.4"W	

Phaeodactylum tricornutum ASYA55	Class: Bacillariophyta classis incertae sedis	ON724163	 67°49'51.9"S 67°14'17.5"W	
Neochloris sp. ASYA56	Class: Chlorophyceae Cells are spherical in shape, and cell size is around 3-7 µm. Chloroplast is single, parietal/cup-shaped with a pyrenoid.	ON782064	 67°48'07.9"S 67°09'52.1"W	
Micractinium sp. ASYA57	Class: Trebouxiophyceae Cells are spherical in shape and cell size around 3-7 µm. Chloroplast is single, parietal with an ovoid pyrenoid.	ON817197	 67°49'24.1"S 67°12'23.9"W	
Coleochlamys sp. ASYA58	Class: Trebouxiophyceae Cells are ellipsoidal, and cells can be small or large. Chloroplast is parietal with a pyrenoid.	ON817222	 67°49'57.6"S 67°14'16.4"W	
Amphora sp. ASYA60	Class: Bacillariophyceae *Amphora* is an asymmetric biraphid diatom. The solitary cells can move and almost always appear as a girdle. Cells appear elliptical with straight-cut ends. The valves are semi-elliptical, with concave ventral margins and sometimes smaller or constricted at both cells ends. Both raphes are on the same side of the valve. Usually, two or more plastids occur at different positions throughout the cell.	NA	 67°49'51.9"S 67°14'17.5"W	
Navicula phyllepta ASYA61	Class: Bacillariophyceae *Navicula* is a symmetric biraphid diatom. The cells are boat-shaped, motile, and solitary. Valves are elliptic to broadly lanceolate. The raphe is straight.	ON810668	 67°49'51.9"S 67°14'17.5"W	

Navicula phyllepta ASYA62	Class: Bacillariophyceae *Navicula* is a symmetric biraphid diatom. The cells are boat-shaped, motile, and solitary. Valves are elliptic to broadly lanceolate. The raphe is straight.	ON724157	 67°49'51.9"S 67°14'17.5"W	
Amphora sp. ASYA63	Class: Bacillariophyceae *Amphora* is asymmetric biraphid diatom. The solitary cells can move and almost always appear in the form of a girdle. Cells appear elliptical with straight-cut ends. The valves are semi-elliptical, with concave ventral margins and sometimes smaller or constricted at both cell ends. Both raphes are on the same side of the valve. Usually, two or more plastids occur at different positions throughout the cell.	ON724160	 67°49'51.9"S 67°14'17.5"W	

Microalgae phylum, class, order, and family distribution retrieved from the AlgaeBase website are presented below (www.algaebase.org/browse/taxonomy, July-2022).

Table 3.6 Taxonomy of IMU-ASYA species

Species	Family	Order	Class	Phylum/Division	Kingdom
Coccomyxa subellipsoidea ASYA1	Coccomyxaceae	Trebouxiophyceae ordo incertae sedis	Trebouxiophyceae	Chlorophyta	Plantae
Coccomyxa subellipsoidea ASYA2	Coccomyxaceae	Trebouxiophyceae ordo incertae sedis	Trebouxiophyceae	Chlorophyta	Plantae
Coccomyxa subellipsoidea ASYA3	Coccomyxaceae	Trebouxiophyceae ordo incertae sedis	Trebouxiophyceae	Chlorophyta	Plantae
Coccomyxa subellipsoidea ASYA4	Coccomyxaceae	Trebouxiophyceae ordo incertae sedis	Trebouxiophyceae	Chlorophyta	Plantae
Coccomyxa subellipsoidea ASYA5	Coccomyxaceae	Trebouxiophyceae ordo incertae sedis	Trebouxiophyceae	Chlorophyta	Plantae
Coccomyxa subellipsoidea ASYA6	Coccomyxaceae	Trebouxiophyceae ordo incertae sedis	Trebouxiophyceae	Chlorophyta	Plantae
Deuterostichococcus sp. ASYA7	Stichococcaceae	Prasiolales	Trebouxiophyceae	Chlorophyta	Plantae
Stichococcus sp. ASYA8	Stichococcaceae	Prasiolales	Trebouxiophyceae	Chlorophyta	Plantae
Stichococcus bacillaris ASYA9	Stichococcaceae	Prasiolales	Trebouxiophyceae	Chlorophyta	Plantae
Scenedesmus sp. ASYA10	Scenedesmaceae	Sphaeropleales	Chlorophyceae	Chlorophyta	Plantae

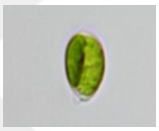
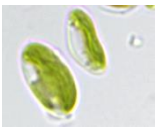
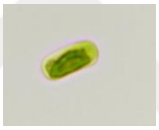
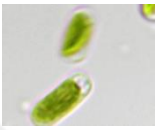
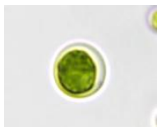
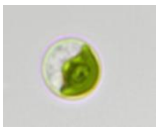
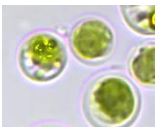
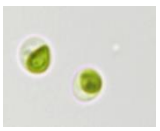
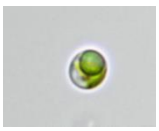
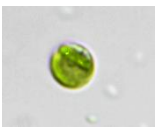
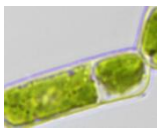
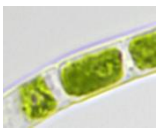
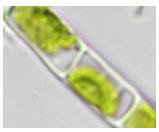
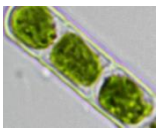
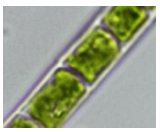
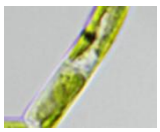
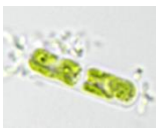
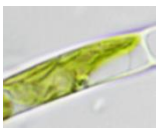
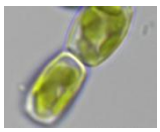
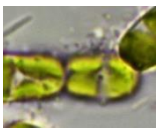
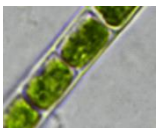
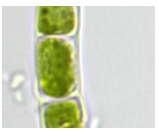
Stichococcus bacillaris ASYA11	Stichococcaceae	Prasiolales	Trebouxiophyceae	Chlorophyta	Plantae
Nannochloris sp. ASYA12	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Nannochloris sp. ASYA13	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Planophila sp. ASYA14	Planophilaceae	Ulotrichales	Ulvophyceae	Chlorophyta	Plantae
Planophila sp. ASYA15	Planophilaceae	Ulotrichales	Ulvophyceae	Chlorophyta	Plantae
Planophila sp. ASYA16	Planophilaceae	Ulotrichales	Ulvophyceae	Chlorophyta	Plantae
Klebsormidium sp. ASYA17	Klebsormidiaceae	Klebsormidiales	Klebsormidiophyceae	Charophyta	Plantae
Klebsormidium flaccidum ASYA18	Klebsormidiaceae	Klebsormidiales	Klebsormidiophyceae	Charophyta	Plantae
Klebsormidium sp. ASYA19	Klebsormidiaceae	Klebsormidiales	Klebsormidiophyceae	Charophyta	Plantae
Klebsormidium sp. ASYA20	Klebsormidiaceae	Klebsormidiales	Klebsormidiophyceae	Charophyta	Plantae
Koliellopsis inundata ASYA21	Trebouxiophyceae incertae sedis	Trebouxiophyceae ordo incertae sedis	Trebouxiophyceae	Chlorophyta	Plantae
Xanthonema sp. ASYA22	Tribonemataceae	Tribonematales	Xanthophyceae	Ochrophyta	Chromista
Chloroidium saccharophilum ASYA23	Watanabeaceae	Watanabeales	Trebouxiophyceae	Chlorophyta	Plantae
Chloromonas sp. ASYA24	Chlamydomonadaceae	Chlamydomonadales	Chlorophyceae	Chlorophyta	Plantae
Chlamydomonas sp. ASYA25	Chlamydomonadaceae	Chlamydomonadales	Chlorophyceae	Chlorophyta	Plantae
Micractinium sp. ASYA26	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Chlorella vulgaris ASYA27	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Chlorella sp. ASYA28	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Chlorella sp. ASYA29	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Micractinium sp. ASYA30	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Chlorella sp. ASYA31	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Chlorella sp. ASYA32	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Chlorella sp. ASYA33	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Chlorella sp. ASYA34	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Chlorella sp. ASYA35	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Micractinium sp. ASYA36	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Chlorella sp. ASYA37	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Micractinium sp. ASYA38	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae

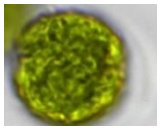
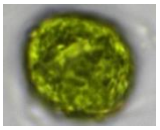
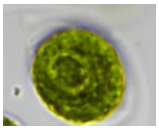
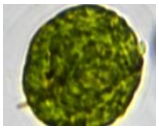
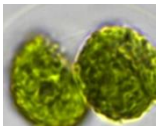
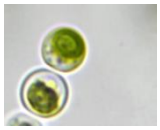
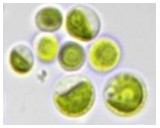
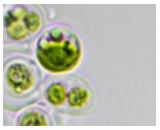
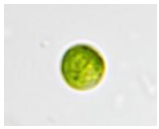
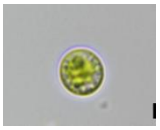
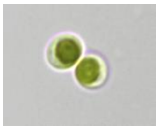
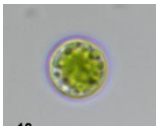
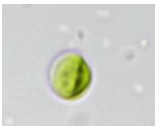
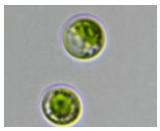
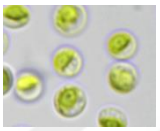
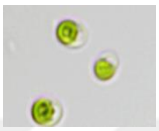
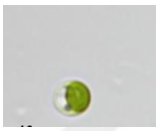
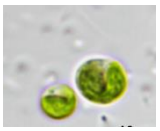
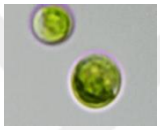
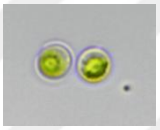
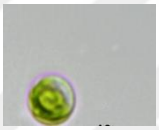
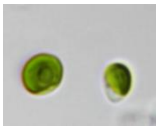
Micractinium sp. ASYA39	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Micractinium sp. ASYA40	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Micractinium sp. ASYA41	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Chlorella sp. ASYA42	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Micractinium sp. ASYA43	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Micractinium sp. ASYA44	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Chlorella sp. ASYA45	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Micractinium simplicissimum ASYA46	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Chlorella sp. ASYA47	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Micractinium sp. ASYA48	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Scenedesmus sp. ASYA49	Scenedesmaceae	Sphaeropleales	Chlorophyceae	Chlorophyta	Plantae
Coccomyxa subellipsoidea ASYA50	Coccomyxaceae	Trebouxiophyceae ordo incertae sedis	Trebouxiophyceae	Chlorophyta	Plantae
Chlorella sp. ASYA51	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Planophila sp. ASYA52	Planophilaceae	Ulotrichales	Ulvophyceae	Chlorophyta	Plantae
Paulbroadya prostrata ASYA53	Ulvales incertae sedis	Ulvales	Ulvophyceae	Chlorophyta	Plantae
Micractinium sp. ASYA54	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Phaeodactylum tricornutum ASYA55	Phaeodactylaceae	Bacillariophyta ordo incertae sedis	Bacillariophyta classis incertae sedis	Bacillariophyta	Chromista
Neochloris sp. ASYA56	Neochloridaceae	Sphaeropleales	Chlorophyceae	Chlorophyta	Plantae
Micractinium sp. ASYA57	Chlorellaceae	Chlorellales	Trebouxiophyceae	Chlorophyta	Plantae
Coleochlamys sp. ASYA58	Microthamniaceae	Microthamniales	Trebouxiophyceae	Chlorophyta	Plantae
Amphora sp. ASYA60	Catenulaceae	Thalassiophysales	Bacillariophyceae	Bacillariophyta	Chromista
Navicula phyllepta ASYA61	Naviculaceae	Naviculales	Bacillariophyceae	Bacillariophyta	Chromista
Navicula phyllepta ASYA62	Naviculaceae	Naviculales	Bacillariophyceae	Bacillariophyta	Chromista
Amphora sp. ASYA63	Catenulaceae	Thalassiophysales	Bacillariophyceae	Bacillariophyta	Chromista

3.3. CRYOPRESERVATION

At the end of the cryopreservation process, samples are removed from the canister and examined under the microscope. As shown in the table below (Table 3.7), their morphology was not deteriorating.

Table 3.7 Microscopic Images of Microalgae 1st and 30th Day of Cryopreservation

Strain	Control	DMSO 1st day	DMSO 30th day	DMSO-Free 1st day	DMSO-Free 30t day
***Coccomyxa subellipsoidea* ASYA5**					
***Stichococcus bacillaris* ASYA11**					
***Nannochloris* sp. ASYA12**					
***Planophila* sp. ASYA14**					
***Klebsormidium* sp. ASYA20**					
***Koliellopsis inundata* ASYA21**					
***Xanthonema* sp. ASYA22**					

***Chlamydomonas* sp. ASYA25**					
***Chlorella vulgaris* ASYA27**					
***Chlorella* sp. ASYA31**					
***Micractinium simplicissimum* ASYA46**					
***Micractinium* sp. ASYA57**					

Upon thawing the cryopreserved microalgae, the growth of microalgae was incubated on a 120 rpm shaker at 15°C under a continuous light intensity of continuous light 50 µmol photon $s^{-1}m^2$ followed by absorbance measurement at 680 nm every 3 days. We used DMSO as a cryoprotectant that protects living cells from freezing damage. DMSO was introduced as a highly effective, rapidly penetrating, and universal cryoprotectant in cryobiology. DMSO was added to cell media before freezing. When DMSO (10%) was added to cell media, it increased the cellular membrane's porosity and allowed water to flow freely through the membrane (Ock & Rho, 2011). Originally, it was used to cryoprotect red blood cells (RBCs) and spermatozoa (Lovelock & Bishop, 1959). DMSO has been used to cryopreserve viruses (Rightsel & Greiff, 1967); bacteria (Gibson et al., 1966; Nash et al., 1963), cyanobacteria (Bodas et al., 1995; J. G. Day, 1998); fungi (Davis et al., 1966; KR & Schmitt, 1967); protozoa (Diamond et al., 1961, 1963); and algae (Daily & Higgens, 1973; J. Day & Brand, 2005; Morris, 1976).

Specific growth rate, doubling time, and the number of generations are shown in Table 3.8.

Table 3.8 Specific growth rate, doubling time, and generation number of species after cryopreservation

	DMSO-FREE						DMSO					
	10 days			30 days			10 days			30 days		
Species	**μ**	**Ω**	**☮**	**μ**	**Ω**	**☮**	**μ**	**Ω**	**☮**	**μ**	**Ω**	**☮**
Coccomyxa subellipsoidea ASYA5	0.088	7.895	2.282	0.078	8.837	1.957	0.075	9.282	2.100	0.119	5.819	2.686
Deuterostichococcus sp. ASYA7	0.135	5.138	2.977	0.149	4.661	2.694	0.077	8.994	2.243	0.116	5.986	2.352
Stichococcus bacillaris ASYA11	0.122	5.678	2.759	0.100	6.952	2.397	0.128	5.403	3.260	0.129	5.394	4.093
Nannochloris sp. ASYA12	0.123	5.657	2.539	0.112	6.161	0.847	0.070	9.877	2.051	0.085	8.150	1.781
Planophila sp. ASYA14	0.119	5.835	2.320	0.112	6.177	1.301	0.095	7.295	2.129	0.106	6.521	3.009
Klebsormidium sp. ASYA20	0.039	17.952	0.110	0.054	12.803	0.598	0.035	19.714	-0.230	0.054	12.839	0.426
Koliellopsis inundata ASYA21	0.076	9.130	0.991	0.072	9.654	1.220	0.080	8.659	1.296	0.102	6.811	1.222
Xanthonema sp. ASYA22	0.073	9.480	1.064	0.081	8.516	1.193	0.064	10.909	1.127	0.100	6.953	1.174
Chlamydomonas sp. ASYA25	0.183	3.790	3.252	0.125	5.544	2.880	0.134	5.190	3.033	0.162	4.272	3.075
Chlorella vulgaris ASYA27	0.112	6.183	2.979	0.094	7.407	2.047	0.150	4.626	2.481	0.128	5.407	2.633
Chlorella sp. ASYA31	0.163	4.252	3.667	0.109	6.346	3.174	0.117	5.949	1.878	0.105	6.620	2.245
Micractinium simplicissimum ASYA46	0.119	5.834	2.339	0.125	5.558	3.030	0.145	4.797	3.288	0.127	5.478	3.085
Micractinium sp. ASYA57	0.130	5.326	3.769	0.119	5.847	3.199	0.107	6.466	3.772	0.094	7.376	2.922

When the results were examined, the cryopreservation procedure was successful in both groups.

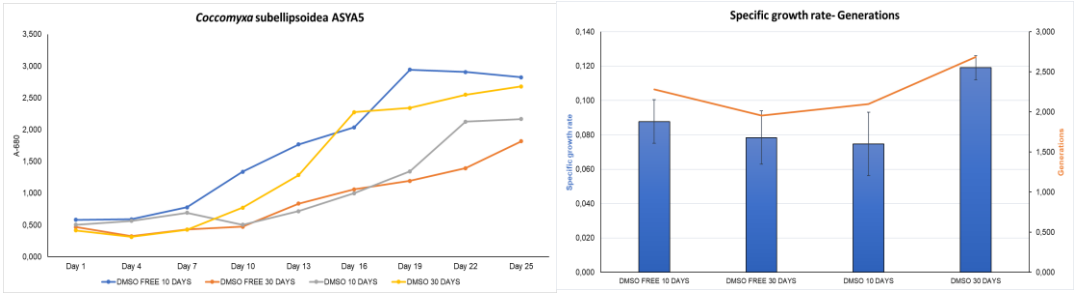


Figure 3.6 Growth analysis of *C. subellipsoidea* ASYA5. Growth curve (left) and Specific growth rate-number of generations (right).

Coccomyxa subellipsoidea ASYA5 entered the exponential growth phase 7 days after cryopreservation. The use of DMSO as a cryoprotectant increased the specific growth rate by approximately 50% on 30 days of cryopreservation, while it did not cause any change when the cryopreservation was on 10 days.

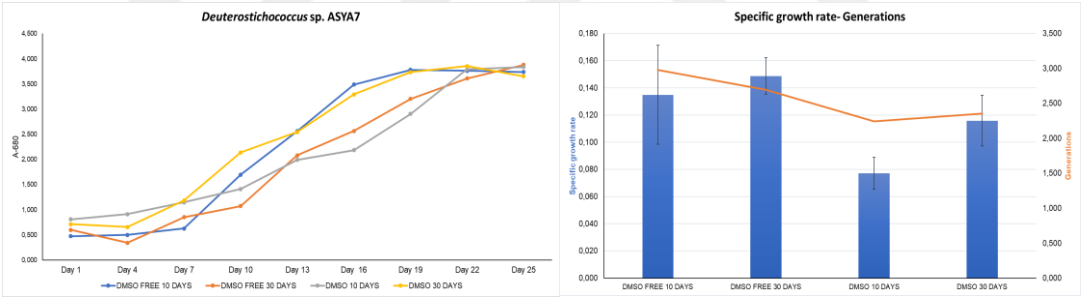


Figure 3.7 Growth analysis of *Deuterostichococcus* sp. ASYA7. Growth curve (left) and Specific growth rate-number of generations (right).

Deuterostichococcus sp. ASYA7 entered the exponential growth phase approximately 10 days after cryopreservation. The use of DMSO as a cryoprotectant was ineffective, and the specific growth rate decreased by around 25-45%.

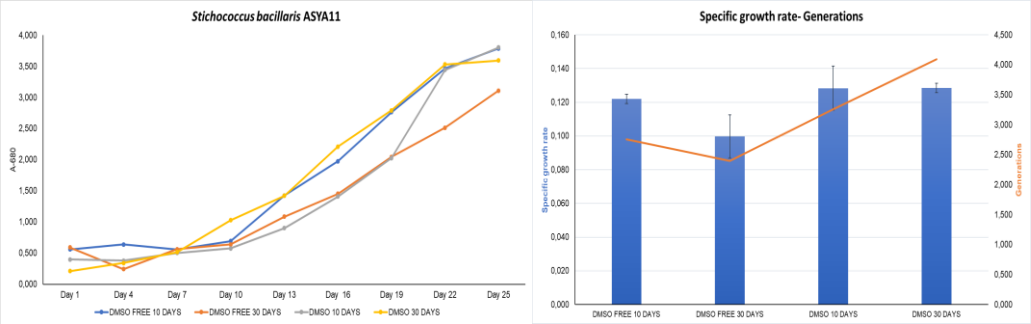


Figure 3.8 Growth analysis of *Stichococcus bacillaris* ASYA11. Growth curve (left) and Specific growth rate-number of generations (right).

Stichococcus bacillaris ASYA11 entered the exponential growth phase around 10 days after cryopreservation. DMSO application had no effect or caused slight increases in specific growth rate, while the number of generations was clearly higher in the DMSO group after 25 days of incubation over 30 days of cryopreservation.

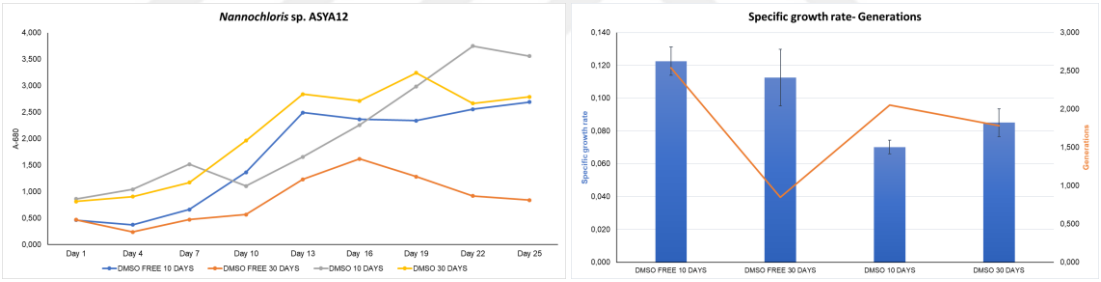


Figure 3.9 Growth analysis of *Nannochloris sp.* ASYA12. Growth curve (left) and Specific growth rate-number of generations (right).

Nannochloris sp. ASYA12 entered an exponential growth phase approximately 10 days after thawing. The use of DMSO as a cryoprotectant was ineffective, and the specific growth rate decreased by around 20-45%.

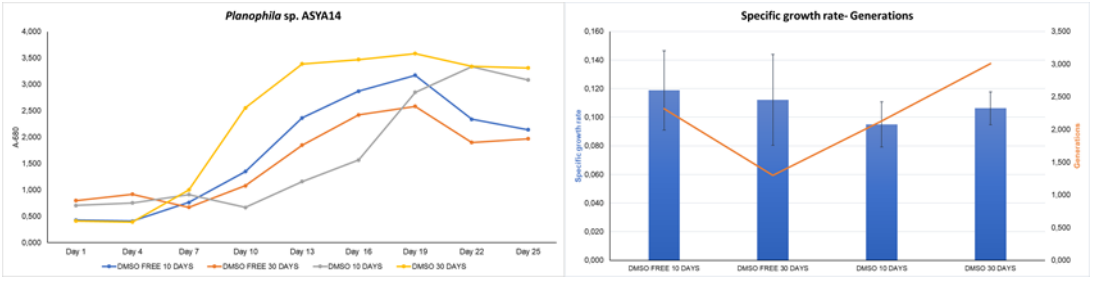


Figure 3.10 Growth analysis of *Planophila sp.* ASYA14. Growth curve (left) and Specific growth rate-number of generations (right).

Planophila sp. ASYA14 entered the exponential growth phase 7 days after thawing. DMSO application did not affect specific growth rates, while the number of generations was clearly higher in the DMSO group after 25 days of incubation on 30 days of cryopreservation.

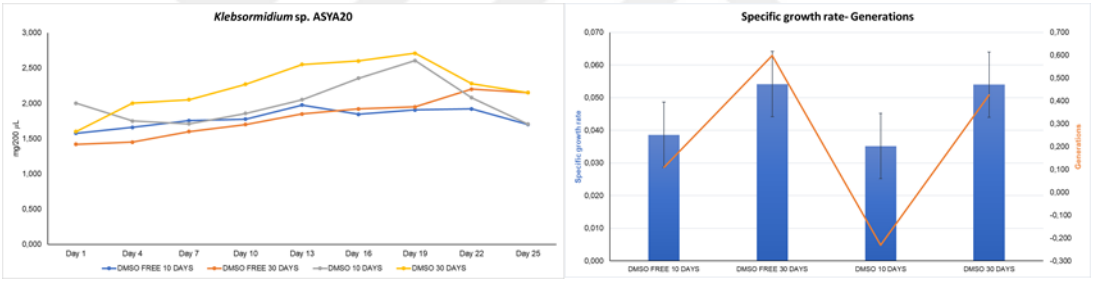


Figure 3.11 Growth analysis of *Klebsormidium sp.* ASYA20. Growth curve (left) and Specific growth rate-number of generations (right).

Klebsormidium sp. ASYA20 is a filamentous microalga; the fresh weight was followed to determine growth dynamics upon cryopreservation. It seems that the cells entered the stationary phase after 19 days of incubation, and cryoprotectant use was ineffective on cryopreservation.

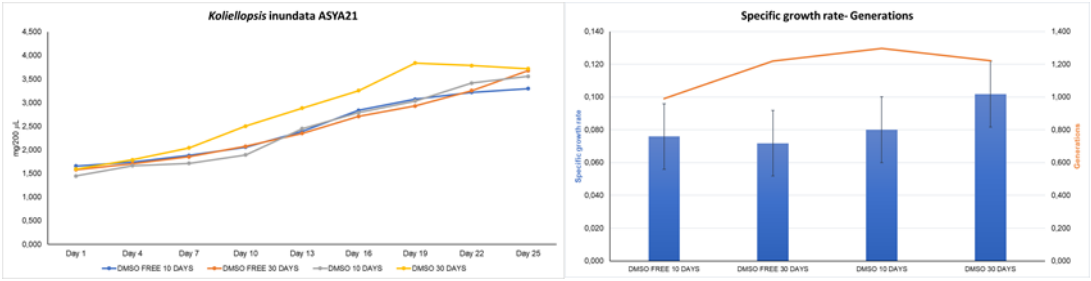


Figure 3.12 Growth analysis of *Koliellopsis inundata* ASYA21. Growth curve (left) and Specific growth rate-number of generations (right).

Koliellopsis inundata ASYA21 is a filamentous microalga; the fresh weight was followed to determine growth dynamics upon cryopreservation. The cells entered the exponential growth phase 7 days after thawing. The use of DMSO as a cryoprotectant was effective, and the specific growth rate increased by around 20-45%.

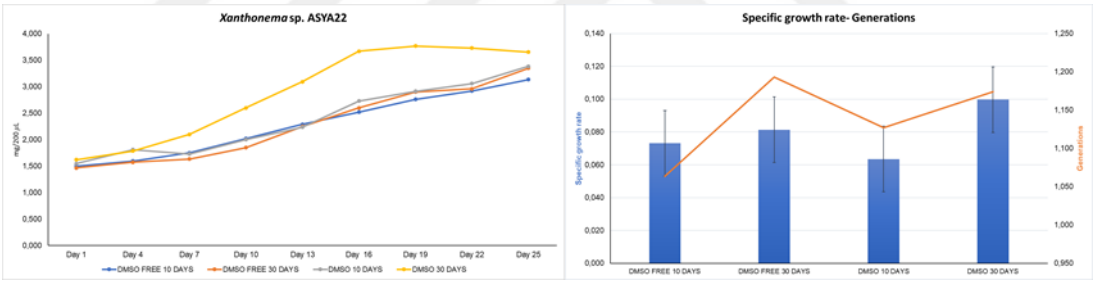


Figure 3.13 Growth analysis of *Xanthonema sp.* ASYA22 Growth curve (left) and Specific growth rate-number of generations (right).

Xanthonema sp. ASYA22 is a filamentous microalga; the fresh weight was followed to determine growth dynamics upon cryopreservation. The cells seemed to enter the exponential growth phase 7 days after cryopreservation. The use of DMSO as a cryoprotectant increased the specific growth rate by approximately 40% on 30 days of cryopreservation, while it did not cause any change when the cryopreservation was on 10 days.

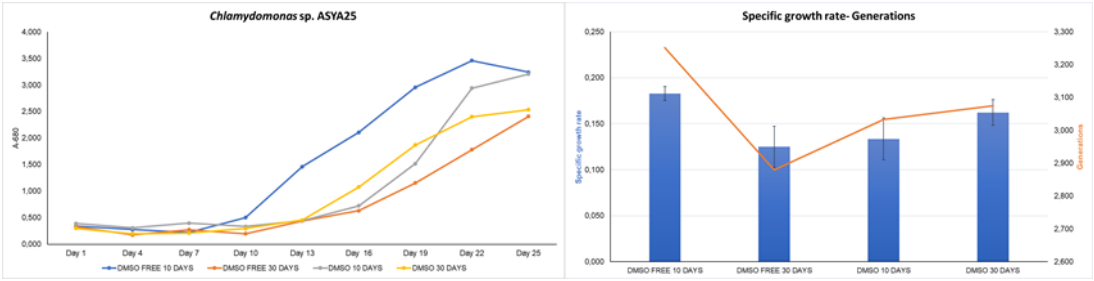


Figure 3.14 Growth analysis *Chlamydomonas sp.* ASYA25 of Growth curve (left) and Specific growth rate-number of generations (right).

Chlamydomonas sp. ASYA25 entered the exponential growth phase after 10 days after cryopreservation. The use of DMSO as a cryoprotectant decreased the specific growth rate on 10 days of cryopreservation, while it did not cause any change when the cryopreservation was on 30 days.

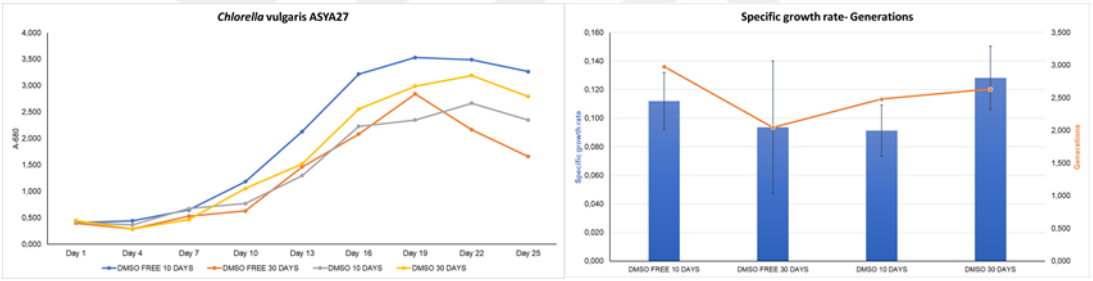


Figure 3.15 Growth analysis of *Chlorella vulgaris* ASYA27.Growth curve (left) and Specific growth rate-number of generations (right).

Chlorella vulgaris ASYA27 entered the exponential growth phase around 7 days after cryopreservation. The use of DMSO as a cryoprotectant increased the specific growth rate by approximately 45% on 30 days of cryopreservation, while it did not cause any change or slightly decreased when the cryopreservation was on 10 days.

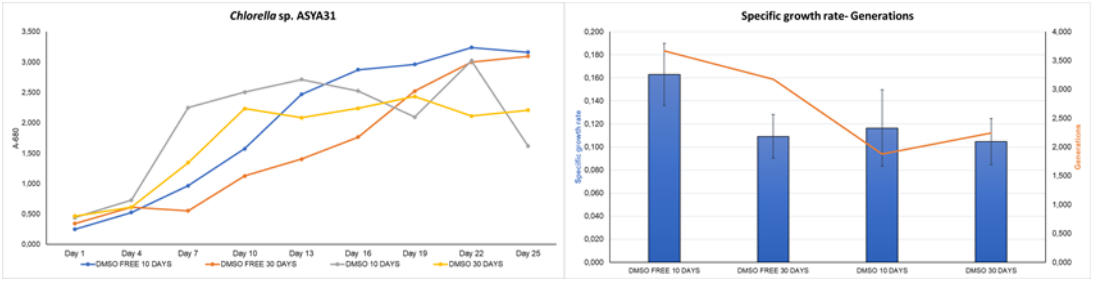


Figure 3.16 Growth analysis of *Chlorella sp.* ASYA31. Growth curve (left) and Specific growth rate-number of generations (right).

Chlorella sp. ASYA31 entered the exponential growth phase around 4 days after cryopreservation. The use of DMSO as a cryoprotectant decreased the specific growth rate on 10 days of cryopreservation, while it did not cause any change when the cryopreservation was on 30 days.

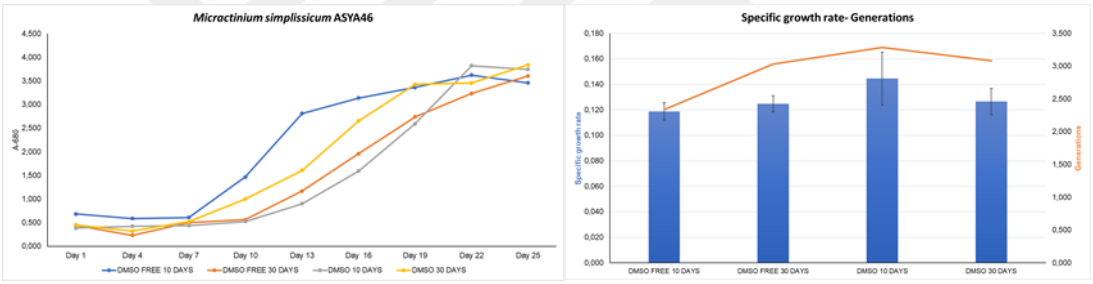


Figure 3.17 Growth analysis of *Micractinium sp.* ASYA46. Growth curve (left) and Specific growth rate-number of generations (right).

Micractinium sp. ASYA46 entered the exponential growth phase around 7 days after thawing. The use of DMSO as a cryoprotectant increased the specific growth rate by approximately 30% on 10 days of cryopreservation, while it did not cause any change when the cryopreservation was 30 days.

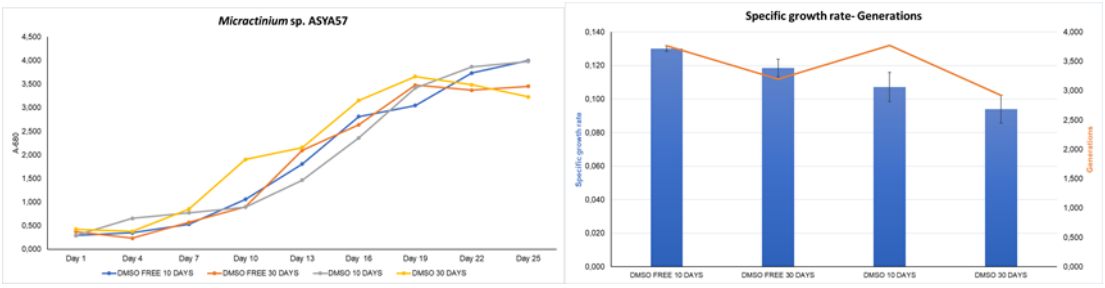


Figure 3.18 Growth analysis of *Micractinium sp.* ASYA57. Growth curve (left) and Specific growth rate-number of generations (right).

Micractinium sp. ASYA57 entered the exponential growth phase 7 days after thawing. The use of DMSO as a cryoprotectant was ineffective, and the specific growth rate decreased by around 15-25%.

IMU-ASYA Culture Collection

The IMU-ASYA collection holds 62 strains, which include eukaryotic microalgae and diatoms. All strains are maintained by subculturing under 50 µmol photon $s^{-1}m^2$ light intensity at 4 °C and 15 °C, 8:16 h light/dark photoperiod. The strains are serially transferred at 30-day to 3-month intervals. Along with subculturing, all microalgae in the IMU-ASYA culture collection have been sustained by cryopreservation (Figure 3.19).

Figure 3.19 IMU-ASYA culture collection

This collection is going to serve as a psychrotolerant microalgae repository for research groups working on the biotechnological evaluation of microalgae and industries that generate income through the production of commercial species.

CONCLUSION

The 11th Development Plan of the Republic of Turkey envisages an economic and social development process that creates more value for our country to increase its efficiency and effectiveness in every field and gain international competitiveness with the national technology move. According to Article 446 in the Development Plan, it is foreseen that the international position of our country will be strengthened in the field of polar research.

This study contributes to microalgae biodiversity information by providing a genomic and morphological evaluation of Antarctic microalgae. In this study, 26 different microalgae species and 62 isolates were cultured and purified from snow, ice, sediment, moss, fresh water, and seawater samples sampled from Dismal Island and Horseshoe Island during the IVth National Antarctic Science Expedition. The isolates were identified morphologically and phylogenetically according to the 18S rDNA gene region. A website where cultured microalgae are presented was published (www.imuasya.org).

Microalgae are primary food chain producers in all ecosystems, and Antarctica is no exception. In order to figure out the current ecophysiology of the food web in an ecosystem, the microalgae motif must be analyzed first. Our results show that green microalgae from *Trebouxiophyceae* and *Chlorophyceae* dominate the terrestrial habitats on the two islands.

Members of *Klebsormidiaceae* include some strains that are of evolutionary importance as they are the only group of terrestrial microalgae having a cutin-like waxy layer covering the cell wall. One of our isolates, *Klebsormidium* sp. ASYA19 from Horseshoe island possesses a cutin-like layer. Seeing this strain on an island in the Antarctic peninsula may refer to UV impact and global warming issues. The density and a year-round identification of the microalgal motif would be helpful to understanding ecological changes in the Antarctic peninsula.

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