REPUBLIC OF TURKEY

YILDIZ TECHNICAL UNIVERSITY

GRADUATE SCHOOL OF SCIENCE AND ENGINEERING

ISOLATION, IDENTIFICATION AND CULTIVATION OF ANTARCTIC MICROALGAE

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MASTER OF SCIENCE THESIS

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Bioengineering Program

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August, 2021

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Arzu ÇELİK

Signature

Dedicated to my family

ACKNOWLEDGEMENTS

First of all, I would like to especially thank Prof. Dr. Didem ÖZÇİMEN, who did not withhold their support from me during the postgraduate period and who always tried to help me, shared her knowledge and experience with me.

I would like to thank Assist. Prof. Dr. Yılmaz KAYA and Assoc. Prof. Dr. Hasan Murat AKSOY from Ondokuz Mayıs University, for providing the experimental environment required for microalgae genetic identification part and to shared their knowledge with me.

Afterward, I would like to thank my laboratuary friends, especially Benan İNAN, Anıl Tevfik KOÇER and Meyrem Vehapi, who tried to help me in all matters and do not hesitate to share their knowledge and experience with me at every stage of my thesis study. And also I thank to one of my friend Bekir Can ALTINDİŞOĞULLARI for his help and motivation in developing this thesis.

Finally, I would like to thank my family for supporting me spiritually and financially throughout writing this thesis and my life in general.

Arzu ÇELİK

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LIST OF SYMBOLS

A	Absorbance
α	Alpha
atm	Atmospheric pressure
β	Beta
cm	Centimeter
m^3	Cubic metre
°C	Degree Celsius
t_d	Doubling Time
g	Gram
μ	Growth Rate
km	Kilometer
L	Liter
m	Meter
μg	Microgram
μL	Microliter
μm	Micrometer
mg	Milligram
mL	Milliliter
mm	Millimeter
min	Minute
M	Molarity
nm	Nanometer
N	Normality
%	Percentage
rpm	Revolutions per minute
S	Second
w/w	Weight by weight

LIST OF ABBREVIATIONS

ATP	Adenosine Triphosphate
bp	Base pair
BLAST	Basic Local Alignment Search Tool
BG-11	Blue-Green Medium
BHT-500	Butylated hydroxytoluene-50
BHT-1000	Butylated hydroxytoluene-1000
DPPH	2,2-diphenyl-1-picrylhydrazyl
DNA	Deoxyribonucleic acid
DMS	Dimethyl Sulfide
DMSP	Dimethylsulfoniopropionate
DMSO	Dimethyl Sulfoxide
DHA	Docosahexaenoic Acid
EPA	Eicosapentaenoic Acid
FID	Flame Ionization Detector
FTIR	Fourier Transform Infrared Spectroscopy
GAE	Gallic Acid Equivalent
GR	Growth Rate
HCT	Human colon cancer cell line
IBP	Ice-binding protein
ITS-2	Internal Transcribed Spacer-2
LEA	Late Embryogenesis Abundant
mRNA	Messenger RNA
NCBI	National Center for Biotechnology Information
NA	Nutrient Agar
OD	Optical Density
PAR	Photosynthetic Active Radiation
PCR	Polymerase Chain Reaction
PUFA	Polyunsaturated Fatty Acids
PDA	Potato Dextrose Agar
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
spp.	Several species
SSU	Small Subunit
sp.	Species
UV	Ultraviolet
UVA	Ultraviolet A
UVB	Ultraviolet B
UVR	Ultraviolet Radiation
UV-Vis	Ultraviolet Visible

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ABSTRACT

Isolation, Identification and Cultivation of Antarctic Microalgae

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Department of Bioengineering

Master of Science Thesis

Supervisor: Prof. Dr. Didem ÖZÇİMEN

Antarctica is a continent which is surrounded with Southern Ocean in the South Pole. This continent is the coldest, driest, windiest region in the world. It is also one of the few places on earth that can show the most obvious extreme conditions. Therefore, it is observed that the ecosystem has a different and complex structure. The photosynthetic primary producers of the region are microalgae. Thanks to their strong adaption mechanisms, these microorganisms can continue to survive even in extreme conditions in Antarctica. During the adaptation process, they produce many valuable metabolites. Therefore, polar microalgae have different biochemical contents compared to microalgae in other regions. In this study, it was aimed to examine, identify and characterize the viability in water samples collected from the West Antarctic peninsula. For this purpose, isolation and identification studies of species were carried out on water samples which was collected in the 3rd National Antarctic Science Expedition from the coasts of Horseshoe Island Skua Lake.

As a result of 16S rRNA gene region sequence analysis of the sample isolation, *Chlorella variabilis* was determined. Results of the analysis were uploaded to NCBI GenBank as *Chlorella variabilis* YTU.ANTARCTIC.001 (GenBank Accession number: MN372092). After this, microalgae growth was performed in BG-11 medium at continuous illumination and two different temperatures, 4°C and 27°C, and characterization studies were carried out. As a result of the thesis study, the growth rates at 4°C and 27°C were determined as 0.02 and 0.18 day^{-1}, respectively. The highest lipid content was determined as 49% of the dry mass at 27°C growth condition. The highest protein content was observed at 4°C growth condition as 31% of dry mass. As a result of the antimicrobial analysis, it was seen that the new identified microalgae species has antioxidant, antibacterial and antifungal properties. In line with the findings, it can be reported that *Chlorella variabilis* YTU.ANTARCTIC.001, which has bioactive substances, can be used in food applications together with potential biotechnological applications in the field of cosmtic and health.

Keywords: Antarctica, Microalgae, *C. variabilis*, Isolation, Identification

ÖZET

Antarktik Mikroalglerinin İzolasyonu, Tanımlanması ve Kültivasyonu

Arzu ÇELİK

Biyomühendislik Anabilim Dalı

Yüksek Lisans Tezi

Danışman: Prof. Dr. Didem ÖZÇİMEN

Antarktika, Güney Okyanusu ile çevrili Güney kutbunda bulunan kıtadır. Bu kıta dünyadaki en soğuk, en kuru, en rüzgarlı bölgedir. Aynı zamanda yeryüzünde bulunan en belirgin ekstrem şartları gösterebilen sayılı yerlerinden biridir. Bu yüzden ekosisteminin farklı ve karmaşık bir yapıya sahip olduğu görülebilmektedir. Bölgenin fotosentetik birincil üreticileri mikroalglerdir. Bu mikroorganizmalar yüksek uyum mekanizmaları sayesinde Antarktika'daki ekstrem koşullarda bile canlılığı sürdürmeye devam edebilirler. Adaptsayon sürecinde bünyesinde değerli birçok metabolitler üretirler. Bu yüzden kutup mikroalgleri diğer bölgelerdeki mikroalglere kıyasla daha farklı biyokimyasal içeriğe sahiptirler. Bu çalışmada, Batı Antarktika yarımadasından toplanan su ve buz örneklerindeki canlılığın incelenmesi, tanımlanması ve karaterizasyonu hedeflenmiştir. Bu amaçla 3. Ulusal Antarktika Bilim Seferi ile Horseshoe Adası

Skua Gölü kıyılarından toplanan su örnekleri üzerinde izolasyon ve tür tanımlaması çalışmaları gerçekleştirilmiştir.

İzolasyon sonucu elde edilen örneğin 16S rRNA gen bölgesinin dizi analizi sonucunda *Chlorella variabilis* olduğu tespit edilmiştir. Analiz sonuçları NCBI Gen Bankası'na *Chlorella variabilis* YTU.ANTARCTIC.001 olarak yüklenerek literatüre kazandırılmıştır. Bu aşamanın ardından, mikroalg büyümesi BG-11 mediumunda sürekli aydınlatma ile 4°C ve 27 °C olmak üzere iki farklı sıcaklıkta yapılmış ve çeşitli karakterizasyon testleri gerçekleştirilmiştir. Yapılan tez çalışması sonucunda 4°C ve 27°C'de büyüme oranları sırasıyla 0.02 ve 0.18 gün^{-1} olarak belirlenmiştir. En yüksek lipid içeriği kuru kütlenin %49'u olarak 27°C büyüme koşulunda belirlenmiştir. En yüksek protein içeriği, kuru kütlenin %31'i olarak 4°C büyüme koşulunda gözlenmiştir. Yapılan analizler sonucunda tanımlanan yeni türün antioksidan, antibakteriyal ve antifungal özelliği olduğu görülmüştür. Elde edilen bulgular doğrultusunda, biyoaktif maddelere sahip olan *Chlorella variabilis* YTU.ANTARCTIC.001'in, sağlık ve kozmetik alanındaki potansiyel biyoteknolojik uygulamalar ile gıda uygulamalarında da kullanılabileceği düşünülmektedir.

Anahtar Kelime: Antarktika, Mikroalg, *C. variabilis*, İzolasyon, Tanımlama

1
INTRODUCTION

1.1 Literature Review

Today, algal biotechnology continues to develop rapidly and its importance is increasing gradually. Algae have been utilized in many different fields for decades because of the proteins, carbohydrates, fatty acids, vitamins, minerals, pigments and many other important metabolites which is accumulated in the cell. Utilization of antioxidants obtained from algae are important in terms of preventive treatment and protection from degenerative diseases. Many of these compounds (oleic acid, linoleic acid, vitamin E, β-carotene, lutein and zeaxanthin) have antimicrobial, antioxidant, antifungal, antiviral and anti-inflammatory properties and play an important role in reducing and preventing diseases (Karakaş, C.Y., 2017).

The accumulation of antioxidant substances such as β-carotene, zeaxanthin and lutein can be carried out in the microalgae cell when they grown under various stress conditions (nutrient stress, high light intensity, high salinity and low temperature) (Karakaş, C.Y., 2017).

By changing these parameters in microalgae culture cultivation, stress conditions are created on the cells, and with this method, the cultured cells can be provided to produce more of the desired product. It is known that even various types of algae grown in temperate conditions have antimicrobial, antioxidant and anticancer properties (Lyon & Mock, 2014; Stamenković et al., 2020; Vehapi et al., 2020).

1.2 Objective of the Thesis

In this thesis study; within the scope of the 3rd National Antarctic Science Expedition, organized with the local resources of Turkey, it is aimed to isolate

microalgae in water samples collected from the coastal of the West Antarctic Horseshoe Island Skua Lake, and to molecularly identify the isolate. After molecular identification, it is aimed to grow the species in different temperatures, to perform and interpret all necessary experiments for biochemical content analysis, and to determine the biotechnological application area that can be used according to the biochemical content of the obtained species.

1.3 Original Contribution

Extremophilic microalgae can survive even in extreme conditions such as the Antarctic pole, thanks to their superior adaptability. These microorganisms can be evaluated in many important areas such as health and pharmaceutical industries. The fact that the biomass source of microalgae obtained from water and ice samples brought to our country with the 3rd National Antarctic Science Expedition can be used to obtain many valuable products with the biorefinery approach shows that this source is very important both environmentally and economically. Studies on the diversity of microorganisms in the poles under extreme conditions are of great importance for Turkey to have the right to comment on this field. In line with its vision of being among the leading countries with its original scientific studies on polar sciences in the world, it is of great importance that our country be included in Antarctic studies. With this thesis, it is aimed to contribute to literature, and future studies in Turkey, in the polar research and application areas.

2
GENERAL INFORMATION

2.1 General Characteristics of Antarctica

Antarctica, which is located at the heart of the South Pole, is the most isolated continent surrounded by the Southern Ocean (Figure 2.1) (Aronson et al., 2011). This continent is also known as the coldest, driest, windiest, highest (on average), darkest (half of the year) and icy continent on Earth. In many places, water is only found in the form of ice. The thickness of the ice is more than 2700 m. The Antarctic ice sheet covers 99 % of the Antarctic continent and stores 70% of the fresh water found on Earth in about 30 million m^3 of ice (Correction Convey et al., 2009a). For this reason, Antarctica is sometimes called the "frozen" continent (Walton, 2013). This continent also experiences 24 hours of continuous daylight in summer and 24 hours of continuous darkness in winter (Correction Convey et al., 2009a). Temperatures as low as –89.2°C and wind speeds of 248 km per hour, sudden temperature drops of up to 36°C in 12 minutes have been recorded by scientists. Because of these challenging conditions, Antarctica is the most challenging place in the world where continuous logistics support has ever been attempted (Augustine, 2012).

The geographic isolation of Antarctica and all its harsh conditions result in low levels of terrestrial biodiversity in this continent (Engelbertz, 2015). For this reason, the continent, which does not allow the formation of a natural human habitat, does not have any indigenous human population (Chapin et al., 2005). The continent is predominantly inhabited by large populations of seabirds (penguins, shearwaters, gulls, terns, skuas) and seals. Additionally, few native plant and insect species can be found on the continent. Surrounding the Southern Ocean, it forms one of the most productive marine ecosystems in the world (Engelbertz, 2015).

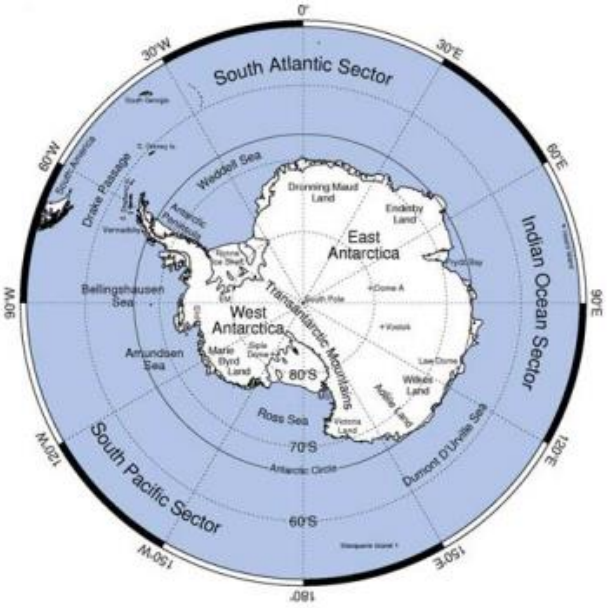


Figure 2. 1 Overview map of Antarctica (Correction Convey et al., 2009b)

2.2 Microorganism Biodiversity in Antarctica

The Antarctic continent, has one of the harshest environments on earth for life with its extreme conditions. Extremely low temperatures, low levels of liquid water resources, low atmospheric humidity, long dark and light cycles and high radiation periods from the sun cause the continent to be generally unsuitable for living populations throughout the continent (Cowan & Tow, 2004). However, the continent has also created some different areas within itself. From Antarctic deserts to lush grasslands and eutrophic lakes, different ecosystems have been formed, including the ice itself, freshwater, saltwater lakes and ice caps on the continent (Kırkıncı et al., 2021). Of these ecosystems, the ice-free zone, which constitutes approximately 0.18% of the surface area, is the main habitat of the majority of organisms on the continent (Burton-Johnson et al., 2016). Despite having to share such a narrow area and these harsh conditions, the Antarctic continent has a wide range of biodiversity.

A variety of animals live on the Antarctic continent, especially on the coastlines and open seas. In this environment, where many single-celled and multi-celled

organisms live, rich microbial life can take place in regions where suitable niches are provided (Cowan & Tow, 2004). Along with these, there are also advanced eukaryotic organisms on the continent. There are 35 penguins, 12 whales, 200 fish and dozens of bird species across the continent (Kemal, 2003). This continent is not as productive in terms of plant population as animal population, and it is home to only 2 plant species that can bloom (Kırkıncı et al., 2021). For this reason, the animals living here have adapted to the carnivorous feeding system in order to survive. Cyanobacteria and eukaryotic algae are the primary producers in the ecosystem (Hirose et al., 2020). Then come the krill and fish that feed on these creatures and feed the polar flora. Krill are the main food source of whales, the world's largest creatures. In the future, it is thought that it can be used to meet the food needs of food deficient countries (Kemal, 2003).

While some 400 plant species live in the Arctic region, there are only 2 plant species blooming in the South pole, Antarctica (Amosova et al., 2015); *Deschampsia antarctica Desv.* (Antarctic hairgrass) and *Colobanthus quitensis*. *Deschampsia antarctica Desv*. is the monocot of two flowering plants found in Antarctica (Amosova et al., 2015). It produces antifreeze proteins during the growth period to avoid freezing despite the extreme conditions it lives in (Romero et al., 1999). *Colobanthus quitensis* is a dicotyledon plant from the clove family that grows naturally in Antarctica (Cuba-Díaz et al., 2017). It developed a similar method for cold resistance. Especially cold resistance of these two plants is widely studied and their commercial applications are considered. Apart from these 2 plant species throughout the continent, there are also some sub-plant forms that can survive in these extreme conditions. Seaweeds, liverworts, lichens and fungi are among the sub-plant species living here (*Plants*, n.d.).

2.3 Antarctic Microalgae

In the limited terrestrial ecosystems of Antarctica, all photosynthetic organisms make a significant contribution to the ecology of their habitats. The ice-free ground makes up only 0.18% of the Antarctic continent (Gray et al., 2020).

However, photosynthetic life is not limited to this area. Green and red colors are seen in certain regions due to algae blooms in coastal snowy regions. Snow algae in Antarctica were first described by discoveries in the 1950s and 1960s, and various types of algae found in Antarctica have been studied ever since (Gray et al., 2020). Considering that a single snow algae explosion can cover thousands of square meters, snow algae is potentially region's one of the most important photosynthetic primary producers (Gray et al., 2020).

Color change occurs in snow or ice when the concentration of algal cells reaches a population in thousands of cells mL^{-1}. Its color and intensity are determined by the pigment composition and population density. Green snow appears when chlorophyll dominates, while golden-brown snow appears if primary carotenoids such as fucoxanthin dominate. Snow and glacier algae are examples of how life can adapt to extreme environment conditions such as limited sun radiation, cold temperatures, and food deficiency. Carotenoids and phenols (astaxanthin in snow algae and purpurogallin in glacial algae) protect the photosynthetic mechanism by absorbing visible wavelengths that dissipate excess radiant energy as heat. This heat is thought to melt the proximal ice crystals providing liquid water at 0°C and release nutrients bound to the frozen water (Hoham & Remias, 2020).

Diatoms are the most abundant microalgal taxa in Arctic and Antarctic sea ice (*Bacillariophyceae*). More than 550 species of diatoms have been described in the Arctic alone (Ilyash & Zhitina, 2009). In the Arctic, *Fragilaria*, *Cylindrotheca* and *Achnanthes* are relatively common unicellular diatom genera, while in Antarctica the presence of species *Amphiprora, Pinnularia*, *Pleurosigma*, *Synedra* and *Tropidoneis* has been reported (Arrigo, 2014). Algae are common in first-year ice, bottom ice, interstitial layers in the interior of sea ice, and ice voids. Microalgal blooms in sea ice are typically short-lived. Algal biomass is usually low in winter due to insufficient light, low temperature and high sea ice salt salinity. Algal concentrations rise rapidly in the spring as light and temperature rise while salinity falls. When the ice is nearly isothermal and increasing brine volume allows for increased nutrient exchange, the algal bloom usually peaks in late spring or early summer, just before ice melt and subsequent breakup. A secondary ice algae

bloom may develop in the fall as ice begins to form again. However, these blooms are usually short-lived and accumulate little biomass before environmental factors become too harsh to support algae growth (Arrigo, 2014).

The presence of ice algae in Antarctic seawater and sea ice was first reported by Bunt and Wood (Bunt & Wood, 1963). Since this date, various researchers have conducted and continue to carry out studies investigating the diversity, physiological adaptations and ecology of algae. Ice algae are the main contributors to primary productivity in sea ice ecosystems associated with their ability to grow rapidly despite low temperature conditions. There is no doubt that low temperatures are a serious threat to living beings. For example, cell membranes become more rigid as temperature drops, thus limiting their ability to take in nutrients and release byproducts of cellular metabolism. In addition, cell integrity and functioning can be severely compromised by the formation of water crystals that can damage cell membranes. In addition, the diffusion of solutes is also markedly limited by the increase in water viscosity (Yarzábal, 2016). Regarding cold adaptation, polar algae have a number of metabolic strategies including: a) maintaining the fluidity of biological membranes by having unsaturated fatty acids that prevent membrane lipids from solidifying; b) molecular adaptations of enzymes to maintain adequate rates of enzyme-catalyzed reactions of key metabolic processes; c) development of cold shock and antifreeze proteins; and d) adaptations for the photosynthetic electron transport chain to function at cold temperatures (Gómez et al., 2009). Most of the microalgae isolated from the polar regions are psychrophilic, able to grow at 0°C or lower. Ice algae can also reproduce and photosynthesize in light-limited environments, especially in snow-covered areas with only less than 1% photosynthetically active radiation (PAR) transmittance. This photosynthetic adaptation under both low temperature and light conditions requires rapid upregulation of photosynthetic pigments as well as composition and structure change of thylakoid membranes (Staehelin, 2003).

Algae contain a wide variety of valuable chemicals with bioactivity such as polysaccharides (alginate, carrageenan, agar, fucoidan and laminarin), polyunsaturated fatty acids, sterols, proteins and peptides, chlorotannins and

pigments (fucoxanthin, astaxanthin, beta-carotene, chlorophylls) (Dominguez, 2003). They can be used in food, animal feed and energy fields thanks to their high lipid, protein and carbohydrate content. In addition to these ingredients, these microalgae synthesize many valuable metabolites such as fucoxanthin, astaxanthin, beta-carotene, chlorophylls, polyunsaturated fatty acids, sterols and peptides inside and outside the cell in order to adapt to the conditions of their environment. These intracellular and extracellular metabolites show antibiotic, antiviral, anticancer, antifungal, antibacterial, anti-inflammatory and hypocholestrolemic properties. For this reason, microalgae can also be used in the fields of medicine, cosmetics and food additives (De Morais et al., 2015).

Microalgae can synthesize many important substances used in pharmaceutical and nutraceutical fields in order to adapt to environmental conditions and protect themselves, many of them which are very difficult to synthesize chemically (Catarina Guedes et al., 2011). When the studies in the literature are examined, it has been seen that microalgae synthesize antimicrobial components (Ghasemi et al., 2007; Vehapi et al., 2019), neuroprotective products (Jha et al., 2017), cosmetological components (Priyadarshani & Rath, 2012) and drug candidates (Kharkwal et al., 2012) used in the pharmaceutical industry. Microalgae also synthesize bioactive nutraceutical components such as carotenoids (Kleinegris et al., 2011; Mendes et al., 1995), anticancer agents (Sadovskaya et al., 2014), anti-inflammatory agents (Robertson et al., 2015) and antioxidant substances (El-Baky et al., 2010). *Chlorella* sp., *Chlamydomonas* sp., *Scenedesmus* sp., *Spirulina* sp. and *Microcystis* sp. type microalgae are one of the most known and used bioactive substances producer microorganisms (Das & Pradhan, 2010).

Secondary metabolites synthesized by Antarctic microalgae can be the basis for the development of important therapeutic agents to treat chronic non-communicable diseases such as cancer, diabetes and cardiovascular diseases (Yarzábal, 2016). In this context, when the studies in the literature are examined, there are many studies examining the effect of cold-adapted microalgae on cancer. Suh et al. reported that *Micractinium* sp. and *C. reticulata* species microalgae

isolated from Antarctica showed anti-inflammatory and anticancer effects on RAW 264.7 macrophages and HCT116 human colon cancer cells (Suh et al., 2019).

PUFA is one of the most important nutraceutical agents synthesized by Antarctic microalgae. PUFAs play an important role in the treatment of obesity, Parkinson's, arthritis and heart disease. EPA and DHA, which are the most important derivatives of PUFAs, are effective in lowering blood cholesterol and fetal brain development, respectively (Jha et al., 2017). It has been proven by many studies that many microalgae isolated from Antarctica are quite rich in PUFA. These Antarctic microalgae are *Chlamydomonas* sp., *Navicula* sp., (Teoh et al., 2004, 2013), *Chlorella* sp. (Teoh et al., 2013), *Nannochloropsis* sp., *Phaeodactylum* sp and *Pseudopleurochlors* sp. (Schulze et al., 2019) can be given as an example.

Microalgae, which have high potential for utilizing various bioactive compounds, can be affected by environmental factors such as light, temperature and pH. By altering these parameters, the biochemical characteristics of algae can be changed (Zullaikah et al., 2019).

Factors Affecting the Growth of Microalgae

Microalgae growth rate is controlled by different factors such as temperature, carbon dioxide, salinity, pH, light intensity and nutrient availability (N, P, etc.).

Light

Light is an important factor for the growth of microalgae. Microalgae use light for photosynthesis, but light energy cannot be stored by microorganisms, so light must be supplied constantly. The most efficient light for photosynthesis is wavelengths which is between 600-700 nm (Anderson & Kommareddy, 2003). Each microalgae species has an optimal light requirement, and when this is exceeded, the growth slows down. Above a certain value, light intensity decreases the biomass growth rate. This is also known as “photo-inhibition” and occurs when microalgae are inhibited at light intensities higher than their specific growth rate peaks (Bitog et al., 2011; Özcimen et al., 2012). Avoiding photo-inhibition can help increase the daily growth rate of microalgae biomass (Chisti, 2007).

Carbon dioxide

Carbon dioxide is the carbon source that microalgae photosynthetic culture needs to use during photosynthesis. As carbon is depleted, oxygen is produced from the photolysis of water and released into the culture broth. Since algae live in high carbon dioxide concentrations, greenhouse gases from different sources, nitrogen dioxide (NO_2) and pollutants in the atmosphere become a food source for algae (İnan, 2019). For this purpose, algae production facilities can be fed with exhaust gases from the combustion of fossil fuels in order to make the process economical. The amount of CO_2 required for growth varies according to the microalgae species and the type of photobioreactors. Some species can survive at very high CO_2 concentrations (Bitog et al., 2011; Özcimen et al., 2012).

Temperature

One of the factors affecting algal growth is temperature. Temperature is an environmental factor that indirectly affects the growth of microalgae (Huang et al., 2008). Algae need an optimal temperature for maximum growth. Optimal temperature for microalgae cultures is usually between 20-24°C, but this may vary according to the composition of the culture medium, species and culture strain. Temperature also determines the activity and reaction rates of the intracellular enzyme, which will limit the growth and distribution of microalgae (Tan et al., 2009). Most common cultured microalgae species can tolerate temperatures between 16-27°C. Temperatures below 16°C slow down growth, and temperatures above 35°C are fatal for the species (Bitog et al., 2011; Özcimen et al., 2012).

pH and Salinity

Another important factor affecting microalgae growth is the pH of the culture. Different species require different pH conditions. Availability of CO_2 at high pH can limit growth and photosynthesis of microalgae. The pH range for most cultured algal species is between 7 and 9. Appropriate pH can be achieved by chemical methods, aeration of the culture and gassing (Bitog et al., 2011).

Salinity is another parameter that affects the growth of microalgae. For example, marine microalgae can tolerate higher salinity than freshwater microalgae (Zhu et al., 2003). Studies have shown that microalgae have their own optimum growth salinity. When salinity is higher or lower than that value, it will be detrimental to algae growth rate. In case the salinity is higher than 6 g/L, the growth rate of microalgae will be inhibited (Liu et al., 2006).

Nutrient

Microalgae need certain proportions of elements according to their needs. The main substances that should be present in the medium of microalgae are nitrogen, phosphorus and carbon compounds (Gülyurt et al., 2016; Juneja et al., 2013; Özcimen et al., 2012). Nitrogen and phosphate are two important macronutrients required for the growth and metabolism of algal cells. Nitrogen is an essential element for the formation of proteins and nucleic acids. Phosphate, which is an integral part of basic molecules such as ATP, is an energy carrier in cells, and is a very important nutrient like nitrogen. Phosphate is also part of the backbone of DNA and RNA, macromolecules essential for all living cells. In natural environments, algae are nutrient-limited (Juneja et al., 2013). The restriction of these essential nutrients changes the metabolic pathway of the organism. For example, the low amount of nitrogen and phosphorus diverts lipid metabolism from membrane lipid synthesis to neutral lipid storage. This increases the total lipid content of algae (Q. Hu, 2004). In other words, changing the ratios of nitrogen and phosphorus-containing compounds in the media changes the biochemical content of microalgae (Moisander et al., 2002).

Researches on Antarctic microalgae has carried out until today are summarized as follows;

Guilherme et al. aimed to develop a voltammetric method with macroalgae in Antarctica for the determination of selenium. *Rhodophyceae* (*C. racovitzae*, *G. confluens*, *I. cordata*) and *Phaeophyceae* (*C. jacquinotii*, *A. utricularis*, *D. anceps*, *A. mirabilis*, *H. grandifolius*) macroalgae were used in the study. In this study, it is aimed to digest the algae by microwave. As a result of the study, it was stated

that Antarctic macroalgae are a promising source for selenium production and can be used in sectors such as food and health sectors (Guilherme et al., 2020).

Soto et al. investigated an innovative genetic sequencing technique which was used to examine the connection between the amount of microorganisms in the marine ecosystem and their functionality in the Fildes Peninsula of King George Island in Antarctica. As a result of the investigation, it was found that green algae such as *Trebouxiophyceae* and *Chlorophyceae* were dominant in the environment on King George Island (Soto et al., 2020).

Jo et al. examined microalgae which are isolated from King George Island. They determined the phylogenetic places of these microalgae by using molecular markers and morphological features. ITS-2 secondary structure prediction and small subunit (SSU) rRNA sequence analysis were performed to determine the phylogenetic position. As a result of the molecular, biochemical and morphological analysis, it was understood that the isolate belonged to the *Chlorella vulgaris* microalgae species. And it is named as *C. vulgaris* KNUA007 temporarily. With specific studies on this strain, it has been understood that the strain can live in the range of 5 to -30^{0}C and it is rich in polyunsaturated fatty acids (PUFAs). Thanks to these properties, it has been suggested that it may have the potential as an alternative to fish and/or vegetable oils as a source of omega-3 PUFAs and may attract attention in the pharmaceutical industry due to its temperature tolerance (Jo et al., 2020).

Caspermeyer et al. investigated the survival mechanism of the algae strains under the extreme cold of Antarctica. For this, a NJ-7 algae strain living in Antarctica and a UTEX259 algae strain living in a temperate region were examined. With this study, it was determined that while there were problems in metabolism of UTEX259 strain at temperatures below 4°C, but NJ-7 algae strain could live unaffected at temperatures down to -20°C thanks to its antifreeze capacity. They found that in the formation of this antifreeze capacity, NJ-7 in some proteins and LEA (Late embryogenesis abundant) proteins protected other proteins from cold stresses (Caspermeyer, 2020).

Hoffmann et al. investigated the bonds and formation processes between an ice alga *Fragillariopsis cylindrus* and microplastic spheres. As a result of the study, it was determined that the extracellular polymeric deposits produced by ice algae have an important function in the regulation of the surface binding structures of microplastics (L. Hoffmann et al., 2020).

Buda et al. examined the cryoconite sediments and holes on the Ecology Glacier (King George Island). The aim here is to identify microbial eukaryotes and prokaryotes in these regions. As a result of the study, it was understood that these holes were algae-dominated regions. A total of 17 algae and cyanogenic bacteria were detected in the sediments. Identified species; 7 Bacillariophyceae species, 4 Cyanobacteria species, 4 Chlorophyta species, and 2 unidentified cysts. The predominant cyanobacteria of these species are; Bacillariophyceae *Microcostaus* sp. and *Pseudanabaena frigida* (Buda et al., 2020).

Vila et al. isolated the *Flavobacterium* strain which is living in Antarctica and they investigated its zeaxanthin and total carotenoid production. They also evaluated the effect of temperatures on carotenoid production. With repeated experiments at different temperatures, it was observed that the most efficient production was at 25°C. They reported that the *Flavobacterium* strain is a promising strain for zeaxanthin production. In addition, as an output from the study, it was determined that peptone, yeast extract and NaCl were used as the basis for carotenoid production of *Flavobacterium* strain (Vila et al., 2020).

Kosugi et al. investigated the chlorophyll absorption band developed by *Prasiola crispa*, a green alga that can be found in large colonies in Antarctica. A long wavelength chlorophyll-α structure capable of absorbing up to 710 nm has been detected in the thallus of *P. crispa* isolated from the terrestrial regions of Antarctica. It has been suggested that long wavelength chlorophylls support photosynthetic production and contribute to the regional superiority of the algae they are found in. As a result of the study, it was explained that the long wavelength, red light absorbing chlorophylls of *Prasiola crispa* are of critical importance for the algae species adapted to Antarctica (Kosugi et al., 2020).

Suh et al. examined *Chloromonas reticulata*, an Antarctic freshwater microalga. The cytotoxic and anti-inflammatory effects of this microalgal on cancer cells were evaluated. They conducted trials on HCT116, a human colon cancer, and observed the dosage-dependent cytotoxic effects of microalgae and proved that they provided significant reductions in the proliferation of cancer cells (Suh et al., 2019).

Sheehan et al. studied Southern Ocean phytoplanktons and their share in DMSP and DMS production in high altitude regions. For that, 15 Antarctic phototrophic phytoplankton species were examined in this study. As a result of the study, cyanobacteria *Synechococcus* proved to be an important producer for DMSP mass (Sheehan & Petrou, 2020).

Wang et al. conducted research on the metabolic activities of an Antarctic strain of *Chlorella vulgaris* (green algae) coded as NJ-7. As a result of the study, it was found that these green algae changed themselves to perform their metabolic activities at 0 ºC and that some genes were revealed and selected with positive selection, increasing their freezing tolerance (Y. Wang et al., 2020).

Vance et al. examined the creatures living in the freezing point and below environments and investigated the structures of these polymers and the creatures that use ice-binding proteins (IBPs) to survive in these environments by eliminating the problem of liquid crystallization (Vance et al., 2019).

Amsler et al. proved that when gastropods in mesocosms are found intensively, they prevent contamination to microscopic algae, especially by diatoms. In addition, they stated that amphipods play an important role in preventing the contamination of macroalgae by diatoms (Amsler et al., 2019).

Hoffmann et al. investigated how the primary production of microphytobenthos, benthic microalgae, was affected by changes in shading patterns and general sedimentation. As a result of the research; it has been proven that there is a decrease in primary production when there is an increase in the variables (R. Hoffmann et al., 2019).

Zhang et al. conducted research on the cryptochrome gene (CiCRY -DASH1) found in the *Chlamydomonas* strain. The response of the gene, which was removed by mRNA analysis, under blue and ultraviolet light was examined and it was proved that the CiCRY -DASH1 gene is a photoreceptor gene (Zhang et al., 2020).

Lai et al. investigated the effects of acute ultraviolet radiation (UVR) stress on the photosynthetic responses of 4 *Chlorella* strains from temperate (18°C), tropical (25°C), Antarctic (4°C) and Artic (4°C) regions. The *Chlorella* strain from the tropical region in the study was more successful in adapting to UVA stress than the temperate and Arctic strains. In addition, UVB stress caused intense photosynthetic dysfunctions in all strains. After the experiments, it was determined that the Arctic and Antarctic strains showed 40% lower inhibition compared to the tropical strain (Lai et al., 2019).

Koppel et al. investigated the cellular accumulation of two Antarctic marine microalgae, *Phaeocystis antarctica* and *Cryothecomonas armigera*, for five metals (cadmium, copper, nickel, lead, and zinc). The aim of this research was to reveal the dangers and risks of cellular metal accumulations for secondary consumers in the Southern Ocean. As a result of the study, it was proven that microalgae can accumulate non-lethal amounts of environmental metals in their bodies and thus cause bioaccumulation of metals and toxic accumulations in plankton fed with microalgae (Koppel et al., 2019).

Králová investigated the role of fatty acids in the adaptation of Antarctic *Flavobacterium* spp. to cold environmental conditions. As result of the study, it was observed that this species has more types of fatty acids than the thermophilic and mesophilic species belonging to the genus it belongs to. In addition, it was observed that the cell membrane of this species mostly consists of branched and unsaturated fatty acids (Králová, 2017).

Caamano et al. investigated the isolation and molecular characterization of the *Thraustochytrium* strain isolated from the Antarctic Peninsula and its usability for the production of fatty acids. As a result of molecular characterization, it was determined that the isolated species is *Thraustochytrium kinnei*. It was

determined that this species has the most palmitic acid in terms of saturated fatty acids and it contains DHA and EPA as long chain unsaturated fatty acids (Caamaño et al., 2017).

Coad et al. investigated that the effect of CO_2 concentration on microalgae in the Antarctic region. As a result of the study, it was stated that the microalgae in this region have a strong tolerance level against high CO_2 concentrations and it was suggested that these microalgae may not be adversely affected by the predicted changes in CO_2 concentration in the next century (Coad et al., 2016).

Teoh et al. conducted a study in which they compared the responses of algae in similar taxa originating from Antarctica, temperate and tropical regions to heat stress. As a result of this comparison, it is aimed to find out how algae in different temperature regimes are affected and it will be affected by global warming. The observations in the studies were derived by monitoring the fatty acid profiles and lipid contents of microalgae. As a result of the study, it was found that as a result of global warming, microalgae in Antarctica will be badly affected. In this microalgae, the percentage of polyunsaturated fatty acids (PUFA) decreased as temperature increased. This microalgae has been analyzed as an effect that may reduce the nutritional value and have severe consequences on the aquatic food chain (Teoh et al., 2013).

Hu et al. isolated two *Chlorella* species of microalgae (NJ-7 and NJ-18) from Antarctica. These isolated microalgae were studied for their antifreeze abilities. It is aimed to determine how Antarctic *Chlorellas* have physiological characteristics to adapt to the harsh weather conditions in their habitats. As a result of the study, it was found that NJ-7 microalgae increased freezing tolerance by increasing the C18:3-fatty acid content and up-regulating two antifreeze protein genes. These physiological outcomes shed light on the development and evolution of the antifreeze mechanisms of photosynthetic microorganisms adapted to Antarctica (H. Hu et al., 2008).

Wong et al. measured and compared the responses of similar taxonomic algae to ultraviolet radiation (UVR) stress in tropical, temperate, and Antarctic regions. In

this way, they aimed to find out the effects of global warming and increasing UVR level on Antarctic microalgae. In studies, it has been observed that UVA applied at different levels does not create a disadvantage in the growth of microalgae even at the highest dose. However, in UVB applications, negative effects were observed in microalgae except Antarctic *Chlorella* microalgae and significant decreases were noted in the ratio of polyunsaturated fatty acids (Wong et al., 2007).

Teoh et al. investigated that the effect of temperature parameter on the growth, biochemical content and fatty acid profile of six different Antarctic microalgae species. The growth, biochemical composition and fatty acid profiles of six Antarctic microalgae cultured at different temperatures were compared between 4, 6, 9, 14, 20 and 30°C. As a result of the study, it was observed that the effect of temperature on the species was different and it was observed that most of the microalgae species studied at low temperatures showed adaptation to cold environmental conditions by increasing their protein content. Among the species, only *Navicula* UMACC 231 had an increased unsaturated fatty acid content at low temperatures (Teoh et al., 2004).

Estevez et al. examined that what can trigger oxidative stress (ascorbyl radical content / ascorbate content ratio in cells) in *Chlorella* sp cells. Study made by exposing some of the cells to UV-B radiation and observing their chlorophyll content and growth rate in the log and stationary phases. As a result of the study, it was found that the significant reductions in the amount of UV-B radiation and both lipid and water-soluble antioxidants caused the development of oxidative stress in *Chlorella* sp cells (Estevez et al., 2001).

Xu et al. searched that the modification of the fatty acid composition of microalgae from the Antarctic region. The amount of fatty acids in the study was modified by partially or completely changing the molecular osmotic pressure (sorbitol) and ionic osmotic pressure (Na^+, K^+, Cl^-) in the growth medium. The results showed that high salt concentrations are not suitable for the growth of these species and the osmotic pressure of the medium should be at a certain level (Xu et al., 1998).

Ryan et al. investigated the effect of UV radiation on the growth of Antarctic sea ice microalgae. In the study, it was reported that UV rays cause changes in the pigment content and pigment composition of microalgae and cause a decrease in the activities of some enzymes (Ryan et al., 1992).

3
MATERIALS AND METHODS

3.1 Isolation and Identification

In this study, experiments were carried out using water samples which were collected from the West Antarctic peninsula by Prof. Dr. Ersan Başar from Karadeniz Technical University, who went to the region within the scope of the 3rd National Antarctic Science Expedition. Samples taken from the coasts of Antarctic Horseshoe Island, Skua Lake were brought to the laboratory in 15 mL Falcon tubes (Figure 3.1). The location and coordinate of the water samples taken from Antarctica are shown in Table 3.1. The isolation of microalgae was carried out in Algal Biotechnology and Bioprocess Laboratory in Yıldız Technical University-Bioengineering Department.

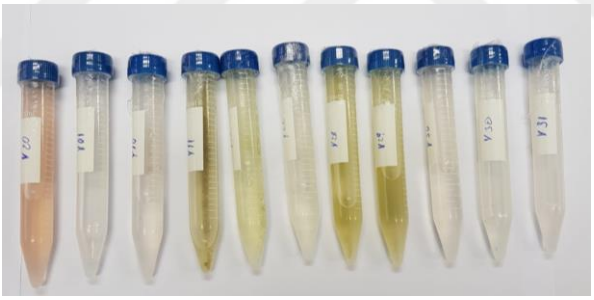


Figure 3. 1 Samples from Antarctica

Table 3. 1 Location and coordinate of water sample from Antarctica

Sample No	Region	Date	Coordinate		Sample Type
Y29	Horseshoe Island, Skua Lake	February 22, 2019 Friday	67º 48' 43,14" S	67º 18' 11,63" W	Green algae from the lake side of the glacier

In order to determine whether the microalgae is freshwater or marine microalgae, f/2 and BG-11 mediums were used to cultivate the microalgae from collected

samples in an incubator. It was seen that, microalgae were started to grow in the BG-11 medium which showed that the species were freshwater microalgae. After that, incubation was conducted in petri dishes on solid agar using BG-11 medium.

Molecular species identification was carried out from microalgae grown in petri dishes. DNA isolation process was performed and then samples were prepared for sequencing.

For the molecular identification of the species isolated from the Antarctic region, the relevant gene region was amplified by colony PCR method. To amplify the target region to be sequenced, the 16S rRNA gene region was targeted and the 27F/1492R 16S rRNA gene region primers given in Table 3.2 were used for this purpose.

Table 3. 2 Primers used in colony PCR

Primer Name	**Sequence**
27F	AGAGTTTGATCCTGGCTCAG
1492R	GGTTACCTTGTTACGACTT

The PCR reaction for colony PCR was set up with the reagents as indicated in Table 3.3 in a reaction volume of 100 μL. A sample taken from Antarctic microalgae grown on solid agar medium with the help of a pipette tip was used as a template DNA.

Table 3. 3 PCR reaction

Content	**Amount**
BioMix Red Kit	50 µL
Forward Primer	5 µL
Reverse Primer	5 µL
Distilled Water	40 µL
Template DNA	Up to the pipette tip

Colony PCR was performed by placing the prepared PCR mixture in the BioRad T100 thermal cycler under the thermal cycling conditions specified in Table 3.4. PCR products were loaded onto the electrophoresis gel, respectively. BIOLINE HyperLadder 100 bp DNA Ladder was used for detection of DNA band in gel electrophoresis.

Table 3. 4 Thermal cycling conditions

Status	**Cycle**	**Temperature**	**Time**
Initial Denaturation	1	95 º C	240 s
Denaturation	9	95 º C	30s
Annealing		65 º C	30 s
Elongation		72 º C	90 s
Denaturation	24	95 º C	30 s
Annealing		56 º C	30 s
Elongation		72 º C	90 s
Cooldown Unlimited	1	16 º C	∞

Colony PCR products were run on the electrophoresis gel and the corresponding band region retrieved from the electrophoresis gel. Using PCR, the SSU regions amplified from the isolated DNA and the PCR products sent to a company for sequencing. Sequencing results had been edited with the CHROMASPRO program. The results were analyzed via BLAST on the NCBI website (https://blast.ncbi.nlm.nih.gov/). After the results obtained from molecular identification, various biochemical tests were performed to support molecular identification.

3.2 Microalgae Cultivation

Isolated and identified Antarctic microalgae were cultivated using BG-11 medium in an incubator under continuous illumination. In order to compare their response to the different temperatures, culture were grown at the temperatures of 4°C and 27°C. To prepare BG-11 medium, the compounds in the Tables 3.5 and 3.6 were added to 829 ml of distilled water, and the pH value was optimized to 7.1 with 1 M NaOH and HCl. For solid media, 15 g/L agar was added (Sukatar, 2002). The prepared media were sterilized by autoclaving at 1 atm 121°C for 15 minutes, then poured into petri dishes and left for cooling before cultivation. Microalgae in petri dishes were left to grow within 15-20 days. In this study, both solid and liquid media were used. For liquid culture, the same procedure were applied without using agar, and the cultures were grown in flasks in shaking incubator.

Table 3. 5 Composition of BG-11 medium stock solution

Volume (mL)*	Stock Solution	Concentration (g/L)
100	$NaNO_3$	15.0
10	K_2HPO_4	4.0
10	$MgSO_4.7H_2O$	7.5
10	$CaCl_2.2H_2O$	3.6
10	Citric Acid	0.6
10	Ferric Ammonium Citrate	0.6
10	Na_2EDTA	0.1
10	Na_2CO_3	2.0
1	Trace Metal Solution*	Prepared as described the Table 3.6.

* The amount of compounds added to 829 mL of distilled water (mL)

Table 3. 6 The composition of trace metal solution for BG-11 medium

Compound	Amount (g)
H_3BO_3	2.86
$MnCl_2.4H_2O$	1.81
$ZnSO_4.7H_20$	0.222
$Na_2MoO_4.2H2O$	0.39
$CuSO_4.5H_2O$	0.079
$Co(NO_3).6H_2O$	0.0494

Algal growth was recorded daily, as samples were taken from each condition in each day and algal growth was monitored by optical density measurement at 680 nm using the PG Instruments P60 Model UV-Vis Spectrophotometer. The growth rate and doubling time were calculated from (3.1) and (3.2) equations:

$$GR = (\ln OD_t - \ln OD_0)/t - t_0 \quad (3.1)$$

$$t_d = \ln 2 / \mu \quad (3.2)$$

where OD_0 is the optical density at the initial day, OD_t is the optical density measured on day t, μ is the growth rate (L. Wang et al., 2010).

Microalgae grown in the shaking incubator were harvested by centrifugation at 5000 rpm for 10 minutes. Centrifuged microalgae were dried in an oven at 60°C for 18 hours.

3.3 Characterization of Microalgae

3.3.1 Carbohydrate Content

The phenol-sulfuric acid technique was used to evaluate the total carbohydrate content of microalgae. In this method, the phenol reagent was prepared by adding 80 g of phenol to 20 g of distilled water, at 80% (w/w). As a standard for analysis,

glucose was utilized. In order to create the standard curve, 0.05 mL of phenol reagent was added to the glucose solutions prepared in different concentrations in the tubes, and then 5 mL of H_2SO_4 was added. After the tubes were kept at room temperature for 15 minutes, the absorbance of the samples were measured at 490 nm in UV spectrophotometer. The total amount of carbohydrate in the microalgae was calculated by plotting a standard curve graph with the data obtained (Dubois et al., 1951).

3.3.2 Protein Content

Standards in the range of 70-140 mg protein/L were obtained when the amount of standard BSA solution specified in the experiment was placed in glass test tubes and added to distilled water and 1 N NaOH solution. 1 N NaOH was added to the samples taken from the cultures and kept in a 100°C water bath for 5 minutes, then the tubes were cooled by taking them into a cold water bath. Reagents C and D were added and mixed by vortexing quickly, after 30 minutes of incubation at room temperature, absorbances at 750 nm were read with a spectrophotometer (Lowry et al., 1951). The amounts of standards and reagents used are given in Table 3.7.

Reagents used in protein analysis:

- Reagent A: 5% Na_2CO_3
- Reagent B: 0.5% $CuSO_4.5H_2O$ in 1% K-Na-tartarate (always freshly prepared, reagents are dissolved separately)
- Reagent C: 50 mL Reagent A + 2 mL Reagent B
- Reagent D: 1 N Folin-Ciocalteau (prepared by diluting 2 N Folin reagent 1:1 with distilled water)

The PG Instruments T60 model UV spectrophotometer used in the experiments is shown in Figure 3.2.



Figure 3. 2 UV-Visible spectrophotometer

Table 3. 7 Standards and reagents used in the Modified Lowry Method

(Unit: mL)	**Blank**	**St1**	**St2**	**St3**	**St4**	**St5**	**St6**	**Sample**
Standard BSA solution	0	0.05	0.1	0.15	0.2	0.25	0.3	-
Distilled water	0.5	0.45	0.4	0.35	0.3	0.25	0.2	-
1 N NaOH	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Sample	-	-	-	-	-	-	-	0.5
Reagent C	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Reagent D	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5

3.3.3 Lipid Content

Microalgae samples were ground into powder and extracted using ethanol as a solvent. For this, the samples were extracted with 100% ethanol solvent for 3 hours. The obtained extract was removed by evaporation at 78°C under vacuum in the evaporator to evaporate the ethanol. The amount of oil was determined by weighing. The extract was stored in a closed and dark environment against any possibility of oxidation.

Gas chromatography was used to determine the content of microalgae oil. Analyzes were performed on YL Instruments 6100 GC brand gas chromatography device. The gas chromatography device includes a flame ionization detector (FID)

and a 30 m x 0.32 mm x 0.25 μm ZB-FFAP column. The column temperature program starts at 50°C and rises to 175°C at a rate of 15°C/min, and then to 230°C at a rate of 5°C/min. The temperature of the injector was set to 230°C, and the flow rate was set at 1.8 mL/min. The detector temperature was held constant at 280°C. As a carrier gas, hydrogen gas was utilized. Methyl heptadeconate (C17:0) was used as internal standard and samples were prepared for gas chromatography by mixing with methyl heptadeconate and n-heptane (Bligh & Dyer, 1959).

3.3.4 Chlorophyll-α and β-Carotene Contents

The following procedure was applied to determine the amounts of β-carotene and chlorophyll-α, which are important bioactives.

5 mL sample was taken into tubes and centrifuged, then excess water was poured out. After being treated with 5 mL of acetone, it was placed in an ultrasonic bath and homogenized for 5 minutes. The obtained sample was kept in water at 50-60°C for 5 minutes and then mixed for 15 seconds with vortex. The resulting extract was separated again by centrifugation. The liquid remaining on the samples coming out of the centrifuge was read at 666 nm and 475 nm in the spectrophotometer, and the amount of chlorophyll-α and β-carotene was determined with (3.3) and (3.4) the formulas given below (Macías-Sánchez et al., 2005; Zou & Richmond, 2000).

$$Chlorophyll\ \alpha\ (\mu g/ml) = 13{,}9 \times A_{666} \qquad (3.\ 3)$$

A_{666}= absorbance reading at 666 nm

$$\text{Carotene}\ (\mu g/ml) = 4{,}5 \times A_{475} \qquad (3.\ 4)$$

A_{475}= absorbance reading at 475 nm

3.3.5 FTIR Analysis

Functional groups of microalgae were determined using Fourier-transformed infrared (FT-IR) spectroscopy (Figure 3.3). Bruker brand Alpha series FTIR

spectrometer was used to determine functional groups and measurements were carried out in the wave number range of 4000-600 cm^{-1}.



Figure 3. 3 FTIR device

3.3.6 Total Phenolic Content

The Folin-Ciocalteu method was used to determine the total phenolic content of microalgae. In the analysis, 200 μL of the extract was mixed with 1 mL of Folin-Ciocalteu reagent in test tubes and then 800 μL (75 g/L) of sodium carbonate was added. After 30 minutes of incubation at room temperature in the dark, the absorbance at 765 nm was measured using a UV spectrophotometer (Li et al., 2007).

3.3.7 Antioxidant Activity

Total antioxidant activity of algal extracts was determined by using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) free radical scavenging method. The method is based on the ability of the extracts to donate a proton or electron, to lighten the purple colored DPPH solution. The decrease in absorbance in the reaction mixture is indicative of the high free radical scavenging activity (Brand-Williams et al., 1995).

DPPH radical scavenging activity was calculated with the help of the following formula (3.5):

$$\text{DPPH Scavenging Activity (\%)} = [(A_0 - A_1) / A_0] \text{ x } 100 \qquad (3.5)$$

where, A_0 represents absorbance value of the control and A_1 represents absorbance value of the sample or standard.

3.3.8 Antimicrobial Activity

For antimicrobial activity analysis, dried microalgae were extracted with DMSO at concentrations of 4%, 2%, 1% and 0.5% (40, 20, 10, 5 mg mL^{-1}) for 24 hours at 60°C. DMSO is considered as a solvent with low toxicity. To remove all non-extractable materials containing cellular material, Whatman no. 1 filter paper was used.

For antimicrobial activity of Antarctic microalgae, agar disk diffusion method was used. Certain pathogenic bacteria strains (*Escherichia coli* ATCC 43888, *Bacillus cereus* ATCC 11778 and *Salmonella typhimurium* ATCC 14028), pathogenic yeast strain (*Candida albicans* ATCC 10231) and, fungi strain (*Colletotrichum gloeosporioides* DSM 62136) were used to test antimicrobial activity. The agar disk diffusion method was applied to the in vitro susceptibility testing of pathogenic microorganisms. For bacterial strains application, a single colony was taken from the bacterial strains incubated at 37 °C for 1 night in Nutrient Agar (NA) medium. They were inoculated into 5 ml of Nutrient Broth, and cultures incubated at 37 °C for 18 hours were used to determine the antibacterial effect. Nutrient Agar (NA) was used to purify and multiply bacterial strains and to determine the antibacterial effect. An antimicrobial disc impregnated with the 20 µl volume of microalgae extract at different concentrations was placed on an agar plate, and the extract was diffused from the disc into the agar. The plate was inoculated with a standardized suspension of bacteria and incubated for 24 h at the temperature of 37°C (Vehapi, 2018). As for the fungal microorganisms, petri plates containing sterile Potato dextrose agar (PDA, 20 ml plate-1) were inoculated with 6 mm agar plugs from previous 7 days old fungi. Then, algae extracts were introduced onto the upper layer of the agar plate impregnated with 20 µL volume of algal extracts with a pipette onto antimicrobial discs. Plates were incubated for 6 days at 25±2ºC (Vehapi et al., 2019). DMSO was used to make negative controls. After the incubation period of bacteria with antimicrobial disk impregnated with algal extract, the growth inhibition zones around the discs were measured. For the determination of growth of fungi, zones were measured on the 3^{rd}, 4^{th}, 5^{th} and 6^{th} days. The diameter of the zone is depending on the antimicrobial

susceptibility of microorganisms (Table 3.8) (Behera et al., 2009; Waitz, 1990). Analysis of variance was performed using the JMP (release 6.0.0, SAS) package program. Data were presented as means with ± standard deviations (Vehapi et al., 2019).

Table 3. 8 Interpretation of antimicrobial susceptibility test (Behera et al., 2009; Waitz, 1990)

Susceptibility	Zone diameter (mm)
Susceptible (S)	≥20
Intermediate (I)	15–19
Resistant (R)	≤ 14

4
RESULTS AND DISCUSSION

4.1 Isolation and Identification

In the thesis study, microalgae in the water sample were isolated and transferred from solid agar medium to liquid cultures. Cultured microalgae were evaluated morphologically under microscope (Figure 4.1), and the shape of the cells were found as similar to *Chlorella* species (Hoshina et al., 2010; Leliaert et al., 2012). Growth was carried out in 250 mL Erlenmeyer flasks in a shaking incubator set at 150 rpm in continuous illumination (Figure 4.2).

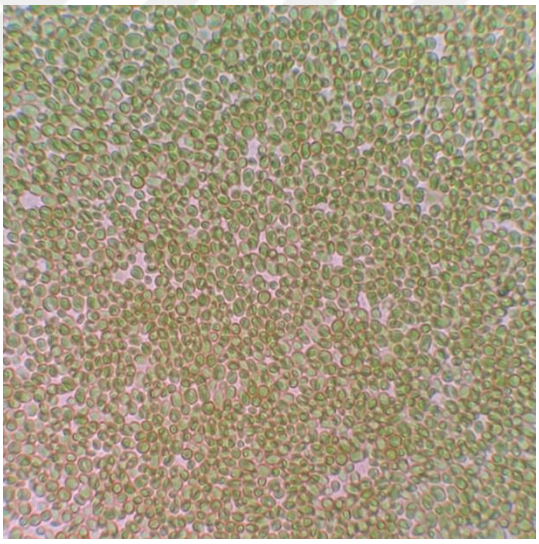


Figure 4. 1 Microscope image of the isolated microalgae (40x)

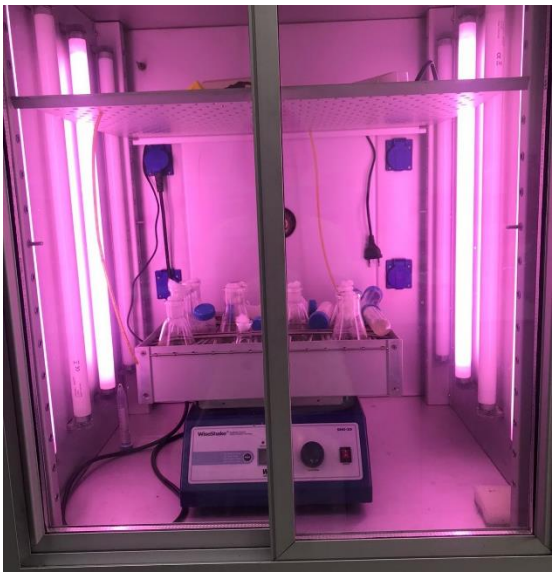


Figure 4. 2 Microalgal growth in a shaking incubator

Figure 4.3 showed the image obtained by running the colony PCR-applied isolate on an electrophoresis gel. It was seen that the target microorganism DNA band around 1500 bp was positioned at the right point relative to the ladder DNA. The isolates loaded into the wells worked successfully in PCR.

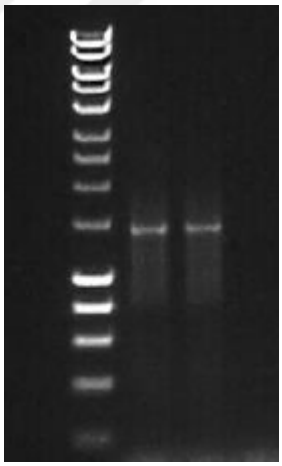


Figure 4. 3 Colony PCR gel electrophoresis image

The 16S rRNA (SSU rRNA) gene region, which is approximately 1500 bp in size and used for species identification, was sequenced and the sequencing results were subjected to BLAST analysis on the NCBI website. Figure 4.4 showed the score values of the BLAST analysis. According to BLAST analysis, the isolate has been determined *Chlorella variabilis* strain and named as YTU.ANTARCTIC.001.

SSU rRNA partial sequence of *Chlorella variabilis* strain

TGCAGTCGCACGAATACATTCTAGCTTGCTAGAATGAGATGAGTGGCGGACGGGTGAGTAACACGTAAGA
ACCTGCCTTTTGGTGAGGGACAACCATTGGAAACGATGGCTAATACCTCGTATTGCTGAGAAGTGAAAGA
TGAAAGTCGCCAAGAGATGGGCTTGCGGCTGATTAGCTAGTTGGTGGGGTAAAAGCTTACCAAGGCGATG
ATCAGTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGG
CAGCAGTGAGGAATTTTCCGCAATGGGCGAAAGCCTGACGGAGCAATGCCGCGTGAAGGATGACGGCCTA
TGGGTTGTAAACTTCTTTTCTCAGAGAAGAATTTTTGACGGTATCTGAGGAATAAGCATCGGCTAACTCT
GTGCCAGCAGCCGCGGTAAGACAGAGGATGCAAGCGTTATCCGGAATGATTGGGCGTAAAGCGTCTGTAG
GTGGCTTAAAAAGTCTCCTGTCAAAGATCAGGGCTTAACCCTGGGCCGGCAGGAGAAACTCTTAGGCTAG
AGTTTGGTAGGGGCAGAGGGAATTCCCGGTGGAGCGGTGAAATGCGTAGAGATCGGGAGGAACACCAAAG
GCGAAGGCACTCTGCTGGGCCAGAACTGACACTGAGAGACGAAAGCGAGGGGAGCAAAAGGGATTAGATA
CCCCTGTAGTCCTCGCCGTAAACGATGGATACTAGATGTTGGATAGGTTAAAGCATTCAGTATCGTAGCT
AACGCGTGAAGTATCCCGCCTGGGGAGTATGCTCGCAAGAGTGAAACTCAAAGGAATTGACGGGGGCCCG
CACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCAGGACTTGACATGCCACT
TTTTCCCTGAAAGGGGAAGTTCCAGAGTGGACACAGGTGGTGCATGGCTGTCGTCAGCTCGTGTCTTGAG
ATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGTTTTGAATTGCCAATTATGGGAAATTCAAAAGAC
TGCCGGTGATAAGCCGGAGGAAGGTGAGGATGACGTCAAGTCAGCATGCCCCTTACGTCCTGGGCGACAC
ACGTGCTACAATGGCCGGGACAAAGAGATGCAAACCCGCGAGGGCTAGCCAACCTCAAAAACCCGGCCTC
AGTTCGGATTGCAGGCTGCAACTCGCCTGCATGAAGTCGGAATCGCTAGTAATCGCAGGTCAGCCATACT
GCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCATGGAAGCTGGCTATGCCCAAAGTC
GTTACTCTAACCTGTAAAGGAGGAGGGCG

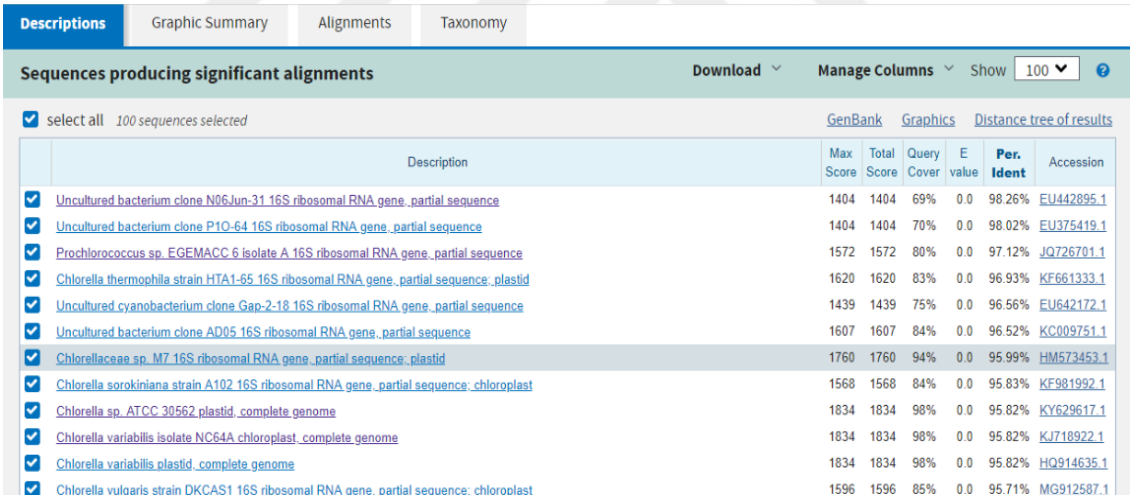


Figure 4. 4 BLAST analysis result on NCBI website

The systematic classification of *Chlorella variabilis* is given below:

Domain: Eukaryota

Kingdom: Viridiplantae

Division: Chlorophyta

Class: Trebouxiophyceae

Order: Chlorellales

Family: Chlorellaceae

Genus: Chlorella

Species: *Chlorella variabilis*

Chlorella variabilis was first identified by Shihira and Krauss (1965). *Chlorella variabilis* cells always have a spherical shape. It has a size of 4-12 μm in diameter. When grown in glucose media, it has a diameter of close to 12 μm. The chromatophore is shallow bowl-shaped and green. But in ancient cultures, it was seen that the color turned white. *Chlorella variabilis* is so named because of its extreme variability (Shihira & Krauss, 1965).

4.2 Microalgae Cultivation

During the cultivation of *Chlorella variabilis* YTU.ANTARCTIC.001 in BG-11 medium at the temperature of 4 and 27°C, growth of this species was observed each day with the UV spectrophotometer at 680 nm and algal growth curve was plotted depending on these data (Figure 4.5). It can be seen that, there is no specific growth at 4°C, while microalgal growth was observed at 27°C. In the study of Reay et al., nitrogen uptake of various microalgae and bacteria at low temperatures was investigated (Reay et al., 1999). It was found in the study that that nitrate affinity was highly dependent on temperature and nitrate uptake decreased continuously at temperatures below the optimum temperature. In addition to that, pH was adjusted to 7-7.2 at the start of the cultivation, yet it decreased to 6.5-6.7 over time (Reay et al., 1999). Manhaeghe et al. examined the effect of light and temperature on microalgae respiration and growth, and reported that microalgae respiration increased more below the optimum temperature range (Manhaeghe et al., 2019). To the same effect, Liao et al. found that the pH of the environment where microalgae were grown decreases with increasing respiration (Liao et al., 2018). For this reason, it is thought that low pH also slows growth in cultivation at 4°C.

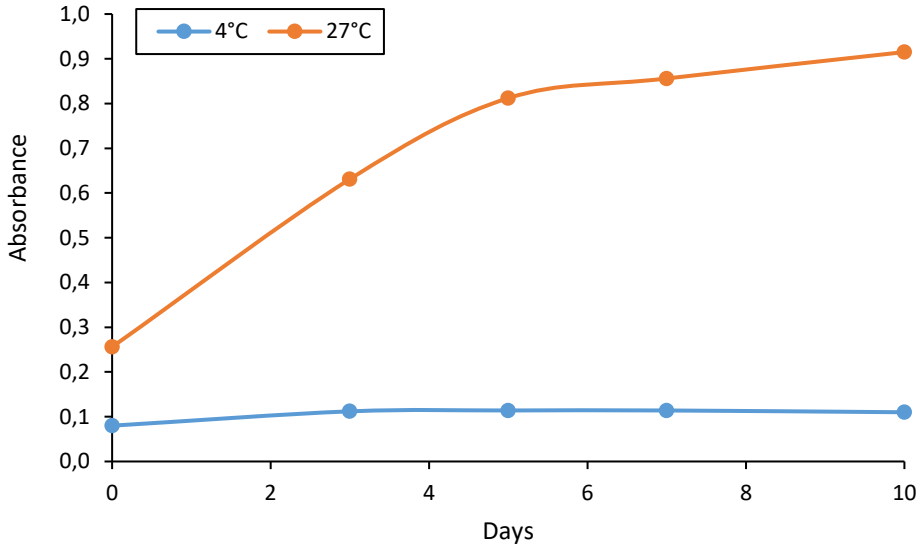


Figure 4. 5 The growth curve of *Chlorella variabilis* YTU.ANTARCTIC.001

After the analysis of these data, the specific growth rate and doubling time of microalgae cultivated at two different temperatures were calculated and presented in Table 4.1.

Table 4. 1 The specific growth rate (d^{-1}) and doubling time (t_d) of *Chlorella variabilis* YTU.ANTARCTIC.001 cultures grown at two different temperatures

Temperature(°C)	Specific growth rate (d^{-1})	Doubling time (t_d)
4°C	0,02	nd*
27°C	0,18	3.85

*This value was not determined. Because there was no growth observed in this experiment.

For *Chlorella variabilis* YTU.ANTARCTIC.001, growth rates were determined as 0.02 and 0.18 day^{-1}; and doubling time was 3.85 days at 27°C. Since there was no evident growth at 4°C, the doubling time was not determined. The specific growth rate of the microalgae species was low at cold conditions than at

temperate temperatures. In the study of Tarhan et al., growth rate of *Chlorella minutissima* microalgae which cultivated in BG11 medium is 0.124 day^{-1} (Tarhan et al., 2021). Öztay reported that *Chlorella minutissima'* growth rate is 0.0776 day^{-1} under 27°C temperature condition (Öztay, 2016). Sert et al. reported the growth rate of *C. minutissima* which was cultured at 25°C under continuous illumination, is 0.0879 day^{-1} (Sert et al., 2018). Teoh et al. found the growth rates of Antarctic origin *Chlorella* sp. were between 0.23-0.26 day^{-1} (Teoh et al., 2013). Morgan-Kiss et al. reported the growth rate of *Chlorella* sp. which was Antarctic origin and cultured in BG-11 medium, is 0.33 day^{-1} (Morgan-Kiss et al., 2008). Barati et al. studied growth of *Chlorella* sp. which was isolated from Antarctica and, when they increased temperature up to 25°C, they observed the specific growth rate positively impacted; however, further increase over 25°C negatively affected the growth. For Antarctic *Chlorella sp.*, the maximum growth rate was recorded at 25°C 0.553 day^{-1}, the lowest rate was observed at 35°C 0.111 day^{-1} (Barati et al., 2018).

In the study conducted by Schulze et al., the growth trends of cold-adapted Arctic and Antarctic microalgae cultures at 8 and 15°C were examined and biomass increase was observed with increasing temperature (Schulze et al., 2019). The Antarctic microalgae studied showed different growth trends in response to culture temperature. This conclusion is due to the fact that Antarctic algae are psychotrophic rather than psychrophilic organisms (Teoh et al., 2004). These data indicate that the growth rate of *C. variabilis* YTU.ANTARCTIC.001 microalgae species in temperate condition (27°C) was similar with the other studies in the literature.

4.3 Characterization of Microalgae

4.3.1 Biochemical content of microalgae

Microalgae produce significant biomolecules such as proteins, polysaccharides, lipids, polyunsaturated fatty acids, vitamins, pigments and enzymes. These substances are known as biologically active substances. They have antioxidant,

antibacterial, antiviral, antitumor, regenerative, neuroprotective, and immunostimulant properties (Gürlek, Yarkent, Köse, Oral, et al., 2020). These compounds are utilized in various applications area such as medicine, pharmacology, energy industry, feed production in agriculture field and functional foods (Bhattacharjee, 2016).

After cultivation of *C.variabilis* YTU.ANTARCTIC.001 in BG-11 medium, carbohydrate, protein, lipid, chlorophyll-α and β-carotene contents of the microalgae were determined (Table 4.2).

Table 4. 2 Biochemical content of *Chlorella variabilis* YTU.ANTARCTIC.001 cultivated at the temperature of 4°C and 27°C

	Carbohydrate(%)	Protein (%)	Lipid (%)	Chlorophyll-α (μg/mL)	β-Carotene (μg/mL)
4°C	12±1.2	31±1.0	33±1.2	0,90±0.1	0,58±0.01
27°C	28±1.3	21±1.2	49±1.1	3,14±0.1	1,51±0.01

In microalgae, carbohydrate types consist of mainly starch, sugar, polysaccharide and cellulose. These carbohydrate compounds can be used as storage or structural components in algae. Microalgal carbohydrate content shows that it has potential as a raw material for bioethanol production or in the food and pharmaceutical industry (Becker, 2007; Sert et al., 2018).

In this study, the carbohydrate content was found to be 12% of the dry mass at 4°C and 28% at 27°C. A higher carbohydrate content was observed at temperate temperatures. Because microalgae growth is increased at these temperatures. Renaud et al. reported that high growth temperature was associated with a significant increase in carbohydrates (Renaud et al., 2002). Loganathan et al. reported that they found a carbohydrate content of 34% in their study with *C. variabilis* at mild temperatures (Loganathan et al., 2020). In different studies with *C. minutissima*, the carbohydrate ratio was recorded as 26% and 33.05%,

respectively (Koçer & Özçimen, 2018; Sert et al., 2018). When compare the results of our study with the literature, it was seen that the carbohydrate content of *C.variabilis* YTU.ANTARCTIC.001 grown at mild temperatures is similar to the values in the literature.

As for the protein content, it was observed that the protein content of *C.variabilis* YTU.ANTARCTIC.001 microalgae were 31% and 21% of the dry mass at 4°C and 27°C conditions, respectively. It was observed that *C.variabilis* YTU.ANTARCTIC.001 microalgae have a higher protein content at low temperatures. The reason for this may be the proteins and enzymes which are produced with the adaptation mechanisms when algae develops adapting to environmental conditions (Piligaev et al., 2019). There is no study in the literature on the % protein content of Antarctic microalgae in dry mass. Generally, protein studies in the literature are on amino acid sequencing. Therefore, the protein content was compared with the data studies grown under temperate temperature conditions. Loganathan et al. reported *C. variabilis* microalgae protein content as 39% in their study (Loganathan et al., 2020). Sert et al. determined the protein content rate as 24.69% for *C. minutissima* microalgae (Sert et al., 2018). Teoh et al. declared that most of the microalgae studied adapt by increasing their protein content at low culture temperatures (Teoh et al., 2004). Increases in microalgal cellular proteins at low temperatures may result from increased enzyme production as an adaptive mechanism to maintain photosynthesis rates (DeNicola, 1996). Production of specific proteins, such as RNA-binding proteins, in response to cold stress may also have contributed to this increase (Los & Murata, 1999). In general, the effect of culture temperature on the biochemical composition of Antarctic algae studied varies according to the species (Schulze et al., 2019; Teoh et al., 2004).

Growing in cold and harsh environments, microalgae produce ice-binding proteins (IBPs) that inhibit the growth of ice crystals inside and outside the cells (Mangiagalli et al., 2017). Ice crystal growth during cold storage creates a quality problem in frozen foods. Ice-binding proteins (IBPs) have great application potential as ice crystal growth inhibitors. The ability of IBPs to delay the growth

of ice crystals suggests that IBPs can be used as a natural ice conditioner for a variety of frozen products (Chen et al., 2020). Therefore, *C.variabilis* YTU.ANTARCTIC.001 microalgae have a high protein content potential at low temperature grown condition to use in the food field.

Microalgae can produce a variety of lipids, including triacylglycerols, phospholipids, and glycolipids. The lipid content of microalgae ranges between 20% and 50% by dry weight. These lipids play important roles in energy storage, energy substrates, cell membrane structural components, and metabolic processes (Bellou et al., 2014).

Lipid analyses showed that *C.variabilis* YTU.ANTARCTIC.001, which was grown at 27 °C in BG-11 medium, have 49% lipid content. On the other hand, lipid content at 4°C was lower than temperate conditions. Renaud et al. in their study reported that high growth temperature has been related to significant decrease in protein content, together with increases in lipids and carbohydrates (Renaud et al., 2002). Loganathan et al. reported that the lipid content of *C. variabilis* microalgae was 22% in their study (Loganathan et al., 2020). Sert et al. determined the lipid content as 42.26% for *C. minutissima* microalgae which is cultivated at 25°C and under continuous illumination (Sert et al., 2018). Öztay reported *C. minutissima'* lipid content as 23.4% (Öztay, 2016).

Microalgae contain variety of pigments: including carotenoids (orange color), xanthophylls (yellowish tint) and chlorophylls (green color). Carotenoids and chlorophyll levels in microalgae are typically higher than in plants (Koller et al., 2014). Carotenoids are generally found in low concentrations (0.5% μg^{-1} dry weight) (Mulders et al., 2013). Carotenoids play an important role in oxygen photosynthesis as a direct quencher of reactive oxygen species. Microalgae carotenoids can also be used as antioxidant molecules that can quench free radicals, thereby protecting cells and tissues from oxidative damage, including preventing oxidative degradation of food products (D'Alessandro & Antoniosi Filho, 2016). Chlorophylls are green pigments that play an active role in photosynthetic processes (Cuellar-Bermudez, 2014). The chlorophyll content in

microalgae ranges from 0.5% to 1.0% of dry weight, mainly chlorophyll-α (D'Alessandro & Antoniosi Filho, 2016).

As a result of chlorophyll-α determination in the thesis study, 0.9 μg/mL and 3.14 μg/mL were observed at 4 °C and 27 °C, respectively. Lee et al. found the chlorophyll-α content of *Chlorella* sp., which is originated in Antarctica and cultured at 25°C, is 1.86 μg/ml (Lee et al., 2018). Loganathan et al. determined chlorophyll-α pigment at temperate temperature for *C. variabilis* microalgae that the pigment amount was 2.60 μg/mL in their study (Loganathan et al., 2020). Öztay reported the chlorophyll-α content of *C. minutissima* as 3.11 μg/mL in their study (Öztay, 2016). It is clear that *C. variabilis* YTU.ANTARCTIC.001, an Antarctic microalgae, has more chlorophyll-α pigment than the same microalgae species at temperate temperature conditions.

Similar to chlorophyll content, β-carotene content of *C. variabilis* YTU.ANTARCTIC.001 increased with increasing temperature. 0.58 μg/mL β-carotene was observed at 4°C, while 1.51 μg/mL obtained at 27°C. Öztay reported the β-carotene content of *C. minutissima* as 1.12 μg/mL in her study (Öztay, 2016). Lee et al. reported the ctotal carotenoids content of Chlorella sp. which is originated in Antarctica and cultured at 25°C, is 0.72 μg/mL (Lee et al., 2018). β-carotene production at low temperature (4°C) was lower than the results found in the literature. It is clearly seen that the production of β-carotene increases with increasing temperature.

FTIR analysis

FTIR spectrometer was used to determine the functional groups contained in Antarctic microalgal biomass samples to be used in characterization study. The FTIR spectra of the raw materials used in the experiments are shown in Figure 4.6.

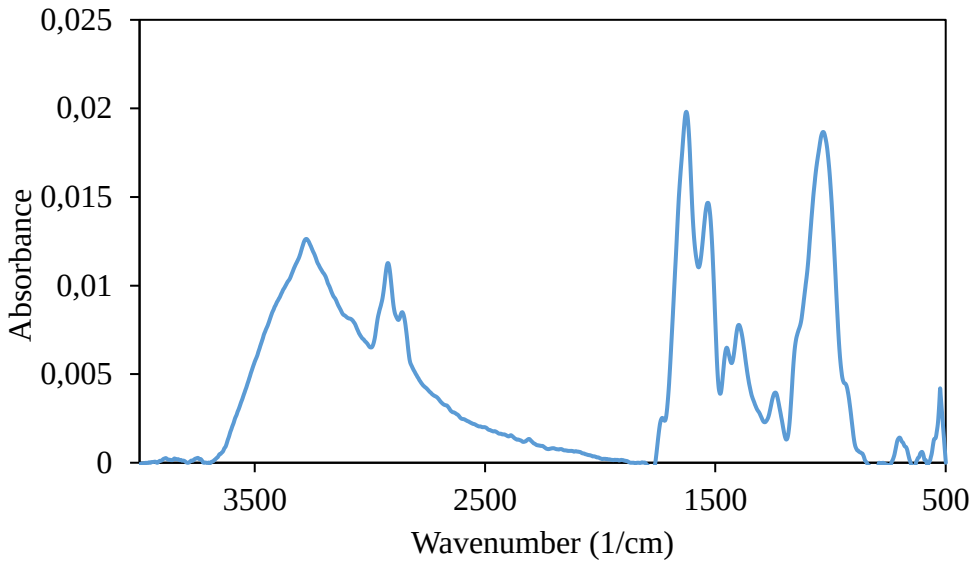


Figure 4. 6 FTIR spectrum of *Chlorella variabilis* YTU.ANTARCTIC.001

The functional groups defined by reading from the spectra is given in Table 4.3.

Table 4. 3 FTIR results of *Chlorella variabilis* YTU.ANTARCTIC.001

Wave Number (cm^{-1})	Functional Groups	Wave Number Range (cm^{-1})
3275	O-H stretching	3500-3200
2921	Aliphatic C-H stretching	3000-2850
1626	C=O stretching	1670-1600
1532	Aromatic ring C=C stretching	1600-1500
1500	C-N stretching	1600-1400
1400	C-C stretching	1500-1400
1239	P=O stretching	1250-1020
1031	C-O-C stretching	1200-1000
700	C-Cl stretching	790-700
524	C-Br stretching	700-515

Biomass contains carbohydrate, lipid and protein biomolecules. When the studies in the literature related to FTIR analysis were examined, it was observed that these biomolecules generally peaked between in 3100–2800 cm^{-1} and 1800-800 cm^{-1} (Duygu, 2012).

Absorption bands in the 1800-800 cm^{-1} region show its intensity in protein and carbohydrate content. This region is specific for proteins and carbohydrates (Dumas & Miller, 2003). Absorption intensity in the range of 3100–2800 cm^{-1} is indicative of the lipid content (Phukan et al., 2011).

Antioxidant content of microalgae

Among natural resources, microalgae, which isolated from extreme habitat regions, produce secondary metabolites as a mechanism for adaptation for maintaining survival against environmental stress. Various studies have shown that these bioactive compounds also have antioxidant properties (Suh et al., 2018; Teoh et al., 2004). The antioxidant activity of the extracts obtained from *Chlorella variabilis* YTU.ANTARCTIC.001 grown under temperate conditions was determined by using the DPPH free radical removal test. As a result of the experiments, it was determined that 1000 μg/ml *C. variabilis* extracts had 32-37% radical scavenging activity (Figure 4.7).

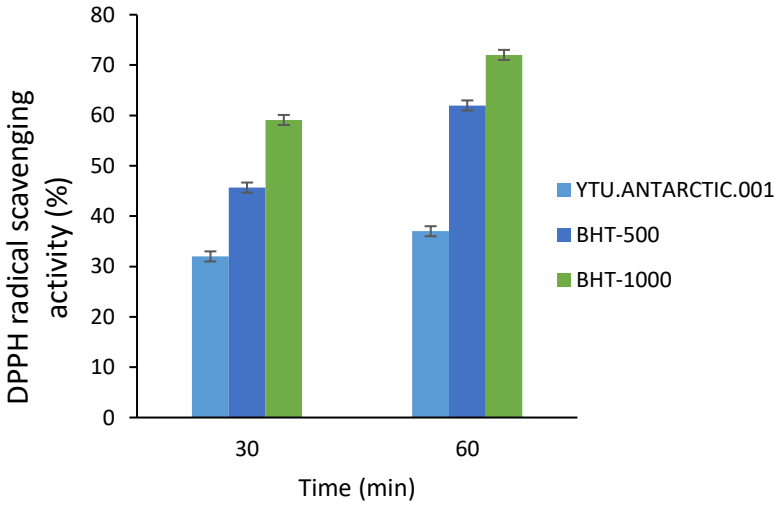


Figure 4. 7 Antioxidant activity of *C. variabilis* YTU.ANTARCTIC.001 microalgae

Total phenolic content

The total phenol content was found 107 mg GAE (gallic acid equivalent) / mg extract. Comparison of antioxidant activities of *C. variabilis* and other *Chlorella* species is presented in the Table 4.4. When these data are examined, it is seen that *C. variabilis* YTU.ANTARCTIC.001 has the potential to be evaluated in the pharmaceutical applications and shows promise.

Table 4. 4 DPPH radical scavenging activity and total phenol content of *C. variabilis* YTU.ANTARCTIC.001 species grown at 27°C and comparison with other *Chlorella* sp.

	DPPH radical scavenging activity (%)	**Total Phenolic Content (mg GAE $mg^{-1)}$**	**Reference**
C. minutissima	41	175	(Gürlek, Yarkent, Köse, Tuğcu, et al., 2020; Vehapi et al., 2020)
C. vulgaris	37	78	(Gürlek, Yarkent, Köse, Tuğcu, et al., 2020; Vehapi et al., 2020)
C. sorokiniana	14	75	(Ani Azaman et al., 2017)
C. variabilis	37	107	This study

Antimicrobial activity of microalgae

Chlorella variabilis YTU.ANTARCTIC.001 extract showed antibacterial activity against all of the bacteria tested. Table 4.5 shows the size of inhibition zones (mm) of polar microalgae extracts against Gram-positive bacteria *B. cereus* and Gram-negative bacteria *E. coli* and *S. typhimurium*. Polar algae extract exhibited stronger antibacterial activity against *B.cereus* and lower antibacterial activity against *S. typhimurium*. *Chlorella variabilis* YTU.ANTARCTIC.001 extract at 4%

concentration exhibit the strongest antibacterial activity against *B. cereus* and similar antibacterial activity against *E. coli* and *S. typhimurium*. Because of this, *B. cereus* was considered as sensitive bacteria. *E.coli* and *S. typhimurium* were considered as intermediate susceptible bacteria in this research. Increasing the concentration (0.5, 1, 2 and 4 %) of *Chlorella variabilis* YTU.ANTARCTIC.001 extracts exhibited stronger antibacterial activity against pathogenic bacteria.

Table 4. 5 The size of the inhibition zone of *Chlorella variabilis* YTU.ANTARCTIC.001 extract and control against bacterial strains

Sample	*Escherichia coli (mm)*		*Bacillus cereus (mm)*		*Salmonella typhimurium (mm)*	
Control	$08.50\pm0.70^{A,b}$	R	$12.00\pm2.82^{A,a}$	R	$08.00\pm0.00^{A,b}$	R
0.5 %	$11.50\pm0.70^{B,b}$	R	$14.50\pm3.53^{B,a}$	I	$9.00\pm0.00^{B,c}$	R
1 %	$13.00\pm0.00^{C,b}$	R	$17.50\pm2.12^{C,a}$	I	$11.50\pm0.70^{C,c}$	R
2 %	$13.25\pm0.35^{C,b}$	R	$18.00\pm0.00^{C,a}$	I	$12.00\pm0.00^{C,c}$	R
4 %	$15.50\pm0.70^{D,b}$	I	$20.00\pm3.53^{D,a}$	S	$15.00\pm1.41^{D,b}$	I

* Data are presented as means ± standard deviations(n=4) ($p < 0.05$).
A–D: Within each column, different superscript uppercase letters show differences between the algae concentration and control samples.
a–c: Within each row, different superscript lowercase letters show differences between the bacterial strains.

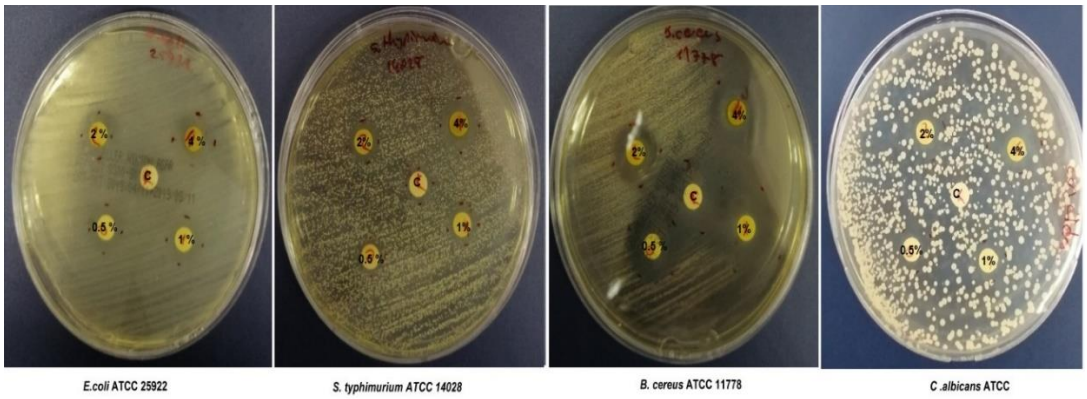


Figure 4. 8 The antimicrobial activity of *C. variabilis* YTU.ANTARCTIC.001 extracts against Gram-positive; *B. cereus* and Gram-negative; *E. coli*, *S. typhimurium* and pathogenic yeast *C. albicans*

In disc diffusion assay, *Chlorella variabilis* YTU.ANTARCTIC.001 extracts exhibited stronger antimicrobial activities against bacterial, fungal and yeast strains (Fig. 4.8 and 4.9). This result might be due to the phenolic compounds that are found in polar microalgae in high amounts (Abdu, 2019; Biondi et al., 2008).

Table 4.6 shows the size of inhibition zones (mm) of *Chlorella variabilis* YTU.ANTARCTIC.001 extracts against *C. albicans* pathogenic yeast. Increasing the concentration (0.5, 1, 2 and 4 %) of polar microalgal DMSO extracts exhibited stronger antimicrobial activity against *C. albicans* pathogenic yeast. *Chlorella variabilis* YTU.ANTARCTIC.001 extracts exhibit between 11.50 – 16.25 mm inhibition zones against *C. albicans*.

Table 4. 6 Size of inhibition zone of *Chlorella variabilis* YTU.ANTARCTIC.001 extract and control against *C. albicans*

Sample	Inhibition zone (mm)	
Control	06.00 ± 0.00^{A}	R
0.5 %	11.50 ± 0.70^{B}	R
1 %	11.75 ± 1.06^{B}	R
2 %	13.75 ± 0.35^{C}	R
4 %	16.25 ± 1.06^{D}	I

* Data are presented as means ± standard deviations(n=4) ($p < 0.05$).
A–D: Within each column, different superscript uppercase letters show differences between the algae concentration and control samples.

Table 4.7 shows the size of inhibition zones (mm) of polar microalgae extracts against *Colletotrichum gloeosporioides* fungal pathogen. Results of fumigation application via polar microalgae extracts, which were determined using the variance analysis JMP package program, showed that the highest inhibition rates at 4% concentration, for the mycelial growth of *C. gloeosporioides* (Figure 4.8). Increasing the concentration from 0.5 to 4 % of *Chlorella variabilis* YTU.ANTARCTIC.001 extracts resulted in weaker growth of *C. gloeosporioides*

(Table 4.7). This might be due to the phenolic compounds that are found in polar microalgae in high amounts.

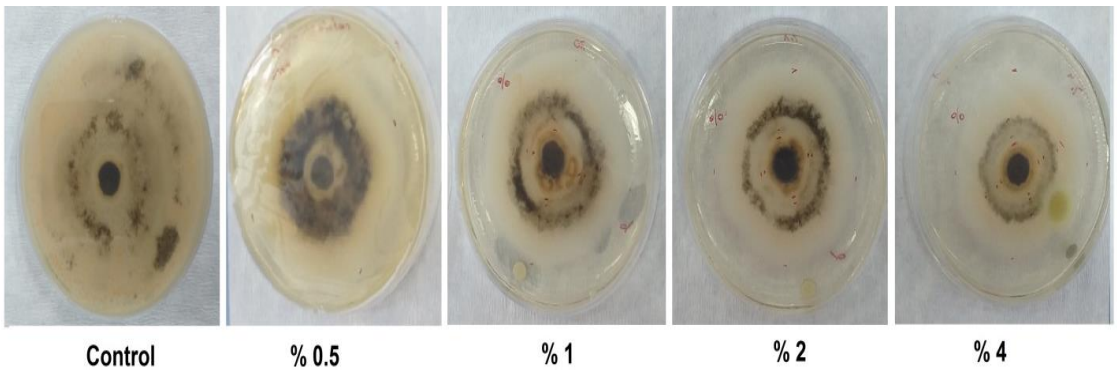


Figure 4. 9 The antifungal activity of *Chlorella variabilis* YTU.ANTARCTIC.001 extracts at different concentrations on fungal growth of *C. Gloeosporioides*

Table 4. 7 Inhibitory effects of *Chlorella variabilis* YTU.ANTARCTIC.001 extracts at different concentrations against *C. gloeosporioides*

Sample	Mycelial Growth (mm) *Colletotrichum gloeosporioides* Incubation Period (days)			
	3th	4th	5th	6th
Control	46.00±1.41^{A,a}	64.50±0.00^{A,b}	79.00±0.00^{A,c}	90.00±0.00^{A,d}
0.5 %	21.00±0.00^{B,a}	26.50±0.00^{B,b}	38.50±0.00^{B,c}	67.00±0.00^{B,d}
1 %	19.00±1.41^{C,a}	24.50±1.41^{C,b}	38.00±1.03^{B,c}	65.00±1.41^{C,d}
2 %	19.00±1.41^{C,a}	23.00±0.70^{D,b}	36.50±1.41^{C,c}	65.00±1.41^{C,d}
4 %	17.25±1.76^{D,a}	21.50±0.70^{E,b}	35.50±0.70^{D,c}	59.00±1.41^{D,d}

A-E Within each column, different superscript uppercase letters show the statistical difference between the antifungal effects of polar algae extract concentration ($p < 0.05$).
a-d Within each row, different superscript lowercase letters show the statistical difference between incubation periods ($p < 0.05$).

It is known that secondary metabolites have antifungal activity to stop or inhibit the development of mycelia growth of fungal microorganisms (Özçimen, 2018). The results of this study indicate that microalgae species that grown under extreme conditions in the Antarctica pole region exhibit promising antimicrobial

activity against some bacterial species and fungi. In this study, it was observed that cell extracts of the microalgae isolated from Antarctica have higher antibacterial activity against Gram-positive and Gram-negative bacteria and antimicrobial activity against fungal pathogens and yeast compared to other macro and microalgae species. The results obtained in this research clearly demonstrate that microalgae isolated from Antarctica have strong antibacterial and antifungal properties.

As a result of the studies and literature review, it has been determined that the microalgae *Chlorella variabilis* YTU.ANTARCTIC.001, which has recently been added to the literature, has a high potential to be used in many areas such as health and food. In this thesis study, firstly, the isolation and genetic identification of Antarctic microalgae was carried out, then, the effect of two different temperatures on the biochemical content was investigated. In addition, carbohydrate, lipid and protein, chlorophyll-α, β-carotene and total phenol contents were determined. FTIR analysis, antioxidant, antimicrobial analyzes were also applied to the microalgal biomass in order to evaluate their potential in cosmetic and pharmaceutical field. The general results of this study, in which algal biomass was grown under different conditions and its biochemical content was examined, are summarized below:

- Microalgae which is isolated from Horseshoe Island, Skua Lake, Antarctica, is a new species. *Chlorella variabilis* sp. was registered in the NCBI GenBank with the name *Chlorella variabilis* strain YTU.ANTARCTIC.001 (GenBank Accession number: MN372092). This was one of the output of the polar studies carried out by our country and a contribution was made to the literature on Antarctic microorganisms.
- In comparison with the cultivation carried out at 4°C, best growth of *Chlorella variabilis* YTU.ANTARCTIC.001 was determined under the conditions of 27°C using BG-11 medium in a shaking incubator set at 150 rpm with continuous illumination.

- Specific growth rates were determined as 0.02 and 0.18 day^{-1}; doubling times were nd and 3.85 days in 4°C and 27°C in BG-11 medium, respectively. *Chlorella variabilis* YTU.ANTARCTIC.001 was able to tolerate a wide temperature range, showing the higher growth rate at 27°C.
- Carbohydrate content was found 12% of the dry mass at 4°C and 28% at 27°C. These data are in accordance with the literature.
- In the protein content determination study, it was observed that the protein content of *C.variabilis* YTU.ANTARCTIC.001 microalgae were 31% and 21% of the dry mass at 4°C and 27°C conditions, respectively. These data are in accordance with the literature, and it can be suggested that, polar microalgae can be evaluated in food industry by utilizing their protein which have various bioactive properties.
- *C.variabilis* YTU.ANTARCTIC.001 microorganism, which was grown at 27 °C, have 49% lipid content. These data are superior to the literature.
- As a result of chlorophyll-α determination, 0.9 μg/mL was observed at 4 °C, while the data obtained at 27 °C was 3.14 μg/mL. These data obtained at 27 °C show superiority than the literature. In β-carotene determination, 0.58 μg/mL was observed at 4 °C, while the data obtained at 27 °C was 1.51 μg/mL. These data obtained at 27 °C show superiority than the literature. It can be suggested that, *C.variabilis* YTU.ANTARCTIC.001 can be utilized for cosmetic and pharmaceutical applications.
- As a result of the DPPH free radical removal test experiments, it was determined that 1000 μg/mL *C. variabilis* extracts had 32-37% radical scavenging activity. The total phenol content was found 107 mg GAE (gallic acid equivalent) / mg extract. These data are in accordance with the literature.
- *Chlorella variabilis* YTU.ANTARCTIC.001 extracts have high antibacterial and antifungal activity against the bacteria *Escherichia coli* ATCC 43888, *Bacillus cereus* ATCC 11778 and *Salmonella typhimurium* ATCC 14028, and pathogenic yeast strain *Candida albicans* ATCC 10231 and, fungi strain *Colletotrichum gloeosporioides* DSM 62136. With a remarkable bioactive

content and antioxidant and antimicrobial properties, *Chlorella variabilis* YTU.ANTARCTIC.001 can be considered as a promising source for pharmaceutical applications.

Polar microalgae perform the biosynthesis of valuable bioactive compounds as a metabolic adaptation mechanism in order to tolerate extreme environmental conditions. It was seen that the experiment's results data were similar to the literature, and even certain data were higher than the data in the literature. In conclusion, these Antarctic species exhibit promising results and can be recommended as a raw material for their applications in the pharmaceutical or healthcare products.

REFERENCES

Abdu, M. (2019). Phytochemical screening and antimicrobial activities of some green algae from Egypt. *Journal of Medicinal Plants Studies*, *7*(3), 12–16.

Amosova, A. V., Bolsheva, N. L., Samatadze, T. E., Twardovska, M. O., Zoshchuk, S. A., Andreev, I. O., Badaeva, E. D., Kunakh, V. A., & Muravenko, O. V. (2015). Molecular cytogenetic analysis of *Deschampsia antarctica Desv.* (Poaceae), Maritime Antarctic. *PLoS ONE*, *10*(9). https://doi.org/10.1371/journal.pone.0138878

Amsler, C. D., Amsler, M. O., Curtis, M. D., McClintock, J. B., & Baker, B. J. (2019). Impacts of gastropods on epiphytic microalgae on the brown macroalga *Himantothallus grandifolius*. *Antarctic Science*, *31*(2), 89–97. https://doi.org/10.1017/S0954102019000014

Anderson, G., & Kommareddy, A. (2003). Study of Light as a parameter in the growth of algae in a Photo-Bio Reactor (PBR). *American Society of Agricultural Engineers*.

Ani Azaman, S. N., Nagao, N., Yusoff, F. M., Tan, S. W., & Yeap, S. K. (2017). A comparison of the morphological and biochemical characteristics of *chlorella sorokiniana* and *chlorella zofingiensis* cultured under photoautotrophic and mixotrophic conditions. *PeerJ*, *2017*(9). https://doi.org/10.7717/peerj.3473

Aronson, R. B., Thatje, S., Mcclintock, J. B., & Hughes, K. A. (2011). Anthropogenic impacts on marine ecosystems in Antarctica. *Annals of the New York Academy of Sciences*, *1223*(1), 82–107. https://doi.org/10.1111/j.1749-6632.2010.05926.x

Arrigo, K. R. (2014). Sea Ice Ecosystems. *Annual Review of Marine Science*, *6*, 439–467. https://doi.org/10.1146/annurev-marine-010213-135103

Barati, B., Lim, P. E., Gan, S. Y., Poong, S. W., Phang, S. M., & Beardall, J. (2018). Effect of elevated temperature on the physiological responses of marine *Chlorella* strains from different latitudes. *Journal of Applied Phycology*, *30*(1). https://doi.org/10.1007/s10811-017-1198-z

Becker, E. W. (2007). Micro-algae as a source of protein. *Biotechnology Advances*, *25*(2), 207–210. https://doi.org/10.1016/j.biotechadv.2006.11.002

Behera, B., Das, A., Mathur, P., Kapil, A., Gadepalli, R., & Dhawan, B. (2009). Tigecycline susceptibility report from an Indian tertiary care hospital. *Indian Journal of Medical Research*, *129*(4), 446–450.

Bellou, S., Baeshen, M. N., Elazzazy, A. M., Aggeli, D., Sayegh, F., & Aggelis, G. (2014). Microalgal lipids biochemistry and biotechnological perspectives. *Biotechnology Advances*, *32*(8), 1476–1493. https://doi.org/10.1016/j.biotechadv.2014.10.003

Bhattacharjee, M. (2016). Pharmaceutically valuable bioactive compounds of

algae. *Asian Journal of Pharmaceutical and Clinical Research*, *9*(6), 43–47. https://doi.org/10.22159/ajpcr.2016.v9i6.14507

Biondi, N., Tredici, M. R., Taton, A., Wilmotte, A., Hodgson, D. A., Losi, D., & Marinelli, F. (2008). Cyanobacteria from benthic mats of Antarctic lakes as a source of new bioactivities. *Journal of Applied Microbiology*, *105*(1), 105–115. https://doi.org/10.1111/j.1365-2672.2007.03716.x

Bitog, J. P., Lee, I. B., Lee, C. G., Kim, K. S., Hwang, H. S., Hong, S. W., Seo, I. H., Kwon, K. S., & Mostafa, E. (2011). Application of computational fluid dynamics for modeling and designing photobioreactors for microalgae production: A review. *Computers and Electronics in Agriculture*, *76*(2), 131–147. https://doi.org/10.1016/j.compag.2011.01.015

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911–917. https://doi.org/10.1139/o59-099

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, *28*(1), 25–30. https://doi.org/10.1016/S0023-6438(95)80008-5

Buda, J., Łokas, E., Pietryka, M., Richter, D., Magowski, W., Iakovenko, N. S., Porazinska, D. L., Budzik, T., Grabiec, M., Grzesiak, J., Klimaszyk, P., Gaca, P., & Zawierucha, K. (2020). Biotope and biocenosis of cryoconite hole ecosystems on Ecology Glacier in the maritime Antarctic. *Science of the Total Environment*, *724*. https://doi.org/10.1016/j.scitotenv.2020.138112

Bunt, J. S., & Wood, E. J. F. (1963). Microbiology of antarctic sea-ice: Microalgae and antarctic sea-ice. *Nature*, *199*(4900), 1254–1255. https://doi.org/10.1038/1991254a0

Burton-Johnson, A., Black, M., Peter, T. F., & Kaluza-Gilbert, J. (2016). An automated methodology for differentiating rock from snow, clouds and sea in Antarctica from Landsat 8 imagery: A new rock outcrop map and area estimation for the entire Antarctic continent. *Cryosphere*, *10*(4), 1665–1677. https://doi.org/10.5194/tc-10-1665-2016

Caamaño, E., Loperena, L., Hinzpeter, I., Pradel, P., Gordillo, F., Corsini, G., Tello, M., Lavín, P., & González, A. R. (2017). Isolation and molecular characterization of *Thraustochytrium* strain isolated from Antarctic Peninsula and its biotechnological potential in the production of fatty acids. *Brazilian Journal of Microbiology*, *48*(4), 671–679. https://doi.org/10.1016/j.bjm.2017.01.011

Caspermeyer, J. (2020). How Algae Adapt to the Extreme Antarctic Cold. *Molecular Biology and Evolution*, *37*(3), 941. https://doi.org/10.1093/molbev/msaa017

Catarina Guedes, A., Barbosa, C. R., Amaro, H. M., Pereira, C. I., & Xavier Malcata, F. (2011). Microalgal and cyanobacterial cell extracts for use as natural antibacterial additives against food pathogens. *International Journal of Food*

Science and Technology, *46*(4), 862–870. https://doi.org/10.1111/j.1365-2621.2011.02567.x

Chapin, F. S., Sturm, M., Serreze, M. C., McFadden, J. P., Key, J. R., Lloyd, A. H., McGuire, A. D., Rupp, T. S., Lynch, A. H., Schimel, J. P., Beringer, J., Chapman, W. L., Epstein, H. E., Euskirchen, E. S., Hinzman, L. D., Jia, G., Ping, C. L., Tape, K. D., Thompson, C. D. C., … Welker, J. M. (2005). Role of land-surface changes in arctic summer warming. *Science*, *310*(5748), 657–660. https://doi.org/10.1126/science.1117368

Chen, X., Shi, X., Cai, X., Yang, F., Li, L., Wu, J., & Wang, S. (2020). Ice-binding proteins: a remarkable ice crystal regulator for frozen foods. *Critical Reviews in Food Science and Nutrition*. https://doi.org/10.1080/10408398.2020.1798354

Chisti, Y. (2007). Biodiesel from microalgae. *Biotechnology Advances*, *25*(3), 294–306. https://doi.org/10.1016/j.biotechadv.2007.02.001

Coad, T., McMinn, A., Nomura, D., & Martin, A. (2016). Effect of elevated CO2 concentration on microalgal communities in Antarctic pack ice. *Deep-Sea Research Part II: Topical Studies in Oceanography*, *131*, 160–169. https://doi.org/10.1016/j.dsr2.2016.01.005

Correction Convey, P., Bindschadler, R., Di Prisco, G., Fahrbach, E., Gutt, J., Hodgson, D. A., Mayewski, P. A., Summerhayes, C. P., Turner, J., Abram, N., Adams, B., Ainley, D., Anderson, J., Arbetter, T., Arthern, R., Atkinson, A., Barbante, C., Bargagli, R., Bargelloni, L., … Zane, L. (2009a). Antarctic climate change and the environment. *Antarctic Science*, *21*(6), 541–563. https://doi.org/10.1017/S0954102009990642

Correction Convey, P., Bindschadler, R., Di Prisco, G., Fahrbach, E., Gutt, J., Hodgson, D. A., Mayewski, P. A., Summerhayes, C. P., Turner, J., Abram, N., Adams, B., Ainley, D., Anderson, J., Arbetter, T., Arthern, R., Atkinson, A., Barbante, C., Bargagli, R., Bargelloni, L., … Zane, L. (2009b). Antarctic climate change and the environment. In *Antarctic Science*. https://doi.org/10.1017/S0954102009990642

Cowan, D. A., & Tow, L. A. (2004). Endangered antarctic environments. *Annual Review of Microbiology*, *58*, 649–690. https://doi.org/10.1146/annurev.micro.57.030502.090811

Cuba-Díaz, M., Cerda, G., Rivera, C., & Gómez, A. (2017). Genome size comparison in Colobanthus quitensis populations show differences in species ploidy. *Polar Biology*, *40*(7), 1475–1480. https://doi.org/10.1007/s00300-016-2058-z

Cuellar-Bermudez, S. P. (2014). *Extraction and purification of high-value metabolites from microalgae: essential lipids, astaxanthin and phycobiliproteins*.

D'Alessandro, E. B., & Antoniosi Filho, N. R. (2016). Concepts and studies on lipid and pigments of microalgae: A review. *Renewable and Sustainable Energy*

Reviews, *58*, 832–841. https://doi.org/10.1016/j.rser.2015.12.162

Das, B. K., & Pradhan, J. (2010). Antibacterial properties of selected freshwater microalgae against pathogenic bacteria. *Indian Journal of Fisheries*, *57*(2), 61–66.

De Morais, M. G., Vaz, B. D. S., De Morais, E. G., & Costa, J. A. V. (2015). Biologically Active Metabolites Synthesized by Microalgae. *BioMed Research International*, *2015*. https://doi.org/10.1155/2015/835761

DeNicola, D. M. (1996). Periphyton Responses to Temperature at Different Ecological Levels. *Algal Ecology*.

Duygu, D. Y. (2012). Fourier transform infrared (FTIR) spectroscopy for identification of *Chlorella vulgaris* Beijerinck 1890 and Scenedesmus obliquus (Turpin) Kützing 1833. *African Journal of Biotechnology*, *11*(16). https://doi.org/10.5897/ajb11.1863

Dominguez, H. (2003). *Functional ingredients from algae for foods and nutraceuticals*.

Dubois, M., Gilles, K., Hamilton, J. K., Rebers, P. A., & Smith, F. (1951). A colorimetric method for the determination of sugars. *Nature*, *168*(4265), 167. https://doi.org/10.1038/168167a0

Dumas, P., & Miller, L. (2003). The use of synchrotron infrared microspectroscopy in biological and biomedical investigations. *Vibrational Spectroscopy*, *32*(1 SPEC.), 3–21. https://doi.org/10.1016/S0924-2031(03)00043-2

El-Baky, H. H. A., El-Baz, F. K., & Baroty, G. S. E. (2010). Enhancing antioxidant availability in wheat grains from plants grown under seawater stress in response to microalgae extract treatments. *Journal of the Science of Food and Agriculture*, *90*(2), 299–303. https://doi.org/10.1002/jsfa.3815

Engelbertz, S. (2015). Values in Antarctica: Discourse Analyses of Two Topical Issues in Antarctic Policy in Antarctic Studies [University of Canterbury]. In *Gateway Antarctica*. https://doi.org/http://dx.doi.org/10.26021/6631

Estevez, M. S., Malanga, G., & Puntarulo, S. (2001). UV-B effects on Antarctic *Chlorella* sp cells. *Journal of Photochemistry and Photobiology BiBiology*, *62*, 19–25.

Ghasemi, Y., Moradian, A., Mohagheghzadeh, A., Shokravi, S., & Morowvat, M. H. (2007). Antifungal and antibacterial activity of the microalgae collected from paddy fields of Iran: Characterization of antimicrobial activity of *Chroococcus dispersus*. *Journal of Biological Sciences*, *7*(6), 904–910. https://doi.org/10.3923/jbs.2007.904.910

Gómez, I., Wulff, A., Roleda, M. Y., Huovinen, P., Karsten, U., Quartino, M. L., Dunton, K., & Wiencke, C. (2009). Light and temperature demands of marine benthic microalgae and seaweeds in polar regions. *Botanica Marina*, *52*(6), 593–608. https://doi.org/10.1515/BOT.2009.073

Gray, A., Krolikowski, M., Fretwell, P., Convey, P., Peck, L. S., Mendelova, M.,

Smith, A. G., & Davey, M. P. (2020). Remote sensing reveals Antarctic green snow algae as important terrestrial carbon sink. *Nature Communications*, *11*(1). https://doi.org/10.1038/s41467-020-16018-w

Guilherme, J. R., Pacheco, B., Soares, B. M., Pereira, C. M. P. de, Colepicolo, P., & Dias, D. (2020). Phaeophyceae and rhodophyceae macroalgae from the Antarctic: A source of selenium. *Journal of Food Composition and Analysis*, *88*. https://doi.org/10.1016/j.jfca.2020.103430

Gülyurt, M. Ö., Özçimen, D., & İnan, B. (2016). Biodiesel production from *Chlorella protothecoides* oil by microwave-assisted transesterification. *International Journal of Molecular Sciences*, *17*(4). https://doi.org/10.3390/ijms17040579

Gürlek, C., Yarkent, Ç., Köse, A., Oral, İ., Öncel, S., & Elibol, M. (2020). Evaluation of several microalgal extracts as bioactive metabolites as potential pharmaceutical compounds. *IFMBE Proceedings*, *73*, 267–272. https://doi.org/10.1007/978-3-030-17971-7_41

Gürlek, C., Yarkent, Ç., Köse, A., Tuğcu, B., Gebeloğlu, I. K., Öncel, S., & Elibol, M. (2020). Screening of antioxidant and cytotoxic activities of several microalgal extracts with pharmaceutical potential. *Health and Technology*, *10*(1), 111–117. https://doi.org/10.1007/s12553-019-00388-3

Hirose, Y., Shiozaki, T., Otani, M., Kudoh, S., Imura, S., Eki, T., & Harada, N. (2020). Investigating algal communities in lacustrine and hydro-terrestrial environments of East Antarctica using deep amplicon sequencing. *Microorganisms*, *8*(4). https://doi.org/10.3390/microorganisms8040497

Hoffmann, L., Eggers, S. L., Allhusen, E., Katlein, C., & Peeken, I. (2020). Interactions between the ice algae *Fragillariopsis cylindrus* and microplastics in sea ice. *Environment International*, *139*. https://doi.org/10.1016/j.envint.2020.105697

Hoffmann, R., Al-Handal, A. Y., Wulff, A., Deregibus, D., Zacher, K., Quartino, M. L., Wenzhöfer, F., & Braeckman, U. (2019). Implications of Glacial Melt-Related Processes on the Potential Primary Production of a Microphytobenthic Community in Potter Cove (Antarctica). *Frontiers in Marine Science*, *6*. https://doi.org/10.3389/fmars.2019.00655

Hoham, R. W., & Remias, D. (2020). Snow and Glacial Algae: A Review1. *Journal of Phycology*, *56*(2), 264–282. https://doi.org/10.1111/jpy.12952

Hoshina, R., Iwataki, M., & Imamura, N. (2010). *Chlorella variabilis* and *Micractinium reisseri* sp. nov. (Chlorellaceae, Trebouxiophyceae): Redescription of the endosymbiotic green algae of *Paramecium bursaria* (Peniculia, Oligohymenophorea) in the 120th year. *Phycological Research*, *58*(3), 188–201. https://doi.org/10.1111/j.1440-1835.2010.00579.x

Hu, H., Li, H., & Xu, X. (2008). Alternative cold response modes in *Chlorella* (Chlorophyta, Trebouxiophyceae) from Antarctica. *Phycologia*, *47*(1), 28–34. https://doi.org/10.2216/07-28.1

Hu, Q. (2004). Environmental effects on cell composition. In A. Richmond (Ed.), *Handbook of Microalgal Culture: Biotechnology and Applied Phycology* (pp. 83–93).

Huang, Y., Chen, M., & Liu, D. (2008). *Effect of Nitrogen, Phosphorus, Light Formation and Disappearance and Water Temperature on the of Blue-green Algae Bloom* (Nature Sci). Journal of Northwest Science-Technology University of Agriculture and Forest.

Ilyash, L. V., & Zhitina, L. S. (2009). Comparative analysis of sea-ice diatom species composition in the seas of Russian Arctic. *Zhurnal Obshchei Biologii*, *70*(2), 143–154.

İnan, B. (2019). *Mikroalgal Biyoproses Optimizasyonu, Mikroalgal Ekstrakt Yüklü Nanopartiküllerin Üretimi ve Antikanser Etkilerinin Araştırılması*. Yildiz Technical University.

Jha, D., Jain, V., Sharma, B., Kant, A., & Garlapati, V. K. (2017). Microalgae-based Pharmaceuticals and Nutraceuticals: An Emerging Field with Immense Market Potential. *ChemBioEng Reviews*, *4*(4), 257–272. https://doi.org/10.1002/cben.201600023

Jo, S. W., Do, J. M., Kang, N. S., Park, J. M., Lee, J. H., Kim, H. S., Hong, J. W., & Yoon, H. S. (2020). Isolation, identification, and biochemical characteristics of a cold-tolerant *chlorella vulgaris* knua007 isolated from king george island, antarctica. *Journal of Marine Science and Engineering*, *8*(11), 1–11. https://doi.org/10.3390/jmse8110935

Juneja, A., Ceballos, R. M., & Murthy, G. S. (2013). Effects of environmental factors and nutrient availability on the biochemical composition of algae for biofuels production: A review. *Energies*, *6*(9), 4607–4638. https://doi.org/10.3390/en6094607

Karakaş, C.Y. (2017). *Algal biyoaktif yüklü nanopartiküllerin elektrosprey ve mikroemülsiyon teknikleriyle üretimi ve karakterizasyonu*. Yıldız Teknik Üniversitesi.

Kemal, B. (2003). ANTARKTİKA Antlaşmala Sistemi (1961-2001): 40 Yılın Ardından Antarktika'nın Hukuki Rejimi. *Ankara Üniversitesi Hukuk Fakültesi Dergisi*, *52*(2), 1. https://doi.org/10.1501/hukfak_0000000529

Kharkwal, H., Joshi, D. D., Panthari, P., Pant, M. K., & Kharkwal, A. C. (2012). Algae as future drugs. *Asian Journal of Pharmaceutical and Clinical Research*, *5*(4), 1–4.

Kırkıncı, S. F., Maraklı, S., Aksoy, H. M., Özçimen, D., & Kaya, Y. (2021). Antarctica: A review of Life Sciences and Biotechnology Researches. *International Journal of Life Sciences and Biotechnology*. https://doi.org/10.38001/ijlsb.853472

Kleinegris, D. M. M., Janssen, M., Brandenburg, W. A., & Wijffels, R. H. (2011). Continuous production of carotenoids from *Dunaliella salina*. *Enzyme and Microbial Technology*, *48*(3), 253–259.

https://doi.org/10.1016/j.enzmictec.2010.11.005

Koçer, A. T., & Özçimen, D. (2018). Investigation of the biogas production potential from algal wastes. *Waste Management and Research*, *36*(11), 1100–1105. https://doi.org/10.1177/0734242X18798447

Koller, M., Muhr, A., & Braunegg, G. (2014). Microalgae as versatile cellular factories for valued products. *Algal Research*, *6*(PA), 52–63. https://doi.org/10.1016/j.algal.2014.09.002

Koppel, D. J., Adams, M. S., King, C. K., & Jolley, D. F. (2019). Preliminary study of cellular metal accumulation in two Antarctic marine microalgae – implications for mixture interactivity and dietary risk. *Environmental Pollution*, *252*, 1582–1592. https://doi.org/10.1016/j.envpol.2019.06.003

Kosugi, M., Ozawa, S. I., Takahashi, Y., Kamei, Y., Itoh, S., Kudoh, S., Kashino, Y., & Koike, H. (2020). Red-shifted chlorophyll a bands allow uphill energy transfer to photosystem II reaction centers in an aerial green alga, *Prasiola crispa*, harvested in Antarctica. *Biochimica et Biophysica Acta - Bioenergetics*, *1861*(2). https://doi.org/10.1016/j.bbabio.2019.148139

Králová, S. (2017). Role of fatty acids in cold adaptation of Antarctic psychrophilic *Flavobacterium* spp. *Systematic and Applied Microbiology*, *40*(6), 329–333. https://doi.org/10.1016/j.syapm.2017.06.001

Lai, J. W. S., Lim, P. E., Wong, C. Y., Phang, S. M., & Beardall, J. (2019). Photosynthetic response and DNA mutation of tropical, temperate and polar *Chlorella* under short-term UVR stress. *Polar Science*, *20*, 35–44. https://doi.org/10.1016/j.polar.2018.12.004

Lee, K. K., Lim, P. E., Poong, S. W., Wong, C. Y., Phang, S. M., & Beardall, J. (2018). Growth and photosynthesis of *Chlorella* strains from polar, temperate and tropical freshwater environments under temperature stress. *Journal of Oceanology and Limnology*, *36*(4), 1266–1279. https://doi.org/10.1007/s00343-018-7093-x

Leliaert, F., Smith, D. R., Moreau, H., Herron, M. D., Verbruggen, H., Delwiche, C. F., & De Clerck, O. (2012). Phylogeny and Molecular Evolution of the Green Algae. *Critical Reviews in Plant Sciences*, *31*(1), 1–46. https://doi.org/10.1080/07352689.2011.615705

Li, H. Bin, Cheng, K. W., Wong, C. C., Fan, K. W., Chen, F., & Jiang, Y. (2007). Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. *Food Chemistry*, *102*(3), 771–776. https://doi.org/10.1016/j.foodchem.2006.06.022

Liao, Y., Bokhary, A., Maleki, E., & Liao, B. (2018). A review of membrane fouling and its control in algal-related membrane processes. *Bioresource Technology*, *264*, 343–358. https://doi.org/10.1016/j.biortech.2018.06.102

Liu, C., Sun, H., & Zhu, L. (2006). Effects of Salinity Formed with Two Inorganic Salts on Freshwater Algae Growth. *Acta Scientiae Circumstantiae*, *26*(1), 157–161.

Loganathan, B. G., Orsat, V., & Lefsrud, M. (2020). Evaluation and interpretation of growth, biomass productivity and lutein content of *Chlorella variabilis* on various media. *Journal of Environmental Chemical Engineering*, *8*(3). https://doi.org/10.1016/j.jece.2020.103750

Los, D. A., & Murata, N. (1999). Responses to cold shock in cyanobacteria. *Journal of Molecular Microbiology and Biotechnology*.

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry*, *193*(1), 265–275. https://doi.org/10.1016/s0021-9258(19)52451-6

Lyon, B. R., & Mock, T. (2014). Polar microalgae: New approaches towards understanding adaptations to an extreme and changing environment. In *Biology* (Vol. 3, Issue 1). https://doi.org/10.3390/biology3010056

Macías-Sánchez, M. D., Mantell, C., Rodríguez, M., Martínez De La Ossa, E., Lubián, L. M., & Montero, O. (2005). Supercritical fluid extraction of carotenoids and chlorophyll a from *Nannochloropsis gaditana*. *Journal of Food Engineering*, *66*(2), 245–251. https://doi.org/10.1016/j.jfoodeng.2004.03.021

Mangiagalli, M., Bar-Dolev, M., Tedesco, P., Natalello, A., Kaleda, A., Brocca, S., de Pascale, D., Pucciarelli, S., Miceli, C., Bravslavsky, I., & Lotti, M. (2017). Cryo-protective effect of an ice-binding protein derived from Antarctic bacteria. *FEBS Journal*, *284*(1), 163–177. https://doi.org/10.1111/febs.13965

Manhaeghe, D., Michels, S., Rousseau, D. P. L., & Van Hulle, S. W. H. (2019). A semi-mechanistic model describing the influence of light and temperature on the respiration and photosynthetic growth of *Chlorella vulgaris*. *Bioresource Technology*, *274*, 361–370. https://doi.org/10.1016/j.biortech.2018.11.097

Mendes, R. L., Fernandes, H. L., Coelho, J. P., Reis, E. C., Cabral, J. M. S., Novais, J. M., & Palavra, A. F. (1995). Supercritical CO2 extraction of carotenoids and other lipids from *Chlorella vulgaris*. *Food Chemistry*, *53*(1), 99–103. https://doi.org/10.1016/0308-8146(95)95794-7

Moisander, P. H., McClinton, E., & Paerl, H. W. (2002). Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. *Microbial Ecology*, *43*(4), 432–442. https://doi.org/10.1007/s00248-001-1044-2

Morgan-Kiss, R. M., Ivanov, A. G., Modla, S., Czymmek, K., Hüner, N. P. A., Priscu, J. C., Lisle, J. T., & Hanson, T. E. (2008). Identity and physiology of a new psychrophilic eukaryotic green alga, *Chlorella* sp., strain BI, isolated from a transitory pond near Bratina Island, Antarctica. *Extremophiles*, *12*(5), 701–711. https://doi.org/10.1007/s00792-008-0176-4

Mulders, K. J. M., Weesepoel, Y., Lamers, P. P., Vincken, J. P., Martens, D. E., & Wijffels, R. H. (2013). Growth and pigment accumulation in nutrient-

depleted Isochrysis aff. galbana T-ISO. *Journal of Applied Phycology*, *25*(5), 1421–1430. https://doi.org/10.1007/s10811-012-9954-6

Özçimen, D. (2018). Investigation of antifungal effect of *Chlorella protothecoides* microalgae oil against *Botrytis cinerea* and *Aspergillus niger* fungi. *Journal of Tekirdag Agricultural Faculty*, *15*(2), 45–52.

Özcimen, D., Gülyurt, Ö., & İnan, B. (2012). Algal biorefinery for biodiesel production. In *Biodiesel: Feedstocks, Production and Applications* (p. 34).

Öztay, D. (2016). *Nanopartiküllerin Mikroalgal Büyüme Üzerine Olan Etkilerinin Araştırılması*. Yildiz Technical University.

Phukan, M. M., Chutia, R. S., Konwar, B. K., & Kataki, R. (2011). Microalgae *Chlorella* as a potential bio-energy feedstock. *Applied Energy*, *88*(10), 3307–3312. https://doi.org/10.1016/j.apenergy.2010.11.026

Piligaev, A. V., Sorokina, K. N., Samoylova, Y. V., & Parmon, V. N. (2019). Production of Microalgal Biomass with High Lipid Content and Their Catalytic Processing Into Biodiesel: a Review. *Catalysis in Industry*, *11*(4), 349–359. https://doi.org/10.1134/S207005041904007X

Plants. (n.d.). British Antarctic Survey. Retrieved from https://www.bas.ac.uk/about/antarctica/wildlife/plants/ (01.07.2021)

Priyadarshani, I., & Rath, B. (2012). Commercial and industrial applications of micro algae–A review. *Journal of Algal Biomass Utilization*, *3*(4), 89–100.

Reay, D. S., Nedwell, D. B., Priddle, J., & Ellis-Evans, J. C. (1999). Temperature dependence of inorganic nitrogen uptake: Reduced affinity for nitrate at suboptimal temperatures in both algae and bacteria. *Applied and Environmental Microbiology*, *65*(6), 2577–2584. https://doi.org/10.1128/aem.65.6.2577-2584.1999

Renaud, S. M., Thinh, L. Van, Lambrinidis, G., & Parry, D. L. (2002). Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. *Aquaculture*, *211*(1–4), 195–214. https://doi.org/10.1016/S0044-8486(01)00875-4

Robertson, R. C., Guihéneuf, F., Bahar, B., Schmid, M., Stengel, D. B., Fitzgerald, G. F., Ross, R. P., & Stanton, C. (2015). The anti-inflammatory effect of algae-derived lipid extracts on lipopolysaccharide (LPS)-stimulated human THP-1 macrophages. *Marine Drugs*, *13*(8), 5402–5424. https://doi.org/10.3390/md13085402

Romero, M., Casanova, A., Iturra, G., Reyes, A., Montenegro, G., & Alberdi, M. (1999). Leaf anatomy of *Deschampsia antarctica* (Poaceae) from the Maritime Antarctic and its plastic response to changes in the growth conditions. *Revista Chilena De Historia Natural*, *72*(3), 411–425.

Ryan, P., Steele, W., Siegfried, W., & Vogel, J. (1992). Radiocarbon-dates of snow petrel regurgitations can reveal exposure periods for nunataks in Antarctica. *South African Journal of Science*, *88*, 578–580.

Sadovskaya, I., Souissi, A., Souissi, S., Grard, T., Lencel, P., Greene, C. M., Duin, S., Dmitrenok, P. S., Chizhov, A. O., Shashkov, A. S., & Usov, A. I. (2014). Chemical structure and biological activity of a highly branched (1 →3,1 →6)-β-D-glucan from Isochrysis galbana. *Carbohydrate Polymers*, *111*, 139–148. https://doi.org/10.1016/j.carbpol.2014.04.077

Schulze, P. S. C., Hulatt, C. J., Morales-Sánchez, D., Wijffels, R. H., & Kiron, V. (2019). Fatty acids and proteins from marine cold adapted microalgae for biotechnology. *Algal Research*, *42*. https://doi.org/10.1016/j.algal.2019.101604

Sert, B. S., Inan, B., & Özçimen, D. (2018). Effect of chemical pre-treatments on bioethanol production from *Chlorella minutissima*. *Acta Chimica Slovenica*, *65*(1), 160–165. https://doi.org/10.17344/acsi.2017.3728

Sheehan, C. E., & Petrou, K. (2020). Dimethylated sulfur production in batch cultures of Southern Ocean phytoplankton. *Biogeochemistry*, *147*(1), 53–69. https://doi.org/10.1007/s10533-019-00628-8

Shihira, I., & Krauss, R. W. (1965). *Chlorella Physiology and Taxonomy of Forty-one Isolates*. https://img.algaebase.org/pdf/5964B937192003134FPWT36D7BB2/35050.pdf (01.07.2021)

Soto, D. F., Fuentes, R., Huovinen, P., & Gómez, I. (2020). Microbial composition and photosynthesis in Antarctic snow algae communities: Integrating metabarcoding and pulse amplitude modulation fluorometry. *Algal Research*, *45*. https://doi.org/10.1016/j.algal.2019.101738

Staehelin, L. A. (2003). Chloroplast structure: From chlorophyll granules to supra-molecular architecture of thylakoid membranes. *Photosynthesis Research*, *76*(1–3), 185–196. https://doi.org/10.1023/A:1024994525586

Stamenković, M., Steinwall, E., Nilsson, A. K., & Wulff, A. (2020). Fatty acids as chemotaxonomic and ecophysiological traits in green microalgae (desmids, Zygnematophyceae, Streptophyta): A discriminant analysis approach. *Phytochemistry*, *170*. https://doi.org/10.1016/j.phytochem.2019.112200

Suh, S. S., Hong, J. M., Kim, E. J., Jung, S. W., Chae, H., Kim, J. E., Kim, J. H., Kim, I. C., & Kim, S. (2019). Antarctic freshwater microalga, *chloromonas reticulata*, suppresses inflammation and carcinogenesis. *International Journal of Medical Sciences*, *16*(2), 189–197. https://doi.org/10.7150/ijms.30647

Suh, S. S., Hong, J. M., Kim, E. J., Jung, S. W., Kim, S. M., Kim, J. E., Kim, I. C., & Kim, S. (2018). Anti-inflammation and anti-cancer activity of ethanol extract of antarctic freshwater microalga, Micractinium sp. *International Journal of Medical Sciences*, *15*(9), 929–936. https://doi.org/10.7150/ijms.26410

Sukatar, D. D. A. (2002). *Alg Kültür Yöntemleri*. Ege Üniversitesi Basımevi.

Tan, X., Kong, F., Yu, Y., Shi, X., & Zhang, M. (2009). Effects of enhanced temperature on algae recruitment and phytoplankton community succession. *China Environmental Science*, *29*(6), 578–582.

Tarhan, S. Z., Koçer, A. T., Özçimen, D., & Gökalp, İ. (2021). Cultivation of green microalgae by recovering aqueous nutrients in hydrothermal carbonization process water of biomass wastes. *Journal of Water Process Engineering*, *40*. https://doi.org/10.1016/j.jwpe.2020.101783

Teoh, M. L., Chu, W. L., Marchant, H., & Phang, S. M. (2004). Influence of culture temperature on the growth, biochemical composition and fatty acid profiles of six Antarctic microalgae. *Journal of Applied Phycology*, *16*(6), 421–430. https://doi.org/10.1007/s10811-004-5502-3

Teoh, M. L., Phang, S. M., & Chu, W. L. (2013). Response of Antarctic, temperate, and tropical microalgae to temperature stress. *Journal of Applied Phycology*, *25*(1), 285–297. https://doi.org/10.1007/s10811-012-9863-8

Vance, T. D. R., Bayer-Giraldi, M., Davies, P. L., & Mangiagalli, M. (2019). Ice-binding proteins and the 'domain of unknown function' 3494 family. *FEBS Journal*, *286*(5), 855–873. https://doi.org/10.1111/febs.14764

Vehapi, M. (2018). *Antifungal Activities of Chlorella vulgaris and Chlorella minutissima Microalgae Cultivated in Bold Basal Medium, Wastewater and Tree Extract Water Against Aspergillus niger and Fusarium oxysporum*. https://doi.org/10.26327/RBL2018.228

Vehapi, M., Koçer, A. T., Yılmaz, A., & Özçimen, D. (2019). Investigation of the antifungal effects of algal extracts on apple-infecting fungi. *Archives of Microbiology*, *202*(3), 455–471. https://doi.org/10.1007/s00203-019-01760-7

Vehapi, M., Koçer, A. T., Yılmaz, A., & Özçimen, D. (2020). Investigation of the antifungal effects of algal extracts on apple-infecting fungi. *Archives of Microbiology*, *202*(3), 455–471. https://doi.org/10.1007/s00203-019-01760-7

Vila, E., Hornero-Méndez, D., Lareo, C., & Saravia, V. (2020). Biotechnological production of zeaxanthin by an Antarctic *Flavobacterium*: Evaluation of culture conditions. *Journal of Biotechnology*, *319*, 54–60. https://doi.org/10.1016/j.jbiotec.2020.05.014

Waitz, J. A. (1990). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically*. National Committee for Clinical Laboratory Standards.

Walton, D. (2013). Antarctica: global science from a frozen continent. In *Choice Reviews Online* (Vol. 51, Issue 03). https://doi.org/10.5860/choice.51-1503

Wang, L., Min, M., Li, Y., Chen, P., Chen, Y., Liu, Y., Wang, Y., & Ruan, R. (2010). Cultivation of green algae *Chlorella* sp. in different wastewaters from municipal wastewater treatment plant. *Applied Biochemistry and Biotechnology*, *162*(4), 1174–1186. https://doi.org/10.1007/s12010-009-8866-7

Wang, Y., Liu, X., Gao, H., Zhang, H. M., Guo, A. Y., Xu, J., & Xu, X. (2020). Early Stage Adaptation of a Mesophilic Green Alga to Antarctica: Systematic

Increases in Abundance of Enzymes and LEA Proteins. *Molecular Biology and Evolution*, *37*(3), 849–863. https://doi.org/10.1093/molbev/msz273

Wong, C. Y., Chu, W. L., Marchant, H., & Phang, S. M. (2007). Comparing the response of Antarctic, tropical and temperate microalgae to ultraviolet radiation (UVR) stress. *Journal of Applied Phycology*, *19*(6), 689–699. https://doi.org/10.1007/s10811-007-9214-3

Xu, X. Q., Beardall, J., & Hallam, N. D. (1998). Modification of fatty acid composition in halophilic Antarctic microalgae. *Phytochemistry*, *49*(5), 1249–1252. https://doi.org/10.1016/S0031-9422(98)00117-4

Yarzábal, L. A. (2016). Antarctic Psychrophilic Microorganisms and Biotechnology: History, Current Trends, Applications, and Challenges. *Microbial Models: From Environmental to Industrial Sustainability*, 83–118. https://doi.org/10.1007/978-981-10-2555-6_5

Zhang, X., Zheng, Z., He, Y., Liu, L., Qu, C., & Miao, J. (2020). Molecular Cloning and Expression of a Cryptochrome Gene CiCRY-DASH1 from the Antarctic microalga *Chlamydomonas* sp. ICE-L. *Molecular Biotechnology*, *62*(2), 91–103. https://doi.org/10.1007/s12033-019-00225-y

Zhu, M., Zhang, X., & Y, M. (2003). Effects of Temperature, Salinity and Illumination on the Growth of Thalassiosira sp. *Marine Sciences*, *27*(12), 58–61.

Zou, N., & Richmond, A. (2000). Light-path length and population density in photoacclimation of *Nannochloropsis* sp. (Eustigmatophyceae). *Journal of Applied Phycology*, *12*(3–5), 349–354. https://doi.org/10.1023/a:1008151004317

Zullaikah, S., Utomo, A. T., Yasmin, M., Ong, L. K., & Ju, Y. H. (2019). Ecofuel conversion technology of inedible lipid feedstocks to renewable fuel. *Advances in Eco-Fuels for a Sustainable Environment*, 237–276. https://doi.org/10.1016/b978-0-08-102728-8.00009-7

PUBLICATIONS FROM THE THESIS

Papers

1. Özçimen, D., Kaya, Y., Aksoy, H. M. , İnan, B., Çelik, A., Edbeib, M., Koçer, A. T. (2019). Isolation, characterization and identification of Chlorella variabilis isolated from Horseshoe Island, Skua Lake, Antarctica, YTU.ANTARCTIC.001, MN372092. NCBI GenBank , 1.

Conference Papers

1. Çelik, A., Koçer, A., İnan, B., Vehapi, M., & Özçimen, D., (2020). Antarktik Mikroalg Bazlı, Antimikrobiyal Etkili Biyoaktif Yara Örtüsü Malzemesi Üretimi. 4. Ulusal Kutup Bilimleri Çalıştayı ve Birinci Kutup Şenliği (pp.1). Kocaeli, Turkey
2. Özçimen, D., Kaya, Y., Aksoy, H., Koçer, A., Çelik, A., İnan, B., Vehapi, M.(2020). Investigation of antibacterial and antifungal effects of C. variabilis isolated from Horseshoe Island, SkuaLake, Antarctica. SCAR 2020 (pp.1). Hobart, Australia
3. Özçimen, D., Kaya, Y., Aksoy, H., Koçer, A., Çelik, A., İnan, B., Vehapi, M.(2020). Isolation and identification of some microorganism species collected from Horseshoe Island, Skua Lake, Antarctica and microalgae cultivation. SCAR 2020 (pp.1). Hobart, Australia

Projects

1. Production of Antarctic Algae Based, Nanoparticle Containing, Antimicrobial, Bioactive Wound Dressing, TEKNOFEST 2020, Position: Team Leader and Researcher (Qualified to qualify for the final. At the end of the competition, we placed 6th.)