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VALORIZATION OF SOME MICROALGAE FROM DIFFERENT AQUATIC ENVIRONMENTS FOR ANIMAL FEED AND OTHER APPLICATIONS.

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ملـــخـــص

ركزت هذه الدراسة على عزل أنواع من الطحالب الدقيقة المحلية وتنقيتها وتحديد شكلها وتوصيفها الوراثي، ودراسة نموها في أوساط الزراعة عند مستويات ملوحة مختلفة. كما أُجريت تحاليل نوعية وكمية لمركباتها المتعددة الفينول ذات الأنشطة البيولوجية (مضادات الأكسدة ومضادات الميكروبات). واستُخدمت الأنواع المعزولة من الطحالب الدقيقة والخميرة كغذاء لطحالب الأرتيميا (*Artemia franciscana*) ودافنيا ماجنا لدراسة تأثيرها على نموهما.

حيث تم عزل ثلاثة أنواع، وهي *Chlorella sp.* CHL11 و*Chlorella sp.* CHL15 و *Limnospira fusiformis* TL03، وتم تنقيتها و تحديد نوعها، وأُودعت في السجل الوطني لمعلومات الأحياء الدقيقة (NCBI)، بأرقام الوصول MZ970329 و ON799438.1 و ON799439.1 على التوالي. أظهرت نتائج تطور النمو، ومحتوى الكلوروفيل أ، والكلوروفيل ب، والكاروتينات للأنواع الثلاثة عند مستويات ملوحة مختلفة، تحملها لتركيزات ملوحة مختلفة، وأن نموها يتناسب عكسيًا مع زيادة الملوحة.

أظهر محتوى البوليفينول والفلافونويد في المستخلصات الهيدروميثانولية المعزولة أن مستخلصات الطحالب الثلاثة غنية بالبوليفينول والفلافونويدات، مع تسجيل كميات أعلى من إجمالي المحتوى الفينولي (18.41±0.32 ملغ GEA/غ) في *L. fusiformis* TL03، ومحتوى أعلى من إجمالي الفلافونويد (3.59±0.06 ملغ QE/غ DW) في *Chlorella sp.* CHL11. كشف تحليل كروماتوغرافيا السائل عالي الأداء (HPLC) للمستخلصات الهيدروميثانولية عن وجود فئات مختلفة من المركبات الفينولية (حمض الفينول، الفلافانول، الفلافانونات، الهيدروكسي فلافونات، الفلافونولات، والفلافونات).

فيما يتعلق بالنشاط البيولوجي، أظهرت نتائج الأنشطة المضادة للأكسدة لمستخلصات الطحالب الثلاثة، التي تم تقييمها من خلال نشاط إزالة الجذور الحرة DPPH ونشاط تبييض بيتا كاروتين، أن أعلى نسب تثبيط DPPH (86.19 ± 1.61%) وتبييض بيتا كاروتين (60.35 ± 1.10%) سُجلت في TL03 *L. fusiformis*. أظهرت نتائج النشاط المضاد للبكتيريا للمستخلص الهيدروميثانولي الخام للطحالب المدروسة ضد ستة سلالات من البكتيريا المسببة للأمراض (*Bacillus subtilis* و *Staphylococcus aureus* و *Listeria monocytogenes* و *Escherichia coli* و *Pseudomonas aeruginosa* و *Klebseilla pneumoniae*) أن جميع المستخلصات كان لها نشاط قوي ضد *Bacillus subtilis* و *S. aureus*، مع تسجيل تأثيرات مختلفة ضد البكتيريات الأخرى.

أظهرت نتائج تغذية الأرتيميا خلال 12 يومًا، أنه تم تسجيل أعلى قيم لطول الجسم (5030.13 ± 189.82 ميكرومتر) ومعدل البقاء على قيد الحياة (78.33 ± 0.88٪) في الأرتيميا التي تغذت على TL03 *L. fusiformis*. بالنسبة لتجربة تغذية *Daphnia magna* التي استمرت أسبوعين، تم تسجيل أعلى قيم لطول الجسم (2997.63 ± 60.04 ميكرومتر) في تلك التي تغذت على *Chlorella sp.* CHL15، وسُجِّل أعلى معدل بقاء (67 ± 1.15%) في *Chlorella sp.* CHL11.

الكلمات المفتاحية: الطحالب الدقيقة، العزل، التعريف، إجمالي الفينول والفلافونويد، HPLC، النشاط البيولوجي، الأعلاف.

ABSTRACT

This study focused on the isolation, purification, morphology, and molecular characterization of local microalgae species and studied their growth in culture media at different salinity levels. In addition, qualitative and quantitative analyses of their polyphenolic compounds with some biological activities (antioxidants and antimicrobials) were performed. The isolated microalgae species and yeast were used as feed for Artemia (*Artemia franciscana)* and *Daphnia magna* to study their effect on their growth.

Three species, *Chlorella sp*. CHL11, *Chlorella sp*. CHL15, and *Limnospira fusiformis* TL03, have been isolated, purified, identified, and deposited in the NCBI, with accession numbers of MZ970329, ON799438.1, and ON799439.1, respectively.

The results of the growth evolution, chlorophyll a, chlorophyll b, and carotenoid contents of the three species at different salinity levels showed their tolerance to different salinity concentrations, and their growth was inversely proportional to the increase in salinity.

The polyphenol and flavonoid contents of the hydromethanolic extracts showed that the three algal extracts were rich in polyphenols and flavonoids, with higher amounts of total phenolic content (18.41±0.32 mg GEA/g) recorded in *L fusiformis* TL03 and higher total flavonoid content (3.59±0.06 mg QE/g DW) in *Chlorella sp*. CHL11.

High-performance liquid chromatography (HPLC) analysis of the hydromethanolic extracts revealed the presence of different classes of phenolic compounds (phenolic acid, flavanols, flavanones, hydroxy-flavones, flavonols, and flavones).

Regarding the biological activity part, the results of antioxidant activities evaluated by DPPH radical scavenging activity and beta-carotene bleaching activity showed that the highest percentages of DPPH inhibition (86.19 ± 1.61%) and beta-carotene bleaching (60.35 ± 1.10%) were recorded in *L. fusiformis* TL03.

The results of the antibacterial activity of the crude hydromethanolic extract against six strains of pathogenic bacteria (*Bacillus subtilis*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebseilla pneumoniae*) showed that all the extracts had strong activity against *B subtilis* and *S. aureus*, with different effects recorded against other bacteria.

The results of *Artemia* feeding during 12 days ,showed that, the highest values for body length(5030.13 ± 189.82 µm) and survival rate(78.33 ±0.88%) were registred in *Artemia* fed on *L. fusiformis* TL03. For the *Daphnia magna* feeding trial lasting two weeks, the highest body length values (2997.63 ± 60.04 µm) were recorded in those fed on *Chlorella sp*. CHL15, and the highest survival rate (67 ± 1.15%) was recorded in *Chlorella sp*. CHL11.

Keys words: Microalgae, Isolation, Identification, Total phenolic and flavonoid, HPLC, biological activity, feed.

RESUME

Cette étude a porté sur l'isolement, la purification, la morphologie et la caractérisation moleculaire des espèces de microalgues locales et a étudié leur croissance dans des milieux de culture à différents niveaux de salinité. De plus, des analyses qualitatives et quantitatives de leurs composés polyphénoliques présentant certaines activités biologiques (antioxydants et antimicrobiens) ont été réalisées. Les espèces isolées de microalgues et la levure ont été utilisées comme nourriture pour Artemia (*Artemia franciscana*) et *Daphnia magna* afin d'étudier leur effet sur leur croissance.

Trois espèces, *Chlorella sp*. CHL11, *Chlorella sp*. CHL15 et *Limnospira fusiformis* TL03, ont été isolées, purifiées, identifiées et déposées au NCBI, sous les numéros d'accès MZ970329, ON799438.1 et ON799439.1, respectivement. Les résultats de l'évolution de la croissance, des teneurs en chlorophylle a, chlorophylle b et caroténoïdes des trois espèces à différents niveaux de salinité ont montré leur tolérance à différentes concentrations de salinité, et leur croissance était inversement proportionnelle à l'augmentation de la salinité.

Les teneurs en polyphénols et en flavonoïdes des extraits hydrométhanoliques ont montré que les trois extraits d'algues étaient riches en polyphénols et en flavonoïdes, avec des teneurs en phénols totaux plus élevées (18,41 ± 0,32 mg GEA/g) enregistrées chez L. fusiformis TL03 et des teneurs en flavonoïdes totaux plus élevées (3,59 ± 0,06 mg QE/g DW) chez *Chlorella sp.* CHL11.

L'analyse par chromatographie liquide haute performance (CLHP) des extraits hydrométhanoliques a révélé la présence de différentes classes de composés phénoliques (acide phénolique, flavanols, flavanones, hydroxyflavones, flavonols et flavones). Concernant la partie activité biologique, les résultats des activités antioxydantes évaluées par l'activité de piégeage des radicaux DPPH et l'activité de blanchiment du bêta-carotène ont montré que les pourcentages les plus élevés d'inhibition du DPPH (86,19 ± 1,61 %) et de blanchiment du bêta-carotène (60,35 ± 1,10 %) ont été enregistrés chez *L. fusiformis* TL03.

Les résultats de l'activité antibactérienne de l'extrait hydrométhanolique brut contre six souches de bactéries pathogènes (*Bacillus subtilis, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Pseudomonas aeruginosa et Klebseilla pneumoniae*) ont montré que tous les extraits avaient une forte activité contre *B. subtilis et S. aureus*, avec des effets différents enregistrés contre d'autres bactéries.

Les résultats de l'alimentation des Artémias pendant 12 jours ont montré que les valeurs les plus élevées de longueur corporelle (5030,13 ± 189,82 µm) et de taux de survie (78,33 ± 0,88 %) ont été enregistrées chez les Artémias nourries avec *L. fusiformis* TL03. Pour l'essai d'alimentation avec *Daphnia magna* d'une durée de deux semaines, les valeurs de longueur corporelle les plus élevées (2997,63 ± 60,04 µm) ont été enregistrées chez celles nourries avec *Chlorella sp*. CHL15, et le taux de survie le plus élevé (67 ± 1,15 %) a été enregistré chez *Chlorella sp*. CHL11.

Mots clés : Microalgues, Isolement, Identification, Composés phénoliques et flavonoïdes totaux, HPLC, activité biologique, Alimentation.

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General Introduction

General Introduction

Microalgae are microorganisms, unicellular or multicellular, filamentous or siphonous, photosynthetic, which include eukaryotes and prokaryotes (Pereira et *al*., 2020). They have been found in a broad spectrum of habitats, encompassing saline, estuarine and freshwater environments; deserts and ice sheets; soil and rock surfaces; and tree bark (Aburai et *al*., 2013; Hopes & Mock., 2015; Borowitzka, 2018; Jacob-Lopes et *al*., 2020; Cook et *al*., 2020; Williamson et *al*., 2020; Senousy et *al*., 2020; Winkel et *al*., 2022; Arsad et *al*., 2022; Wu et *al*., 2022; Mahmudi et *al*., 2023; Borics et *al*., 2023, Roque *al*., 2023; Yan et *al*., 2024; Li et *al*., 2024).

Some microalgae are extremophiles or tolerant microalgae that prefer or are able to live in extreme conditions such as acidic or alkaline pH, high temperature, light, CO2 level and metal concentration (Varshney et *al*., 2015). Microalgae are characterized by a wide variety of colors and live in different forms of cell organization, individually (single cells), in chains or colonies (aggregate in colonies), and in filamentous forms (Hachicha et *al*., 2022; Stoyneva-Gärtner et *al*., 2024), depending on the species .

Microalgae are a diverse group of photosynthetic microorganisms, comprising both prokaryotic and eukaryotic species (Brasil et *al*., 2017). It is estimated that there are around 800,000 species of microalgae, but only 40,000–50,000 have been described (Hachicha et *al*., 2022).

The large diversity of microalgae and their biochemical compounds have expanded their uses in various biotechnological applications. Microalgae are used in wastewater treatment, clean and renewable energy production, biofertilizers, bioplastics, and some cosmetic and pharmaceutical products production. In addition, microalgae are used in human food as dietary supplements and as feed for aquatic animals such as rotifers, daphnia, brine shrimp, shrimp larvae, mussels, oysters, and larvae of some fish produced in hatcheries.

Algeria has diverse aquatic ecosystems freshwater, brackish water, and hyper saline water. These diverse aquatic environments can be a valuable source of microalgae, which have multiple biotechnological applications and thus provide economic benefits.

Isolating and cultivating local microalgae that are adapted to the local aquatic environment, tolerate brackish and saline water (especially water that is unsuitable for drinking or agricultural use) and have economic value contributes to the development of algae production and economic diversification without the use of fresh water.

In Algeria, limited research has been carried out on the isolation and valorization of local algae. This study therefore addresses this topic through various chapters. This work is divided into two parts: the first is Literature Review part and the second is Experimental part.

The present work aims to isolate, purify, and characterize three local microalgae strains – *Limnospira fusiformis* TL03, *Chlorella sp*.CHL11, *Chlorella sp*.CHL15 from Telamine and Gharabas Lake, and to evaluate their potential for biological activities and aquacultural applications. The experimental part of this study divided in six chapters

In Chapter 1, the sampling, isolation, purification, and preservation of the three local microalgae strains in both solid and liquid BG11 media is focused on. This critical initial phase establishes pure cultures, which are essential for subsequent morphological and molecular characterisation. Morphological identification is based on microscopic features, whereas molecular identification through DNA analysis provides taxonomic confirmation and evolutionary insights.

The effect of different salinity concentrations on the growth performance and pigment content (chlorophyll a, chlorophyll b, and carotenoids) of each microalgae strain is examined in Chapter 2. The Salinity is a key environmental parameter that influences photosynthetic efficiency and metabolite synthesis. Studying growth and pigment variation under stress salinity shows adaptive responses and indicates the potential nutritional and functional benefits of these microalgae for animal feed, particularly in the zooplankton feeding, such as Rotifere Artemia and Daphnia.

Chapter 3 involved assessing the total phenolic and flavonoid content, as well as qualitatively analyzing polyphenolic compoundso of the hydromethanolic extract of the three microalgae using HPLC. The antioxidant capacity is evaluated using DPPH and β-carotene assays, which provide insight into the extracts' free radical scavenging activity. The antioxidant activity of microalgae is strongly related to their ability to increase the antibacterial resistance of their consumers, such as zooplankton, against pathogenic microbes.

Continuous to the biological activities of the isolated microalgae, Chapter 4 examined the antibacterial properties of microalgal extracts against pathogenic bacterial strains.

Finally, Chapter 5 and 6 focused on the use of three isolated microalgae as feed for Artemia and Daphnia magna and investigated their effect on their growth and survival rate.

Part 1

Literature Review

Chapter 1
Classification of Microalgae

1. Introduction

Phycologists regard all organisms with chlorophyll a, without roots, stem, and leaves; can be classified as alga (Lee 1989). The latter term is divided into macroalgae and microalgae (Visuddho et *al*., 2024), which differ in size and organelle content.

Macroalgae or seaweeds, are macroscopic, visible to the naked eye; they can reach tens of meters in length, while microalgae are microscopic, ranging in size from a few micrometers to a few hundred micrometers (Zuccaro et *al*., 2020; Khaligh & Asoodeh, 2022).

Microalgae are characterized by a wide variety of colors and live in different forms of cell organization, individually (single cells), in chains or colonies (aggregate in colonies), and in filamentous forms (Coêlho et *al*., 2019; Hachicha et *al*., 2022; Stoyneva-Gärtner et *al*., 2024), depending on the species.

2. Evolution of algal classification

Harvey is one of the first algologists to describe the first classification of algae, followed by other classifications that have been based on a number of characters, such as morphological, physiological, biochemical, and, more recently, molecular characters (Harvey 1836; Baweja & Sahoo, 2015), on the basis of the color of the thallus, have been classified algae into four groups: Chlorospermae (green), Melanospermae (brown), Rhodospermae (red), and Diatomacea (diatoms).

Eichler (1883) classified algae into five groups: Chlorophyceae (green algae), Phaeophyceae (brown algae), Rhodophyceae (red algae), Cyanophyceae (blue green algae), and Diatomeae (diatoms).

Pascher (1931) presented another classification based on phylogeny and relationships within groups. He divided algae into eight divisions, which were subdivided into different classes.

Fritzsch (1935) classified algae based on various criteria such as pigmentation, the chemical nature of the reserve food material, the arrangement of the flagella, the presence or absence of an organized nucleus in a cell, and the mode of reproduction. He divided algae into 11 classes, each one of them subdivided into various orders.

Chapman (1973), Parker (1982), and Lee (2008), they classified algae into prokaryotes and eukaryotes, which were divided into divisions and classes.

Person (2011), and Levasseur et *al.* (2020) reported that the classification of microalgae is very complex and mysterious. It based on several different criteria, such as organizing

photosynthesis membranes, chemical nature of storage products, and their pigments and other morphological features (Vieira et *al*., 2020).

Hachicha et *al*. (2022), have been summarized the classification of microalgae and cynobacteria (Tab. 1) in 11 phyla in which he based on the classification of Metting in 1996 and Fritsch in 1922. The classification is based on several criteria such as pigments, reserve, flagellation, reproduction and cell envelope

Table 1: Classification of microalgae and cynobacteria (Hachicha et *al*., 2022).

PHYLUM	CLASS	PIGMENTS	RESERVE	FLAGELLATION	REPRODUCTION	CELL ENVELOPE
Cyanobacteria	Cyanophyceae	Chla,β carotene flavacene, Echinenone isozea-, zea-, myxo-, oscillaxanthin APC, C-PC, C-PE	Starch (granule) and glycogen	Unflagellated	Simple division, non-motile endospore, vegetative fragmentation No sexual reproduction	Lack of cellulose
Chlorophyta	Chlorophyceae Trebouxiophyceae Ulvophyceae Ulvophyceae	Chl a, b, α a β-carotene, lutein, zea-, viola-, loro- and neoxanthin	Starch Oil	From two to four flagella (mobile), Isokont	Isogamy, anisogamy, oogamy Motile zoospore, non-motile spore	Cellulose+ mannans+ xylans Sistosterol. Sometimes calcified
Euglenophyta	Euglenophyceae	Some colorless Chl a, b, diadinoxanthin	Paramylon Ergosterol	One or two flagella, one of which contains cilia and the second being reduced	Longitudinal division	No cell membrane Flexible periplast or pellicle
Heterokontophyta/ Ochrophyta	Xanthophycea E Eustigmatophyceae	Chl a and c, β-carotene, heteroxanthin, diadinoxanthin (++)	Oil Leucosin Ergosterol	Two heterokont flagella and sometimes a single flagellum	Zoospore, aplanospores, statospores Isogamy, oogamy (*Vaucheria*	Rich in pectic compounds Silicification during the rest period
Miozoa	Dinophyceae	Chl a, c, β-carotene, diadinoxanthin, dinoxanthin, Peridinins	Starch Lipids	Lateral biflagella	Zoospore Longitudinal oblique division Aniso or isogamy	With or without cellulose
Euglenophyta	Euglenophyceae	Some colorless Chl a, b, diadinoxanthin	Paramylon Ergosterol	One or two flagella, one of which	Longitudinal division	No cell membrane Flexible

				contains cilia and the second being reduced		periplast or pellicle
Heterokontophyta/Ochrophyta	Chrysophycea	Chl a, c, β-carot, Fuco-, Diato-, diadinoxanthin	Chrysolaminarin Fucosterol Porifasterol	Two heterokont flagella	Zoospore, statospore	Less cellulose with silicification or calcification of the membrane
Haptophyta	Coccolithophyceae Pavlovophyceae Rappephyceae	Chl a, c, β-carot, Fuco-, Diato-diadinoxanthin	Chrysolaminarin Fucosterol Porifasterol	Two isokont flagella + haptonema	Zoospore, statospore	Less cellulose with silicification or calcification of the membrane
Bacillariophyta (Diatoms)	Bacillariophyceae	Chl a, c, β-carot, Fuco-, Diato-, diadinoxanthin	Chrysolaminarin Oil	Without flagellum except for male gamete	Cell division ogamie (centered diatoms) Amoeboid isogamy (pinnate diatoms)	Silica + pectic compounds
Cryptophyta	Cryptophyceae	Chl a, c, Biliproteins, α-carot, Allo-, Croco-, Monado-xanthi	Starch (granule) Oil Carbohydrates	Two unequal flagella except for *Bjornbergiella* and *Tetragonidium*	Zoospores	Proteinaceous periplast
Heterokontophyta/ Ochrophyta	Phaeophyceae	Chl a, c, β-carot, violaxanthin Fucoxanthin (++)	Mannitol Laminarin Lipids	Two heterokont flagella	Monospores, tetraspores, non-motile aplanospores From isogamy to oogamy	Cellulose + alginic acid + fucoidin
Rhodophyta	Porphyridiophycea e Bangiophyceae Florideophyceae	APC, C-PC,R-PC, B-PE, R-PE ,phytochrome,α and βcarot, Chl a and d, zeaxanthin, lutein	Starch, floridoside, mannoglycerate, sulfated polysaccharides	No flagella	Oogamy complex	Carbohydrates (xylose, cellulose, galactane)

3. Studied microalgal species

3.1. *Limnospira*

Spirulina is the ancient commercial name for edible filamentous cyanobacteria, which was first attributed to the genera *Spirulina* and *Arthrospira*

Turpin (1827) has identified and described the multicellular spiral Cyanobacterium as Spirulina *ocellarioides*. To distinguish *Spirulina* from *Arthrospira* Castenholz (1989) proposed the use of the following criteria: the degree of inclination of the pitch of the trichome helix (from the transverse axis); the visibility of the cross-walls between the cells in the filament; the distribution of the junction pores in the cell wall.

3.1.1. Morphologic description

The name Spirulina was first given by Wittrock and Nordstedt (1884) to a blue-green microalgae in the form of spiral filaments divided by septa, which was discovered in a stagnant pond near Montevideo, where the microalgae was named “Spirulina”.

Based on the research conducted by Komárek & Anagnostidis, (2005) and Komárek &d Hauer (2011)[Tab 2], Sili et *al*., (2012) reported that *Spirulina* and *Arthrospira* belong to two different genera and also Sinetova et *al*. (2024) summarized the different taxonomic morphological criteria of them as follows: *Spirulina* genus, characterized by its trichome helix, usually nearly closed and permanently motile by rotation; cell cross walls, usually invisible under microscope; cell width range, 2–4 μm. whereas in the *Arthrospira* genus the trichome helix is usually open; cross walls visible under light microscope; gas vesicles usually present; cell width range 6-12 μm.

Limnospira is a recently established genus with three validated species that were reclassified from *Arthrospira* based on morphological, molecular, and ecological differences (Nowicka-Krawczyk et *al*., 2019). *Arthrospira fusiformis, Arthrospira maxima, and Arthrospira indica* were reclassified to *Limnospira fusiformis, Limnospira maxima,* and *Limnospira indica* respectively (Nowicka-Krawczyk et *al*., 2019).

Spirulina (*Arthrospira* or *Limnospira*) is a blue-green microalgae (Spínola et *al*., 2024; Sharma & Rana, 2024) that belongs to the phylum Cyanobacteria (Sinetova et *al*., 2024), which is one of the most ancient living organisms on Earth (AlFadhly et *al*., 2022).

Table 2. Morphology of *Arthrospira* species based on Komárek and Anagnostidis (2005) , Komárek &d Hauer (2011)

SPECIES	Trichome width (µM)	Cell length (µm)	coils width (µm)	coils height (µm)	helix shape	gas vacuoles	morphology of trichome end
Types Without Gas-Vacuoles (Forming Mats)							
A. balkrishnanii	3.5–4 (5)	0.8–1.2	6–7	12–15	-	−	Rounded. no calyptra
A. desikacharyensis		0.85–1.7	51–6.8	10.2–11.5	Regularly loosely spiral coils	-	Rounded
A. gigantean	(2.5) 3–4		(8) 11–16	7–12 (15)	Regularly coiled	−	+/− conical
A. gomontiana	2.5–3	+/− isodiametric	6–6.5	15–19		-	
A. jenneri	(3.7) 4–6 (8)		(7.4) 8–15 (17)	(9.2) 12–25 (31)	Regularly screw-like coils	−	Rounded, no calyptra
A. massartii	5–6	-	-	-	Loosely screw-like	-	Rounded conical
A. pellucidis	-	-	-	-	-	-	
A. platensis	(4) 6–7 (9)	Nearly isodiametric	(20) 26–36	(24) 30–57	+/− regularly loosely spiral coils	-	Rounded or flat calyptra
A. santannae	2.8–3.8	+/− isodiametric	12.5–18.5 (loose) 8.8–10.8 (dense)	20–40 (dense) 5.5–5.8 (loose)	+/− densely or loosely coil	-	Rounded, no calyptra
A. skujae	(2.7) 3.1–4.3	3.1–9.3	7.4–11.8	7–13	Regularly screw-like coil	-	Rounded,
A. tenuis	About 2	2–3	20–35			-	

Types With Gas Vacuoles (Planktonic, Solitary Trichomes)							
A. argentina	9–10.5		33–49	63–75		+	
A. *fusiformis*	(3.4)5–9 (11)	2–3 × shorter than wide	15–50	0–80	Regularly screw-like coiled intensely narrowed or widened towards ends	+	Rounded
A. indica	8–9	1/3–1/2 as long as wide	16–39	16–66	Spirally coiled and distinctly narrowed towards ends	+	Conical calyptra
A. *ghannae*	3–5.2		20–22	20–35	Regularly loosely screw-like coils	+	Slightly attenuated end and capitates
A. joshii	4.2–5.1	2.7–3.4	6.8–7.6	17–19.2	Regularly screw-like coils, not attenuated towards ends	+	Rounded, no calyptra
A.maxima	(6.5) 7–9 (10) 5–7	5–7	40–60	70–80	Regularly loosely screw-like coiled, gradually slightly attenuated at ends	+	Rounded with thickend outer cell wall

3.1.2. Geographical distribution:

The species of *Arthrospira* have been isolated mostly from alkaline, brackish, and saline waters in tropical and semitropical regions (Castenholz, 2001; Komárek & Anagnostidis, 2005). The first discovered of *A. platensis* in Algeria was by Fox (1996) in Tamanrasset Pond, located in the southern region of Algeria.

The main populations *Arthrospira* have been reported in 30 countries worldwide (Table 3), eleven in Africa, nine in Asia, five in America, and five in Europe (Sili *et al*., 2012).

Table 3: Geographical distribution of the main populations of *Arthrospira* (Castenholz 2001; Komárek & Anagnostidis, 2005).

Country	***location***	***Taxon***
Africa		
Chad	Natron lakes (Bodou, Mombolo, Rombou, Yoan) and pools (Latir, Iseirom, Latir, Liva), Kanem region	*A. platensis, A. platensis* f. *minor*
	Lake Kailala, Lake Kossorom	*A. fusiformis*
Kenya	Natron lakes (Bogoria, Crater, Elmenteita, Nakuru)	*A. platensis*
	Rift Valley	*A. fusiformis*
	Lake Bogoria	*A. platensis, A. platensis* f . *minor*
		A. fusiformis
	Lake Simbi	*A. platensis*
		A. platensis = A. fusiformis
		A. fusiformis
	Lake Sonachi	*A. fusiformis*
	Lake Oloidien	*A. fusiformis*
Ethiopia	Lake aranguadi	*A. platensis*
	Lake Chiltu, Green lake	*A. platensis = A. fusiformis*
Egypt	Lake maryut	*A. platensis*
Sudan	Lake dariba, jebel marra	*A. geitleri*
Algeria	Pond tamanrasset	*A. platensis*
Congo	Laka mougounga	*Arthrospira* sp.

	Lake Kivu	*Arthrospira* sp
Zambia	Lake bangweolou	*Arthrospira* sp.
Tunisia	Lake korba	*Arthrospira* sp.
Mozanbique	Wastewater ponds	*A. fusiformis*
South africa	Lake tswaing	*A. fusiformis*
Asia		
India	Ponds	*A. maxima*
	Lonar Lake; ponds; tank	*A. indica*
	(Madurai, MCRC isolate)	
Myanmar	Crater lake	*Arthrospira* sp.
Pakistan	Fish pond, lahore	*Arthrospira* sp.
Sri lanka	Lake beria	*Arthrospira* sp.
China	Fish ponds, nanking	*A. platensis*
	Lake Bayannur	*A. platensis*
Thailand	Tapioca factory ef fl uent lakes, bangkok	*Arthrospira* sp
Russia	Tunatan lake, siberian steppe	*A. fusiformis*
Azerbaijan	Water basin, khumbasha	*Arthrospira* sp.
America		
Mexico	Lake Texcoco solar evaporator	*A. maxima*
Brazil	Mangueira Lagoon	*Arthrospira* sp.
California	Pond, Oakland	*A. maxima*
	Coastal lagoon, Del Mar	*A. platensis*
Peru	Lake Huachachina	*A. platensis*
		A. maxima
Uruguay	Montevideo	*A. platensis*
Europe		
Spain	Lake Santa Olalla	*Arthrospira* sp.
France	Tiny lake, Camargue	*Arthrospira* sp.
Hungary	Adasztevel-Oroshaz	*Arthrospira* sp
Romania	Alkaline pond near Cluj-Napoca	*A. fusiformis*
Serbia	Salty puddles near river Tamiš	*A. fusiformis*

3.2. Chlorella

Chlorella is a genus of microalgae that belongs to the family Chlorellaceae, order Chlorococcale, class Chlorophyceae, division Chlorophyta(Long et *al*.,2024). The National Center for Biological Research (NCBI) classification lists 42 genera in the Chlorellaceae family. Among these genera is the genus Chlorella, which is divided into 25 species (Mócsai et *al*., 2024). This genus was discovered by Beijerinck (1890) when he described the species *Chlorella vulgaris*.
The genera have been the subject of a number of systematic revision studies over the time (Bock et *al*., 2011; Champenois et *al.*, 2015). These revisions have been based principally on their morphological and reproduction studies (Fott, 1969; Nozaki et *al*., 1995).
Over that period, more than a hundred of chlorella species have been identified from different environments (Krienitz et *al*., 2004) and have been classified within the genus Chlorella based on morphological features similarities.

3.2.1 Morphologic description

The Chlorella genus is characterized by its spherical, sub-spherical or ellipsoidal cells, they live in solitary or in colonies form up to 64 cells, with or without mucilage. It reproduces by autospores (Bock et *al.,* 2011).
The term *Chlorella* has been used for a group of small green eukaryotic cells with hardly any obvious visual characteristics to differentiate their species and even genera (Bock et *al*., 2011; Leliaert et *al*., 2014; Krienitz et *al*., 2015; Zou et *al*., 2016; Fawley & Fawley, 2020).
The present challenge in the study of this genus is the difficulty of distinguishing between the many species that resemble *Chlorella* with the limited number of morphological characters and the small sizes of the vegetative cells that hinder identification and distinction between species (Ettl & Gärtner, 1995; Neustupa et *al*., 2009).
Bock et *al*. (2011) modified the general description of Chlorella through a polyphasic approach that included morpholological and phylogenetic characteristics.

3.2.2 Geographical distribution

The genus Chlorella (Trebouxiophyceae) is widespread in almost all regions of the world (Aigner et *al*., 2020), living in freshwater brackish water, seawater, and soil terrestrial habitats,

and some species are also symbionts of lichens, protozoa and invertebrates (Luo et *al*., 2010; Bock et *al*., 2011; Darienko et *al*., 2015).

Chapter 2
Microalgae Culture

1. Introduction

The production of microalgal biomass involves several steps, the most important of which are cultivation, harvesting and drying. The cultivation phase represents the largest cost due to the high cost of the culture medium and the energy required to maintain optimal culture conditions (temperature, pH, aeration and light) (Norsker, 2011; Cabrera-Capitillo et *al*., 2023).

The growth and physiological functions of microalgae are entirely dependent on the presence of essential nutrients in their culture media; macronutrients such as carbon (C), nitrogen (N), phosphorus (P), and for certain species, silicon (Si), in addition to micronutrients such as iron, magnesium, and trace elements like zinc and copper serve as cofactors in enzymatic reactions and are essential for photosynthetic efficiency (Satriaji et *al*., 2016).

2. Nutritional mode of microalgae

The micro-algae can be used three different trophic mode their nutrition, namely autotrophic (photoautotrophic), heterotrophic and mixotrophic mode (Dragone 2022). Each influencing their growth, metabolism, and potential applications (Dolganyuk et *al*., 2020) [Tab. 4].

2.1.Autotrophic mode :

In this mode, microalgae use light as an energy source and inorganic carbon (CO_2) to produce organic compounds essential for growth through photosynthesis. It is the most common mode of microalgal production. It is carried out in both photobioreactors and open ponds.

2.2.Heterotrophic mode

This method consists of using the external organic carbon in the absence of light (in the dark) to produce microalgal biomass.

2.3.Mixotrophic mode :

In this mode, microalgae can use light energy as well as external inorganic carbon to supply required CO_2 for photosynthesis and also take up external organic carbon to supply energy for growth (Cheng et *al*., 2023).

Table.**4**. Characteristics of possible methods for microalgae production (Dolganyuk et *al*., 2020).

Cultivation Method	Energy Source	Carbon Source	Biomass production	Rate Reactor Type	Price	Features
Phototrophic	Light	Inorganic	Low	Photobioreactor/ open waters	Low	The cell density of the culture is low; water evaporation
Heterotrophic	Organic matter	Organic	High	Bioreactor	Medium	The high price of the nutrient medium components; possibility of microbial contamination
Mixotrophic	Light, organic matter	Organic and inorganic	Medium	Closed photobioreactor	High	The high price of the nutrient medium components; possibility of microbial contamination

3. Microalgae production system

Microalgae production is carried out in open culture systems such as basins , circular ponds, racwey ponds or in closed- culture systems called photobioreactors(PBRs) in various designs (Mata et *al*., 2010; Karthikeyan et *al*.,2016; Acién et *al*., 2017; Zohir et *al.,* 2023**)**

3.1. Open culture systems

Open-pond culture systems are one of the oldest, simplest, and easiest methods for microalgae production on a large scale (Tan et *al*., 2020). This production system is widely used due to its advantages in industry such as inexpensive construction, maintenance, operation costs (Novoveská et *al*., 2023) and low energy demand (Costa & de Morais, 2014). On the other hand, this culture system has disadvantages such as water evaporation, limited control of

external contamination (other microalgae, bacteria, and fungi), and change in environmental conditions (Naseema Rasheed et *al*., 2023)

Open culture systems are subdivided into natural water bodies such as lakes and lagoons (Fig 1) and artificial ones such as raceway pond (Fig 1. B and C), circular ponds (Fig 1.D (Borowitzka & Moheimani, 2012). Few species are widely produced in open ponds due to their ability to grow in a selective environment such as high salinity (*Dunaliella*), high alkalinity (*Spirulina, Arthrospira, Limnospira*), and high nutrient levels (*Chlorella*, Scenedesmus, *Phaeodactylum*) [Borowitzka & Moheimani, 2012] in which these harsh conditions help reduce contamination by other microorganisms.

Figure 1. Different culture types of open system for microalgae production (**A**) Kanembu women harvesting *Spirulina* from Lake Boudou Andja, in Chad. (Hamed 2016), (**B**) Production of *Haematococcus* in raceway pond (Zhang *al*., 2009), (**C**) Production of microalgae in raceway pond, (**D**) Microalgae mass production in circular pond (Shen et *al*., 2009).

3.2. Closed photobioreactors:

Closed PBRs can be operated indoors, using natural or artificial lighting, or Outdoor applications will require less energy for lighting; however, depending on the location, this benefit may be eliminated by the need to cool the PBRs to avoid overheating (Wang et *al*., 2012; Sukačová et *al*., 2021); The term closed systems refers to photobioreactors that have no direct exchange of gases and contaminants between the cultivation systems and the outside environment (El-Baz & Abd El Baky, 2018).

Photobioreactors (PBRs) are made of transparent or semi transparent materials (glass, plexiglas, polycarbonate, plastic) that allow natural and artificial light energy to be used by the microorganisms (Amaral et *al*., 2020). There are various categories of PBRs (Fig.2), including horizontal , vertical tubular reactors, flat panel reactors, vertical column reactors, bubble column reactors, air lift reactors, and immobilized reactors (Devi et *al*., 2013; Płaczek et *al*., 2017; Jerney & Spilling, 2018; Pereira et *al*., 2018; Razzak et *al*., 2024).

Figure 2. Different culture types of closed system; (A) Horizontal tubular reactors (Pereira et *al*., 2018). (B) Vertical tubular reactors (Devi et *al*., 2013), (C) vertical column reactors, (D) Flat panel reactors (Jerney & Spilling, 2018).

4. Microalgae production mode:

Batch cultures, semi-continuous, and continuous methods are the three ways for microalgae production (Novoveská et *al*., 2023) in which there are differs in the way and timing of nutrient supply.

4.1.Batch or Discontinuous culture

This is a closed system in which the microalgae are inoculated once at the start of the culture into a nutrient medium containing all the nutrients required for growth. Thereafter, neither algae nor nutrient medium are added to the culture. In the stationary phase, the entire culture is collected, the container are cleaned and a new culture is started.

The growth evolution of microalgae cultured in batch cultures generally involves five successive phases (Fig.3): lag phase, exponential phase, declining relative growth, stationary phase, and death phase.

1. Lag phase: Initial period of slow growth as cells adapt to new environmental culture conditions;
2. Exponential phase: Rapid growth and cell division;
3. Declining Relative Growth phase: Occurs when the growth requirements for cell division are limited;
4. Stationary phase: Cell division slows down due to a lack of resources needed for growth;
5. Death cell phase/ lysis cell phase: Cells begin to die due to a lack of nutrients.

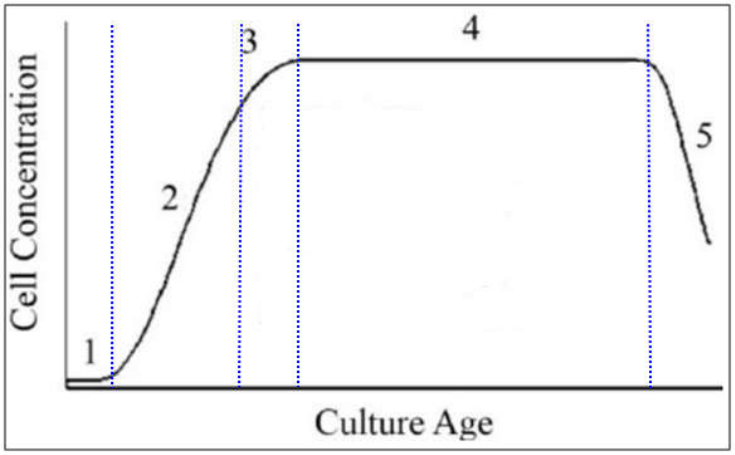

Figure 3. Growth phases in microalgae batch culture; (1) lag phase; (2) exponential phase; (3) (4) stationary phase; and (5) death phase (Price & Farag, 2013)

4.2. Semi-continuous culture

Semi-continuous culture is a culture method between batch and continuous culture, which is diluted periodically by fresh medium to provide sufficient nutrients to maintain an exponential growth state for a long time (Chen & Gao, 2021). In this method, a portion of the culture is periodically harvested, and the same volume of fresh medium is added. It keeps the culture in the exponential growth phase, resulting in higher productivity and longer cultivation time.

4.3. continuous culture :

Continuous culture maintains a constant culture volume by continuously removing a portion of the culture and adding the same volume of fresh medium at the same time.

5. Harvesting techniques

. The harvesting method intensely depends on the physiognomies of the micro algae chosen, density and size of the microalgal cell, specifications of the final product and on allow ability for reuse of the culture medium (Uduman et *al*., 2010; Amaro et *al*., 2011; Rawat et *al*., 2011).

Microalgae harvesting is considered one of the most important steps in industrial production to obtain value-added products. Dewatering microalgae accounts for 20-30% of the total costs associated with microalgae production and processing **(**Molina Grima et *al*., 2003**;** Zitelli et *al*., 2006; Al Hattab et *al*., 2015; Barros, 2015; Sahoo et *al*., 2017) . There are several methods of concentration and harvesting of microalgae, including: sedimentation, centrifugation, flocculation, flotation, and filtration (Wang et *al*., 2012). Each methods has both advantages and disadvantages **(**Table 5). As a strategy to reduce costs and energy consumption, microalgae harvesting is usually done through a combination of two or more methods to increase biomass concentration. (Barros et *al*., 2015)

Table 5. Advantages and disadvantages of different harvesting methods applied to microalgal biomass (Barros et *al*., 2015, Kucmanová & Gerulová, 2019)

Harvesting method	Advantages	Disadvantages
Chemical coagulation/ flocculation	- Simple and fast method - No energy requirements	- Chemical flocculants may be expensive - No ecofriendly (toxic to microalgal biomass - Recycling of culture medium is limited - Need supplemental treatments process to remove the toxicity which add to the production cost
Auto and bioflocculation	- Intensive method - Less cost method (cheap) - Low energy - Allows culture medium recycling - Non-toxic to microalgal biomass	- Changes in cellular composition -Ossibility of microbiological contamination
Gravity sedimentation	- Simple and inexpensive method	- Time-consuming - Low concentration of the algal cake - Possibility of biomass deterioration
Flotation	- Feasible for large scale applications - Short operation times - Low space requirement -Low cost method	-Generally requires the use of chemical flocculants - Unfeasible for marine microalgae harvesting
Electrical based processes	- Applicable to a large variety of microalgal species - Eco-friendly (do not require the addition of chemical flocculants)	- Not largely disseminated - High energetic and equipment costs

Filtration	- High recovery efficiencies (70–90%) - Allows the separation of shear sensitive species - Wide variety of filter and membrane types available, reliable, can handle delicate cells	- The possibility of fouling/clogging increases operational costs - Membrane replacement and pumping represent the major associated costs - Membranes should be regularly cleaned
Centrifugation	- Fast method - High cell recovery efficiencies (over 90%) - Suitable for almost all microalgal species	- Expensive method, energy intensive - Possibility of cell damage due to high shear forces - Suitable only for the recovery of high-valued product

6. Drying:

Drying of microalgal biomass is the most common method for marketing microalgae, as it increases product stability and durability, and facilitates storage and transportation (De Farias et *al*., 2019). There are several types of drying techniques, such as solar drying, convective drying, spray drying, and freeze-drying (De Farias et *al*., 2019) are usually required, depending on the requirements of the final products (Oliveira et *al*., 2009; Brennan et *al*., 2010; Dissa et *al*., 2010).

6.1.Solar drying:

This technique considered as the cheapest method because it based on the use of solar energy, but it requires long drying times and a large drying surface (Brennan et *al*., 2010). Moreover, it is difficult to maintain the quality of the end product with traditional open solar drying methods, and the slow drying rate due to a low temperature can cause biomass degradation and thus a rise in the bacterial count (Prakash et *al*., 1997)

6.2.Connective drying:

This method is one of the popular technique for removing water from microalgae, convective drying, is usually accomplished by distributing the microalgae biomass into thin layers in an oven, tray, or tunnel-type dryer and applying convective hot air , leading to microalgae dehydration (Kim & Kim, 2022).

6.3.Spray drying

Spray drying involves atomizing a liquid microalgae solution into small droplets, followed by a rapid drying process utilizing hot air or gas in a drying chamber (O'Sullivan et *al*., 2019).

6.4.Freeze drying:

Freeze-drying, also known as lyophilization, is an advanced and widely used technology for drying microalgal biomass (De Farias et *al*., 2019; Schmid et *al*., 2022). The microalgae is cryopreserved by subjecting them to low temperatures, and the frozen biomass is then placed in a vacuum environment. The ice sublimates from a solid to a vapor state without passing through the liquid (Foo et *al*., 2023).

Table 6. Common drying methods applied in microalgae processing (Show et *al*., 2015; Ljubic, 2020)

Drying techniques	Advantages	Disadvantages
Solar drying	Cost-effective process, No capital investment	Slow process, Difficult to control, Risk of microbial contamination
Spray drying	Rapid drying process High drying efficiency Produces homogenous powder	High capital and operational costs, Possible deterioration of heat sensitive compounds
Freeze drying	Gentle process, Preserved quality of high-value metabolites	High operating costs
Convective drying	Relatively low capital investment	Low drying efficiency Possible deterioration of heat sensitive compounds due to the long drying time

Chapter 3

Microalgae biotechnology application

1. Introduction

Microalgae are attracting a lot of interest due to its numerous uses in a variety of fields that contribute to both economic growth and environmental sustainability. Microalgae are considered the first link in the food chain in aquatic ecosystems and are used in several fields, such as animal aquaculture, human and animal nutrition (Kamani et *al.*, 2019; Deviram et *al.*, 2020), pharmaceutical and cosmetical application, bioenergy production (Anand et *al.,* 2023; Su et *al.,* 2023.) and wastewater treatment (Peter et *al.,* 2021; Abuhasheesh et *al.,* 2025).

2. Human Food and Nutrition

Microalgae are an important renewable reservoir of bioactive compounds with wide use in human nutrition (Silva et *al.*, 2025). The large diversity of microalgae provides different biochemical compositions, including proteins, polyunsaturated fatty acids (PUFAs), pigments, polysaccharides, vitamins, and antioxidants, making them valuable for functional foods and nutraceuticals (Kaur et *al.*, 2025). The incorporation of microalgae into food products has been shown to enhance nutritional profiles and confer health benefits (Mehwish et *al.*, 2023). Microalgae for human nutrition are commercialized in various forms: powders, tablets, capsules and liquids (Torky et *al.,* 2023) or are used to enrich various food products, such as biscuits, juices, breads, pasta (Hernández et *al.*, 2022), couscous, (Boukhari et *al.*, 2018), spaghetti (Fradique et *al.,* 2010), and yogurt (Albuquerque et *al.,* 2024), enhancing their nutritional value and functional properties (Hernández et *al.*, 2022).

In the last years, the most biotechnological and nutraceutical applications of microalgae have been carried out on different species of microalgae, mainly *Spirulina (Arthrospira* or *Limnospira*), *Chlorella*, *Dunaliella salina*, and *Haematococcus pluvialis* (Hemantkumar & Rahimbhai, 2019).

The *Spirulina* and *Chlorella* microalgae are the most used in nutraceutical application due to their high nutritional value (Abreu et *al.*, 2023; Ang et *al.,* 2024). They possess a high amount of protein, ranging from 52–72 % for spirulina and 42–65.5 % for Chlorella of the dry weight of the two species , with nine essential amino acids as well as saturated and polyunsaturated fatty acids (omega-3 and omega-6)[Ötleş & Pire, 2001; Grosshagauer et *al.*, 2020; Ru et *al.*, 2020], phenolic compounds, vitamins, pigments (such as phycocyanin, carotenoids, and astaxanthin), minerals, and other bioactive compounds (Čabarkapa et *al.*, 2022; Abreu et *al.*, 2023).

More than 90% of the global microalgae biomass consists of two species, *Spirulina sp*. and *Chlorella* sp. (Benemann et *al.*, 2018). With regard to market value, the *Spirulina* market is projected to reach USD 1.1 billion by 2030, exhibiting a compound annual growth rate (CAGR) of 9.4% from 2023 to 2030. The market for *Chlorella sp*. is predicted to increase at a compound annual growth rate (CAGR) of 6.3% from 2021 to 2028, to reach USD 412.3 million by 2028 (Abreu et *al.*, 2023). Some price of microalgae and coproducts were illustrated in the table 7.

Table 7. Market value of some high-value microalgae extract compared to whole microalgae.

Product	**Price range (USD kg−1)**	**References**
Phycocyanin	500 for food-grade, to 100,000 for analytical grade	Rybner, 2016
Astaxanthin	6000 to 7150	Saikia et *al*., 2023
β-carotene	350–7500	Goswami et *al.,* 2022
Astaxanthin	2500–7000	Debowski et *al.*, 2020
β-carotene	300–1500	Borowitzka, 2013
Omega-3 fatty acids	80–160	Borowitzka, 2013
Chlorella biomass	44	Barkia et *al.*, 2019
***Arthrospira* biomass**	42 *	Barkia et *al.*, 2019

3. Microalgae application as animal feed

3.1. Microalgae as feed in aquaculture

Microalgae play a crucial role in aquaculture, they are considered as the first link in the food chain in aquatic ecosystems, they are used directly to feed a wide range of aquaculture animals, especially fish in early growth stages, molluscs, shrimps, sea cucumbers and crab larvae in hatcheries (Zmora et *al.*, 2013), daphnia, artemia, rotifers and other zooplankton (Ma & Hu, 2023) or indirectly through their incorporation in the feed of aquatic animals, pets and farmed animals.

The richness of microalgae in fatty acid, proteins, amino acids and pigments makes them essential for the early growth phases of several aquatic animals, especially in hatcheries. For example, Isochrysis sp. contains high levels of docosahexaenoic acid (DHA), which is essential for feeding Artemia and other zooplankton, and Nanochlopsis is widely used for its

high levels of ecosapentaenoic acid (EPA), which is necessary for the growth and survival of fish larvae and rotifers [Paulo et *al.*, 2020].

Schizochytrium sp. has been successfully used to substitute fish oil in aquafeeds, improving the growth performance of shrimp and tilapia (Kim et *al.*, 2022). Additionally, *Tetraselmis sp*. and *Chlorella vulgaris* have shown promise in enhancing the immune responses and growth rates of a variety of aquaculture species (Vijayaram et *al*., 2024). Incorporating these microalgae into aquafeeds guarantees the supply of superior nutrition necessary for the best possible development of aquatic organisms while also encouraging sustainable aquaculture practices (Kim et *al.*, 2022). The Table 8 summarized the most species of microalgae used in aquaculture.

Table 8. Commonly Used Microalgae in Aquaculture Applications

Aquacluture	**Commonly Used Microalgae**	**References**
Fish **Nile Tilapia (*Oreochromis Niloticus*), Rainbow Trout (*Oncorhynchus Mykiss*), European Seabass (*Dicentrarchus Labrax*),** **Golden Gourami (*Trichopodus Trichopterus*),** **Cichlid (*Cichlidae*) Fish, Wag Swordtail (*Xiphophorus Hellerii*),** **Orange Molly Pink Zebra, Tetras (*Paracheirodon Axelrodi*), And Prawns (*Dendrobranchiata*)**	*Leptolyngbya valderiana*, *L. tenuis*, *Arthrospira maxima*, *Navicula minima*, *Nostoc ellipsosporum*, *Cytoseira,* *Ulva*, *Pavlova*, *Chaetoceros* , *Porphyridium*, *Chlorella*, *Palmaria*, *Gracilaria*, and *Isochrysis*	Vijayaram et *al*., 2024
Shrimps Larvae	Diatoms (e.g., *Skeletonema costatum, P. tricornutum, Conticribra weissflogii,* and *Chaetoceros gracilis*), chlorophytes (e.g., *Chlorella sp. and Tetraselmis chuii), and chrysophytes (e.g., Isochrysis sp*	Ma & Hu, 2023
Artemia	*Tetraselmis, Dunaliella, Cryptomonas, Chaetoceros, Isochrysis,* and *Rhodomona*	Le et *al*., 2019
Copepods	*Rhodomonas, Isochrysis,*	Jones & Flynn,

	Tetraselmis, Cyclotella, and *Heterocapsa*	2005
Bivalves	*Chaetoceros calcitrans, Thalassiosira pseudonana, Isochrysis galbana, Pavlova lutheri,* and *Phaeodactylum tricornutum*	Cheng et *al*., 2020.
Rotifers	example, *Chlorella, Nannochloropsis, Tetraselmis*, and *I. galbana*	Conceição et *al*., 2010

3.2. Microalgae as feed for pets and farm animals

Several recent studies have reported the use of microalgae in the diets of several animal species: cats, dogs, horses, goats**,** poultry, sheep, and cattle (Spolaore et *al.*, 2006; Souza et *al.*, 2019; Saadaoui et *al.,* 2021).The table 9, summarized some application of microalgae as animals feed.

Table 9. Summary of some applications of microalgae as animal feed

Microalgae	Application	References
Chlorella vulgaris	Supplementation with *Chlorella vulgaris* has been shown to improve reproductive performance and milk quality in goats.	Silva et *al.*,2023 Kholif et *al.*, 2016
Spirulina And Chlorella	Meat quality, immunological responses, and growth rates have all increased as a result of poultry diets enhanced with microalgae	Ross & Dominy, 1990; Toyomizu et *al.*, 2001
Schizochytrium Sp.	The use of *Schizochytrium sp.* in dairy cow diets led to omega-3 fatty acid content increasing in milk.	Jiang et *al.*, 2020

4. Microalgae and their potential health benefits

Microalgae have recently been identified as a potential source of bioactive compounds with a wide range of pharmaceutical applications, such as antioxidant, antibacterial, antifungal, antiviral, anti-inflammatory and metabolic regulatory properties(**Tab 10)**.

Table 10. Some application of microalgal biomass and its product in pharmaceutical application

Bioactive Compounds		Microalgae	Use	References
Carotenoids	β-carotene	*Dunaliella salina*	Antioxidant, pro-vitamin A,anti-allergic, anti-inflammatory	Fujitani, et *al.*, 2001 Borowitzka, 1995
			Lipid-lowering	El-Baz et *al.*, 2023
	Astaxanthin	*Haematococcus pluvialis, C. zofingiensis*	Antioxidant, anti-inflammatory	Ciccone et *al.*, 2013
		Haematococcus pluvialis	protecting against neurodegenerative diseases like Alzheimer's	Fakhri et *al.*, 2019; Kang et *al.*, 2023
	Lutein	*Scenedesmus spp., Muriellopsis sp.,C. sorokiniana*	Antioxidant, anti-inflammatory	Del Campo et *al.*, 2001 Sánchez et *al.*,2008 Cordero, 2011
PUFAs	Arachidonic acid(AA)	*Porphyridium purpureum, P. cruentum,Parietochloris incisa*	Improves normal growth, visualand functional developmentin infants	Su et *al.*,2016 Solovchenko et *al.*,2008 Giménez et *al.*,1998
	Eicosapentaenoic acid(EPA)	*Nannochloropsis sp., Phaeodactylumtricornutum, Porphyridium cruentum*	Cardiovascular benefits, mentaldevelopment and support,anti-inflammatory, protectionagainst atherosclerosis	Asgharpour, et *al.*, 2015 Molina Grima, et *al.*, 1999 Sukenik,. 1999
	Docosahexaenoic acid(DHA)	*Crypthecodinium cohnii, Schizochytriumspp., Ulkenia spp*	Cardiovascular benefits, improves nervous system development and function of the brain	Chu, 2012; Horrocks & Yeo, 1999
Other metabolites	Peptides	*Chlorella pyrenoidosa,Nannochloropsis oculata, rthrospira maxima, Tetraselmis*	antioxidant, anti-inflammatory,anticancer, antihypertensive	Wang & Zhang, 2013; Samarakoon,et *al.*, 2013.

	suecica,		
Phenolics	*Botryococcus braunii,Isochrysis sp., Chlorella vulgaris,Nannochloropsis sp*	Antioxidant	Goiris et *al.*, 2012; Cha, 2011 ; El-Baky et *al*.,2009
Phycocyanin	*Arthrospira platensis*	Antioxidant, anti-inflammatory	Romay et *al.*, 2003.
Phycocyanin, Phycobiliproteins	*Spirulina platensis*	protection against oxidative liver damage	Liu et *al.*, 2020
		antidiabetic	Li et *al.*, 2020
Sulfatedpolysaccharide	*C. pyrenoidosa, C. stigmatophora,Porphyridium sp., Phaeodactylumtricornutum*	antioxidant, anti-inflammatory,anti viral, immunomodulatory	Guzmán et *al.*, 2003; Huheihel et *al.*,2002; Tannin-Spitz, et *al.*, 2005
Water-soluble extract"	*hlorella stigmatophora, Phaeodactylumtricornutum, Graesiella sp*	nti-inflammatory, analgesic,antioxidant, antiproliferative	Guzmán et *al.*, 2003;
Methanolic extracts Phenols, Fatty acids	*Chlorella*	antibacterial effects against both Gram-positive and Gram-negative bacteria	Shaima et *al.*, 2022
Fucoidan	brown algae	antiviral activities by inhibiting viral replication and preventing virus-host cell interactions	Ahmadi et *al.*, 2023
Fucoidan, Sulfated galactan	Red algae	Antifungal	El Gamal, 2010

5. Microalgae application in Environment:

Microalgae production utilizing wastewater provides the greatest atmospheric carbon sequestration rate (1.83 kg CO2/kg of biomass) and best biomass productivity (40–50% more than that of land crops), and the pollutant removal varies from 80–100% (Shahid et *al.*, 2020).

Microalgae can be used in the treatment of agricultural, aquacultural, industrial, and pharmaceutical wastewater **(Tab 11)**.

Table 11. Applications of microalgae in environment-protecting technologies (Dębowski et *al.,* 2020)

Sector	**Use**		**References.**
Wastewater treatment	Nitrogen and phosphorus removal from municipal wastewater		Gomez Villa et *al.*, 2005
	Biodegradation of sparingly degradable pollutants		Chojnacka et *al.,* 2005 ; Mùnoz et *al.,* 2006
	Treatment of organic wastewater		Mùnoz et *al.*, 2009
	Treatment of hard-to-manage wastewater produced by	timber and paper industry	Tarlan et *al.*, 2002 Yewalkar et *al.*,2007 ;
		Textile industry	Acuner & Dilek,, 2004
		phenol industry	Lima et *al.*, 2004; Essam et *al.*, 2007
	Ethanol and citric acid production		Valderramaa, et *al.*, 2002
	Removal of heavy metals (copper, nickel, lead) from wastewater		Tien, 2002 Chojnacka et *al.*, 2005,
Gas treatment	Reducing emissions of carbon dioxide and other pollutants (nitrogen and sulfur oxides) from waste and exhaust gases		Jacob-Lopes et *al.*, 2010.
Waste management	Use of waste glycerol as a carbon source in heterotrophic cultivation		Lam et *al.*, 2012
	Microalgae cultivation using industrial waste or low-value feedstocks, such as	Bread crumbs	Thyagarajan, et *al.*, 2014
		brewer's spent yeast	Ryu, et *al.*, 2012
		Coconut water	Unagul et *al.*, 2007
		Empty palm fruit bunche	Hong et *al.*, 2013
Leachate treatment	Biodegradation of landfill leachates		. Lin et *al.*, 2007
	Neutralization of degraded effluent from		Jedynak et *al.*, 2018

	anaerobic fermentation of sewage sludge	
Biogas upgrading	Biological sequestration of CO2 with photosynthetic microalgae (photosynthesis allows producing biogas with 94% methane content)	Yan & Zheng, 2013

6. Microalgae application in Cosmetics and Skin Care

Microalgae are a valuable resource in cosmetic biotechnology, containing a range of bioactive compounds beneficial for skin and hair care and sun protection. Species such as *Spirulina*, *Chlorella*, *Dunaliella*, and *Haematococcus* are among the most well-known algae in these applications due to their richness in antioxidants, pigments, and other bioactive compounds (Borowitzka, 2013).

There are numerous cosmetic products made from microalgae, the most important of which are anti-aging creams, refreshing and regenerating care products, emollients and anti-irritants in peelers, sunscreens, and hair products skincare, hair care, and sun protection face masks, body lotions, face and hand creams (**Tab 12**).

Table 12. Major microalgae used in cosmetically industry and their applications (Kholssi et *al*., 2021).

Species	Applications	References
Spirulina spp.	Improves moisture balance of skin, strengthens skin's immunity, Improves skin complexion and reduces wrinkles, Cosmetics (eye shadow)	Chakdar et *al.*, 2012
Spirulina sp.	Moisturizing, anti-aging, anti-acne, skin pigmentation treatment, wound healing, sunscreen	D'Angelo Costa & Maia Campos, 2024
Chlorella vulgaris	Stimulate collagen synthesis in the skin, give support to skin tissue and increase rebirth of the new-tissue	Yarkent et *al.*, 2020
Chlorella vulgaris	Antioxidant, skin regeneration, collagen synthesis, UV protection	Ganeson, et *al.*,2024
Arthrospira maxima	Strengthens skin's natural protection and regeneration	Yarkent et *al.*, 2020
Dunaliella salina	Photoprotection, anti-aging, antioxidant properties	da Silva et *al.,* 2021 Sousa et *al.*,2024
Haematococcus	Rich in astaxanthin; offers potent	Ruiz-Domínguez et ,

pluvialis	antioxidant properties and UV protection.	2019
Anacystis nidulans	Protects the skin against sun damage and strengthens skin's immunity	Santos et *al.*, 2019
Halymenia durvillei	Improves radiance and skin luminosity	Ryu et *al.*, 2015
Nannochloropsis oculata	Excellent skintightening properties (short- and long-term effects)	Ariede et *al.*, 2017
Dunaliella salina	Stimulates cell proliferation and turnover and positively influences the energy metabolism of skin	Yarkent et *al.*, 2020
Spirulina platensis	Help combat early skin aging, exerting a tightening effect and preventing wrinkle formation	Yarkent et *al.*, 2020
Anacystis nidulans	Strengthens skin's immunity	Yarkent et *al.*, 2020
Porphyridium spp.	Improves the characteristics of dry or aged facial skin and may prolong the effect of moisturizer	Majee et *al.*, 2017
C. vulgaris	Stimulate collagen synthesis in skin supporting tissue regeneration and wrinkle reduction	Ryu et *al.*, 2015
Porphyra spp., Porphyridium spp	Cosmetics (face powder cake, eye shadow)	Ariede et *al.*, 2017

7. Microalgae application in Agriculture

Microalgae have garnered considerable attention in sustainable agriculture due to their numerous advantages, including serving as biofertilizers, biostimulants, sources of phytohormones, agents for soil improvement, tools for biological treatment, enhancers of plant disease resistance, and mitigators of abiotic stress.

Current global agricultural activities highly depend on synthetic fertilizers and pesticides, with environmental and health impacts (Tripathi et *al*., 2020). To reduce the impact of chemical fertilizers on the environment, the use of biofertilizers has emerged as an eco-friendly alternative. Interest in the use of microalgae in the agricultural industry has increased in recent years due to their high potential for biostimulant and biofertilizer properties (Shaaban, 2001; Khan, et *al.* 2009; Shaaban, et *al.* 2010; Garcia-Gonzalez & Sommerfeld, 2016; Ronga, et *al*., 2019). In addition to their using in different applications, such as amendment, foliar application (Suchithra, et *al*., 2022), and seed priming (Elhafiz et *al.*, 2015;

Ronga, et *al.*, 2019), enhanced root development, which facilitates beter water and nutrient absorption (Barone, et *al.*, 2018).

Numerous studies have reported the presence of phytohormones such as auxins, cytokinins, abscisic acid, ethylene, and gibberellins in microalgae extracts, which influence plant growth and development (Karthikeyan, et *al.* 2007; Stirk & Ördög et *al.,* 2013a; Stirket *al.,* 2013b). In addition, these substances have the ability to influence metabolic activities such as photosynthesis, respiration, nucleic acid synthesis, and nutrient absorption (Tarraf et *al.,* 2015).

Microalgae extracts can be used to protect plants from abiotic stresses (Abd El-Baky et *al.*, 2010; Kapoore et *al.*, 2021), reduce salinity stress such as in the bell pepper seed during the germination process (Guzmán-Murillo et *al.*, 2013), improve wheat tolerance to salinity (Abd El-Baky et *al.*, 2010).

8. Microalgae application in Bioenergy production

Microalgae are used as a versatile, renewable and sustainable feedstock for bioenergy production (Demirbas & Demirbas, 2011), offering multiple conversion pathways and environmental advantages. Microalgae have recently been used to produce a variety of bioenergy products, including biodiesel, bioethanol, biogas, biohydrogen, and bioelectricity (Bora, et *al*., 2024). Biodiesel production from microalgae involves lipid extraction followed by transesterification; the favorite microalgae species for diesel production are those with high lipid content (Choudhary et *al.*, 2022). The use of microalgal carbohydrates for bioethanol production involves three main stages: pre-treatment, saccharification, and fermentation (Velazquez-Lucio et *al.*, 2018; Maity & Mallick, 2022). Biogas production from microalgae is involves via Anaerobic digestion , which is a biochemical process, in which specialized anaerobic bacteria decompose organic matter and produce biogas containing methane and carbon dioxide (Harun et *al*.,2010).

Biohydrogen production from microalgae is achieved through several methods, including direct photobiolysis, indirect photobiolysis, photofermentation, and dark fermentation (Li et *al.*, 2022). Bioelectricity may be produced using bioelectrochemical systems, in particular photosynthetic microalgae microbial fuel cell systems (Qin et *al*., 2024).

Part 2 :

Experimental Part

Chapter 1

Sampling, isolation and identification

1. Introduction

Microalgae are a diverse group of photosynthetic microorganisms inhabiting various aquatic environments, from freshwater to hyper saline ecosystems. Local microalgae are often well-adapted to the regional climate and environment, making them ideal candidates for local production (Lee et *al.*, 2014).

Morphological characterisation of purified microalgae using light and electron microscopy reveals cell structure, size and arrangement, which is helpful for species identification. However, environmental conditions can influence morphological features, necessitating molecular approaches for correct classification. Molecular studies, such as 16S rRNA gene sequencing of prokaryotes and 18S rRNA gene sequencing of eukaryotes, are essential in determining the evolutionary relationships among microalgae.

The isolation, purification and identification of local microalgal strains, as well as their preservation, facilitates the establishment of a local microalgal culture collection. This ensures the conservation of endemic species, encourages the exploration of other species, and provides interested researchers and investors with local species for various biotechnological applications.

This study aimed to isolate native microalgal species from two Lakes located in northwest Algeria , and then identify, and preserve three local microalgal strains(*Limnospira fusiformis* TL03, *Chlorella sp.* CHL11, *Chlorella sp*. CHL15) for short and medium term as a first step to establish a culture collection (bank of microalgae) of local microalgae.

2. Materials and methods

2.1. Sampling site description

Water sampling was conducted in April 2018 from two lakes, Telamine (Fig. 4 A-C) and Gharabas Lake (Fig. 4 D-F), located in northwestern Algeria. Samples were collected from less than one metre below the surface of the water bodies using a phytoplankton net with a pore size of 50 μm.

a) Telamine lake

Telamine brackish water Lake is located in northwest Algeria, approximately 20 km East of Oran city and exactly northwest of the Arzew salt flats. It is approximately 8.5 km long and 0.5 to 1.5 km wide (Fig. 4A, Table 13). The Telamine Lake has a surface area of 2.399 ha and a perimeter of 20 km. The majority of its water is alkaline, with a pH range of 7.43 to 8.95

(Hadjadj, 2018) and a salinity was 15 mg/l. Telamine Lake is a protected wetland (Ramsar list on 12/12/2004) due to its high animal and plant biodiversity.

b) Gharabas Lake

Lake Gharabas is located in north-western Algeria, about 27 kilometres south-east of Oran (Fig. 4D-F, Table 13). The pH range of 7.31 to 8.54 and salinity 3 mg/l

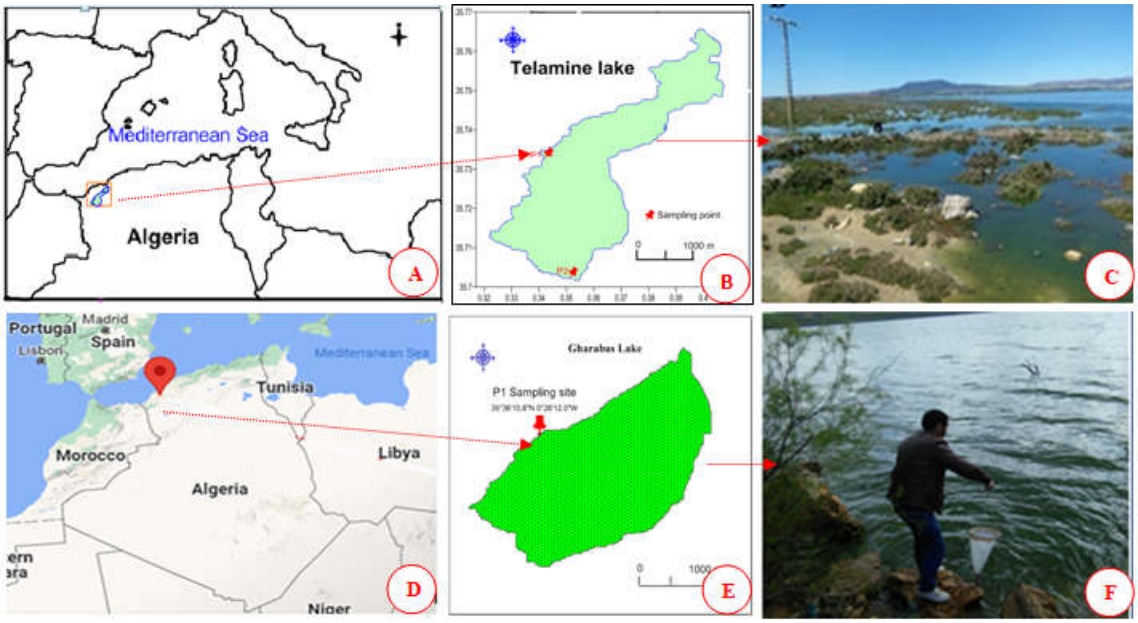

Figure 4. Sampling sites location (A-B) Site geographic location of Telamine Lake. (C) Phytoplankton bloom in Telamine Lake , (D-E) Site geographic location of Gharabas Lake . (F) Phytoplankton bloom in Gharabas Lake (Original, April, 2018).

Table 13. Geographic coordinates of the sampling points

Lake name	**Wilaya**	**Geographical coordinates of the sampling points**
Telamine Lake	Oran	p1 (35°43'53.9 "N 0°23'17.7" W)
		p2 (35°42'12.9 "N 0°23'38.2" W)
Gharabas Lake		35°36'10.8 "N 0°26'12.0" W

2.2.Sampling :

Water samples were collected from approximately 30 cm below the surface using a phytoplankton mesh nets with a mesh size of 50 µm. The geographical coordinates of the sampling points are shown in the Table 13.

The samples were immediately transported to the microbiology laboratory (National Centre for Research and Development of Fisheries and Aquaculture: CNRDPA) for their treatment

2.3. Microalgae isolation:

The samples collected were subjected to physical treatments such as filtration, centrifugation and streak plating on solid BG11 medium (Allen & Stanier, 1968) [see Appendix 1], as well as chemical treatments such as the application of antibiotics, in order to isolate and purify the microalgae.

2.3.1. *Limnospira* fusiformis TL03 isolation

As a first treatment step, the sample taken from Telamine Lake was centrifuged at 1,000 rpm for 2 minutes to remove unwanted microorganisms and solid residues from the bottom of the sample. The supernatant was collected and washed with sterile BG11 medium using a phytoplankton tissue with a 50 µm mesh pore size to allow passage of smaller microorganisms while retaining those larger than the pore size. The filtrate retained in the mesh was added to 20 ml of sterile liquid BG11 medium.

One hundred microliters of the sample were aseptically spread on the surface of Petri dishes containing BG11 sterile solid media with different NaCl concentrations (0, 5, 15, and 25 g/l), and the pH was adjusted to 9.5. The plates were incubated at room temperature and illuminated 24 hours a day with 1 Klux light emitting diodes (LEDs, ABALIGHT 18 W, 220V, 50 HZ).

Individual colonies formed on the surface of the agar medium were picked up and streaked in fresh, similar solid medium under aseptic conditions. Individual colonies were separately transferred to 50 ml Erlenmeyer flasks containing 20 ml of BG11 culture medium and incubated under the same conditions as above

- **Cycloheximide treatment**

In order to eliminate eukaryotic microalgae from the mixed algal culture, chemical treatment was performed by adding the cycloheximide to the liquid culture medium (100 mg/l) for one week (Prasad et *al*., 2013).

- **Streak plating**

An aliquot of 100µL of the diluted *Limnospira* sample was spread with a needle on BG11 solid medium (containing 1.5% agar) and incubated at room temperature with continuous illumination at 1 Klux for 4 weeks. Individual colonies were inoculated into Erlenmeyer flasks containing 20 ml of sterile BG11 at pH 9.5.

- **pH treatment**

pH treatment ; After two weeks under the same incubation conditions as above, a further step was performed to remove Microcystis from the culture using the pH treatment technique (Sena et *al*., 2011).

The cultures were subjected to a pH treatment in which their pH was adjusted to 12.5 by using a 10 M NaOH solution and then incubated for 72 hours. Samples were then taken for microscopic examination to confirm that the cyanobacterium of interest had been isolated.

2.3.2. *Chlorella* (*Chlorella sp*. CHL11 and *Chlorella sp*. CHL15) isolation

Physical and chemical treatments were carried out on the samples taken from Gharabas Lake to isolate *Chlorella sp*. CHL11 and from Telamine Lake to isolate *Chlorella sp*. CHL15

Volume of 100 ml from each sample was filtered with phytoplankton tissue with a 50 µm mesh pore size to remove unwanted microorganisms and large particles of solid debris, and then centrifuged at 1000 rpm for 2 minutes. The upper floating layer was discarded and the lower suspension was washed twice with sterilized BG11 liquid medium by centrifugation at the same speed and time as above.

As a second step; one hundred microliters from each sample was streaked onto the surface of Petri dishes containing BG11 solid media with pH adjusted previously to 8. The plates were incubated for one week at room temperature under continuous illumination with intensity of 1 Klux. When the colonies formed were streaked separately into fresh solid medium .These process were repeated three times in order to isolate targeted species

When the colonies formed were transferred separately to 50 ml Erlenmeyer flasks containing 20 ml of BG11 culture medium and incubated under the same conditions as above.

2.4. Microalgae purification:

2.4.1. *Limnospira* fusiformis TL03

To eliminate bacteria present in the culture, antibiotic treatment was performed for 48 h by adding a cocktail of antibiotics to warm BG11 solid medium at the following concentrations: 112.5 µg/ml for ampicillin, cefoxitin and penicillin G and 37.5 µg/ml for meropenem (Sena et *al*., 2011).

2.4.2. *Chlorella sp.* CHL11 and *Chlorella sp.* CHL15.

The antibiotics cocktail fromed from ampicillin, cefotaxime and carbendazim were added to warm BG11 solid medium at concentrations of 700 µg/ml, 200 µg/ml and 0.1 µg/ml respectively (Mustapa et *al.,* 2016) before being poured into Petri dishes. After the medium solidified, 100 µl of the diluted culture was streaked on the surface in a zigzag pattern to separate individual algal cells and then incubated for 1 week at room temperature with continuous illumination at 1 Klux.

Macroscopic and microscopic observation of the culture was performed after each treatment to verify culture purity.

2.5. Microalgae identification :

Microalgae identification was based on the both morphological and molecular characteristics to identify the isolated strains.

2.5.1. Description and morphological measurements of the isolated strains

2.5.1.1. *Limnospira* fusiformis TL03

Using a Zeiss microscope connected to a camera (CMEX1, DC 1300C), the isolates were observed and examined directly to try to detect the *Limnospira* strain morphologically similar to the TL03-like isolate, as described in the literature (Komárek & Anagnostidis, 2005; Sili *et al.*, 2012; Nowicka-Krawczyk *et al.*, 2019). The morphological properties of 610 filaments (trichomes) were measured using the method depicted in Figure 5.

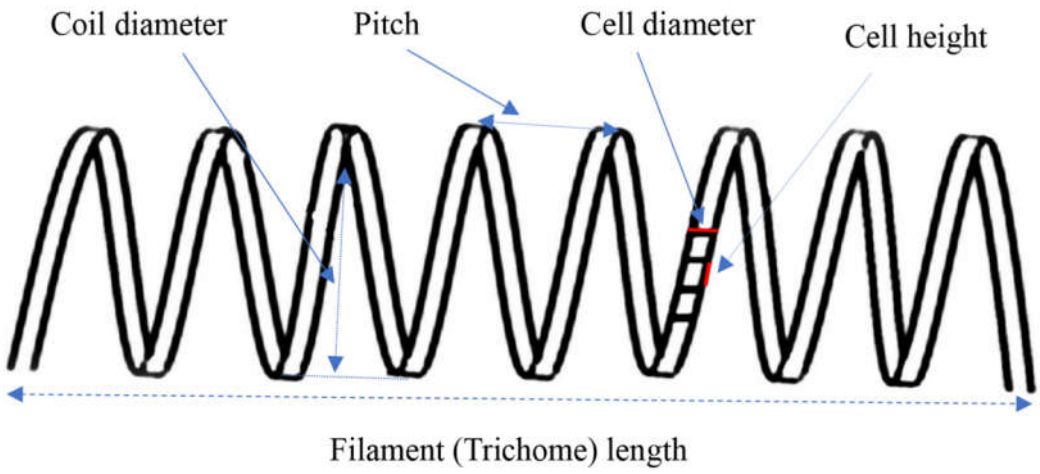

Figure 5. Schematic diagram for cell and filament morphological measurements of *Arthrospira* (Kaggwa *et al.*, 2013).

The diameter and the length of the cell were measured at 1000 X magnification, the coil diameter and pitch at 400 x magnification, and the filament length at 100 X.

2.5.1.2. Morphological identification of *Chlorella sp.* CHL11 and *Chlorella sp.* CHL15

Suspended cultures of the two studied species of *chlorella* sp. were examined at a magnification of ×1,000 using a Zeiss microscope connected to a camera (CMX1, DC 1300C) for morphological identification.

2.5.2. Molecular identification

The DNA extraction, PCR purification, and sequencing were conducted by Roscoff Culture Collections (Sorbonne University).

2.5.2.1. DNA extraction

Two mL of pure microalgae culture were mixed with 2 µL of Poloxamer (https://www.sigmaaldrich.com/catalog/product/sial/p2164009?lang=fr&region=FR). After 10 min of centrifugation at 11,000 g, the supernatant was removed, and the DNA was extracted according to the kit's instructions: http://www.mn-net.com/media/pdf/5b/d0/d9/Instruction-NucleoSpin-Tissue.pdf.

2.5.2.2.PCR amplification and sequencing

- ✓ ***Limnospira* fusiformis TL03**

The PCR amplification (polymerase chain reaction) for 16S rRNA gene fragments was carried out using the universal bacterial primers 27F (5′- AGA-GTT-TGA-TCC-TGG-CTC-AG -3′) and 1492R (5′- GGT-TAC-CTT-GTT-ACG-ACT-T -3′) (Lane, 1991). The PCR reaction mixture contained 15 µl of Phusion, 0.9 µl DMSO, 0.75 µl of each primer, 11.6 µL of deionized water, and 1 µl of DNA genome for a total volume of 30 µl of the reaction mixture. The PCR conditions were as follows: 1 cycle at 98 °C for 30 s, followed by 35 cycles of 98 °C for 30 s, 58 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. PCR was used to amplify the 16SrRNA gene sequence from isolated genomic DNA using the Applied Biosystems GeneAmp PCR 9700 Thermal Cycler. PCR products were examined on 0.8% agarose gel stained with ethidium bromide, and the migration was performed in an electrophoresis chamber. The lecture was done on an ImageQuant Las 4000 (GE HealthCare). The PCR products were purified using Exosap-it PCR product clean-up (https://www.thermofisher.com/fr/fr/home/life-science/sequencing/sanger-sequencing/sanger-sequencing-kits-reagents/exosap-it-pcr-product-cleanup.html) and reamplified using primers specific to cyanobacteria: forward primer 359F (GGG GAA TYT TCC GCA ATG GG) and reverse primer 781R(GAC-TAC-WGG-GGT-ATC-TAA-TCC-CWT-T) (Nubel *et al.*, 1997; Lau *et al.*, 2005).

Exosap mixture for 1 primer contains 2 µl of the PCR gene product, 1 µl ExoSap-IT, and 2 µl Eau millliQ, incubated at 37 °C for 4 min and 80 °C for 1 min and directly sequenced in both directions with primers 27F, 359F, 1492R, 781R. The sequencing was performed by Macrogen (https://dna.macrogen-europe.com/eng/). The sequences were assembled in the software Geneious.

- ✓ ***Chlorella sp.*** **CHL11 and** ***Chlorella sp.*** **CHL15**

The PCR amplification (polymerase chain reaction) for 18S rRNA genes fragments was carried out using forward SA (5′ AAC-CTG-GTT-GAT-CCT-GCC-AGT 3′) and reverse SB (5′ TGA-TCC-TTC-TGC-AGG-TTC-ACC-TAC 3′) primers (Lane, 1991). The PCR reaction mixture consisted of 15 µl of *Phusion* taq polymerase, 0.9 µl DMSO, 0.75 µl of each primer, 11.6 µL of ddH_20 and 1 µl of genomic DNA for a total volume of 30 µl. The PCR program was set as follow: a precyclying heat activation at 98 °C for 30 s followed by 35 cycles of 98°C for 10 s, 56°C for 30 s, 72 °C for 1 min; and a final extension at 72°C for 10 min. PCR products were examined on 0.8 % agarose gel.

The PCR products were purified using Exosap-it PCR product clean-up (https://www.thermofisher.com/fr/fr/home/lifescience/sequencing/sanger.sequencing/sanger-sequencing-kits-reagents/exosap-it-pcr-product-cleanup.html) and re-amplified using the following cyanobacteria-specific primers : forward 359F (5’ GGG-GAA-TYT-TCC-GCA-ATG-GG 3’) and reverse 781R (5’ GAC-TAC-WGG-GGT-ATC-TAA-TCC-CWT-T 3’) (Nubel et *al*., 1997; Lau et *al*.,2005). The Exosap mixture for 1 primer contained 2 µl of PCR gene product, 1 µl ExoSap-IT and 2 µl millliQ water, incubated at 37 °C for 4 min then 80 °C for 1 min before being directly sequenced in both directions. The sequencing was performed by Macrogen (https://dna.macrogen-europe.com/eng/). The sequences were assembled using Geneious ver. 8.0 (http://www.geneious.com).

2.5.2.3. Phylogenetic analysis

- ✓ ***Limnospira*** **fusiformis TL03:**

Using the Basic Local Alignment Search Tool (BLAST), the 16S rRNA gene sequences were aligned with reference sequences obtained from the National Center for Biotechnology Information database (NCBI database, http://www.ncbi.nlm.nih.gov/). The alignments were manually checked and edited in the BioEdit sequence alignment editor (version 7.2.6.1).

The neighbor-joining (NJ) algorithm and bootstrap resampling (1000 replicates) were used for phylogenetic analysis and tree construction. Next to the branches is the percentage of replicate

trees (numbers at nodes) in which the associated taxa clustered together in the bootstrap test (Felsenstein, 1985). The MEGA package database was used to build the phylogenetic tree, and the ClustalW algorithm with the MEGA version was used (Kumar *et al.*, 2018). The evolutionary distances were calculated using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in base substitutions per site (Bar, 0.01 nucleotide substitution per nucleotide position). The tree was established using *Planktothrix agardhii* NIES-204^{T} as an outgroup. The 16S rRNA gene sequences were entered into the GenBank database..

- ✓ ***Chlorella sp.*** **CHL11 and** ***Chlorella sp.*** **CHL15**

The 18S rRNA gene sequences of the selected strains were used for the molecular identification and evolutionary relationships investigation. Each sequence was blasted using MegaBLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) against the NCBI 18S rRNA using type material database. The closest (non-redundant) hits and an outgroup (*Ostreococcus tauri* OTH95) were used for the phylogenetic analysis, using MEGA11 (Tamura et *al*.,2021). 18S rRNA gene sequences were aligned using the Muscle algorithm (Edgar RC, 2004). The multisequence alignment bloc was trimmed by removing flag sequences, prior to phylogenetic analysis. The evolutionary history was inferred by the Maximum Likelihood method based on the Kimura 2-parameter model with a discrete Gamma distribution (+G), assuming that a certain fraction of sites are evolutionarily invariable (+I) (Kimura, 1980).

The tree with the highest log likelihood (-2596.96/-5643.14) was shown. The branch-support values were evaluated based on bootstrapping using 100 re-sampling. Initial tree(s) for the heuristic search were obtained by applying BioNJ and Neighbor-Join algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) method, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites (5 categories (+G, parameter = 0.1234/0.1445)). The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 42.35% / 40.79% sites). The analysis involved 25/30 nucleotide sequences. All positions containing gaps and missing data were eliminated. There were a total of 863/1575 positions in the final dataset. The final tree arranged using iToL (Letunic & Bork, 2019).

2.6. Preservation :

There are various preservation techniques used for the storage of microalgae (prokaryotic and eukaryotic), such as periodic transfer, freeze drying, and cryopreservation (Arguelles et *al.* 2020).

In this study, the periodic transfer technique was used to preserve *L. fusiformis* TL03, *Chlorella sp.* CHL11 and *Chlorella sp.* CHL15.

After checking the purity of the colonies, a single colony was transferred to a 50 ml Erlenmeyer flask containing 20 ml of fresh BG11 medium and incubated for 2 weeks at 25°C under continuous light (2.5 Klux). When the suspension looked green, it was subcultured into fresh solid and liquid media for preservation.

2.6.1. Preservation in solid medium:

Two methods were used, the first being preservation in Petri dishes containing BG11 and the second using glass test tubes containing the same solid medium. The pH of the culture media was previously adjusted to 9.5 for *L. fusiformis* TL03 and 8 for *Chlorella sp.* CHL11 and *Chlorella sp.* CHL15

- ✓ **Preservation in glass test tubes:**

A 10 ml volume of warm sterilized BG11 solid medium was poured into sterile glass test tubes and placed in an inclined position until solidified. A 100 µl aliquot of pure microalgal culture in the exponential growth phase was taken and then spread on the agar surface with a Pasteur pipette under aseptic conditions. The tubes were sealed and incubated for two weeks at 25°C with continuous illumination (2.5 Klux). When the colonies were formed, the culture tubes were stored in the dark at 4°C.

- ✓ **Preservation in petri dishes:** The same process was applied for the preservation in Petri dishes, such as glass test tubes, under the same conditions.

2.6.2. Periodic transfer from liquid to liquid medium (serial subculture)

Conventional serial subculture was performed by transferring a sample of a pure culture in the exponential growth phase to fresh sterilized culture medium (1volume of culture was added to 4 volume of fresh medium) at a subculture rate of one every two weeks. Incubation was carried out under the same incubation conditions as for the microalgae cultured in glass test tubes.

3. Results

3.1. Isolation, purification and microalgae morphology

3.1.1. *Limnospira fusiformis* TL03

3.1.1.1. Isolation

In order to determine the steps to be followed to isolate and purify *Limnospira*, a preliminary macroscopic and microscopic examination of the collected samples was carried out to know their content of microorganisms. The investigation revealed the presence of various suspended organic matter, Artemia, bacteria, and other microorganisms.

Most of the suspended solids and microorganisms were separated from *Limnospira* and other microalgae by low speed centrifugation (1000 rpm), where the bottom layer was discarded while the top layer containing filamentous cyanobacteria, other microalgae species and bacteria was re-centrifuged several times with distilled water. The number of microorganisms smaller than the mesh pore diameter was reduced by washing the upper layer of the sample with sterilized culture medium and its filteration through the net.

Under a photonic microscop we observed a mixture of phytoplankton that included Cyanobacteria (*Arthrospira sp*., *Phormidium sp.*, and *Microcystis sp*.), Diatoms *(Navicula sp*.), Chlorophytes (*Chlorella sp*.) and, others microalgae.

After ten days of incubation of the sample, it was observed with the naked eye that colonies of different colors and shapes were formed on the surface of the solid culture medium. (Fig.6 A), Their examination under the light microscope revealed the presence of a mixture of green, brown microalgae and, filamentous species (Fig. 6 B).

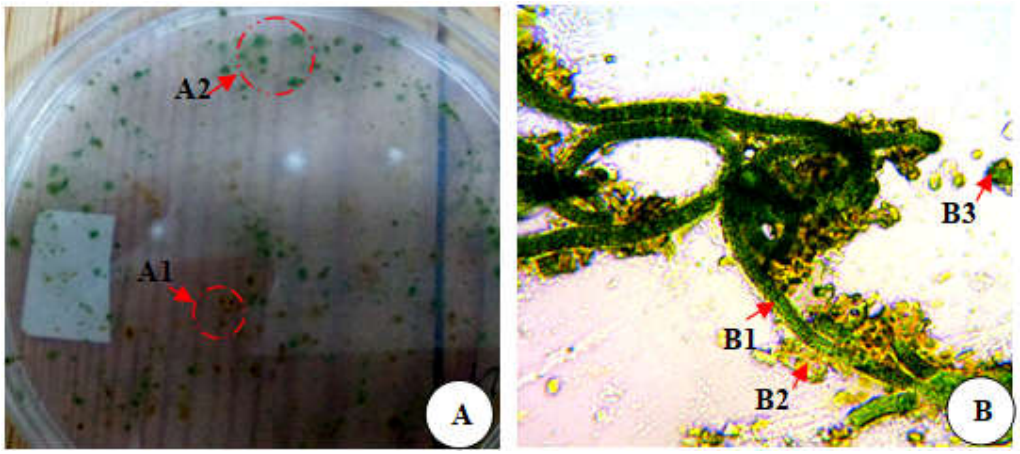

Figure 6. A-B Visual observation of mixed colonies (A1: brown colonies, A2: green colonies) formed on the surface of BG11 solid medium after 10 days of incubation and microscopic observation showed several species (B1) filamentous species, (B2) brown species, and (B3) green species (400 × magnifications).

Microscopic examination showed that many species were eliminated after treating the microalgae culture mixture with cycloheximide; however, two species of algae, *Limnospira sp* and *Microcystis sp*, in addition to bacteria were observed (Fig. 7A).

Based on these results, a further treatment step was carried out by raising the pH to 12.5, which proved to be very effective in eliminating the brown microalgae (*Microcystis sp*.) and reducing the presence of bacteria (Fig. 7B2).in the treated microalgae culture(Fig. 7B).

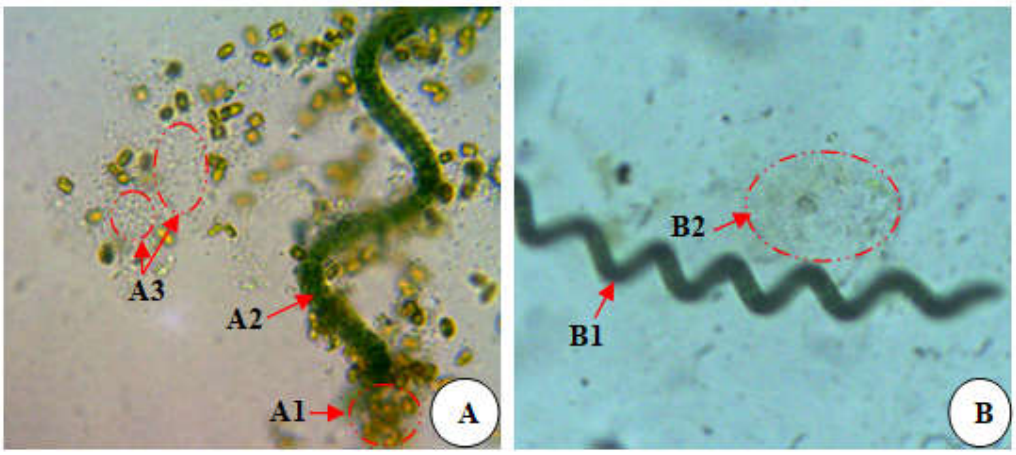

Figure 7. A-B microscopic observation of treated culture after cycloheximide and pH treatment respectively .(A1) *Microcystis sp*, (A2) *Limnospira sp*,(A3) Bacteria,(B1) *Limnospira sp*., (B2) Bacteria(400X)

3.1.1.2. Purification

To obtain a pure culture of *L. fusiformis TL03*, antibiotic treatment was used. Microscopic examination results of culture after treatment with a combination of four antibiotics showed the purity of *L. fusiformis* TL03 culture (Fig 8 A-B).

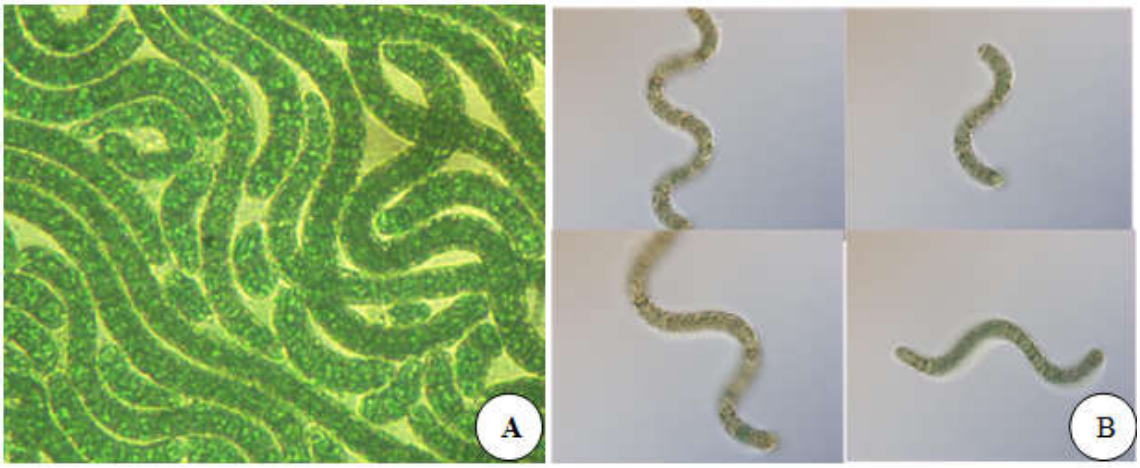

Figure 8. A-B, Pure culture of *L. fusiformis* TL03 after antibiotic treatments (400X)

3.1.1.3. Morphological characteristics of the isolated strain

Based on the microscopic examination of the strain isolated from the current study, we recorded several characteristics and morphological features, among which a blue-green color, filamentous form, and unbranched and free-floating trichomes. The filaments are motile, gliding along their axis. The filaments are divided by the cross-wall of the cell into multiple cylindrical cells arranged in trichomes in an open helix with a regular helical coil, gradually narrowing slightly towards the ends (Fig. 9 A). Morphological modification from loosely coiled trichomes to tightly reversed coils was observed. The isolated strain showed various degrees of coiling, from loosey coiled S-type (Fig. 9 A) to intermediate C-type (Fig. 9 B-C) to highly coiled H-type (Fig. 9 D). Cell cross-walls (Fig. 9 E1), calyptras at the end of the trichome (Fig. 9E2), and gas vacuoles (Fig. 9F1) were visible under a light microscope.

The results of the measurements of 610 filament morphology indicate that the length, diameter, and pitch of the trichome range from 210 to 2027 µm (Fig. 9 G-H), 20 to 60 µm (Fig. 9I1-J1), and 30 to 137 µm (Fig. 9 I2-J2), respectively The diameters and lengths of the trichome's cells range from 7 to 9 µm (Fig.9 K1-L1) and 4 to 6 µm (Fig. 9 M1-N1), respectively.

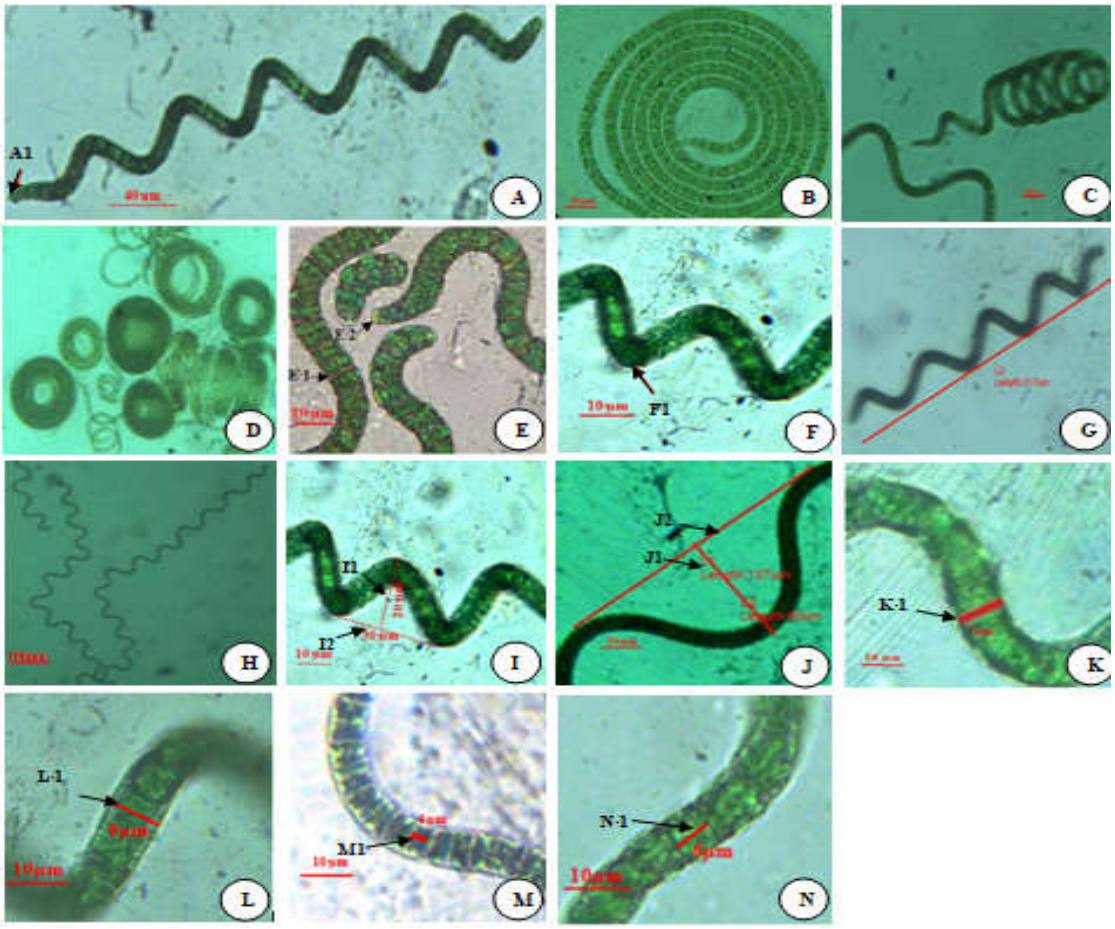

Figure 9. A-N Microscopic view of various morphological features of the isolated *Limnospira fusiformis* TL03 strain. (A.1) Trichome type C with attenuation at the end (100 X). (B-C) Tightly coiled trichome (400 X). (D) Tightly coils H shape (100X). (E.1) Cell cross wall. (E2 2) Caliptra at trichome end (1000 X). (F1) Gas vacuole (1000 X). (G-H) Smallest and tallest trichome with loose coils (100 X). (I1) Small coil diameter (400 X). (I2). Small pitch (400 X). (J1) Large coil diameter (400 X). (J2). Large pitch (400 X). (K1-L1). Cell diameter. (M1. N1) (1000 X) Cell length (1000 X).

3.1.2. *Chlorella sp.* CHL11 and *Chlorella sp.* CHL15

3.1.2.1. Isolation.

Microscopic examination of a samples collected from the two lakes (Telamine and Gharabas Lake) revealed the presence of a numerous microorganisms, including several green microalgae, diatoms and filamentous microalgae. Some filamentous microalgae and particles larger than 50 µm were removed with a net, and some floating microalgae were removed from the upper layer after centrifuging the two samples at low speed.

After a week of incubation of the streaked solid culture medium, variously shaped and coloured colonies were seen on its surface. These included small, circular, brown ones, as well as green ones with white fungal spots and filamentous algae . Individual green colonies were transferred into fresh BG11 solid culture medium twice in succession for the purpose of algal isolation.

During the third streaking of the plate, after 5–10 days of incubation, small green colonies formed along the streak lines. These colonies exhibited the characteristic morphology of Chlorella: small, round and raised with a light green colour for the CH15 strain and a dark green colour for the CHL11 strain. Through examination, it was confirmed that two species of Chlorella were successfully isolated from the two samples taken from Lakes Telamen and Garbas, where a monoalgal culture was obtained in each culture medium.

3.1.2.2.Purification:

Macroscopic and microscopic examination of colonies treated with an antibiotic cocktail showed the absence of the bacterial or fungal contamination in the culture media of CHL11 and CHL15 strains (Fig. 10).

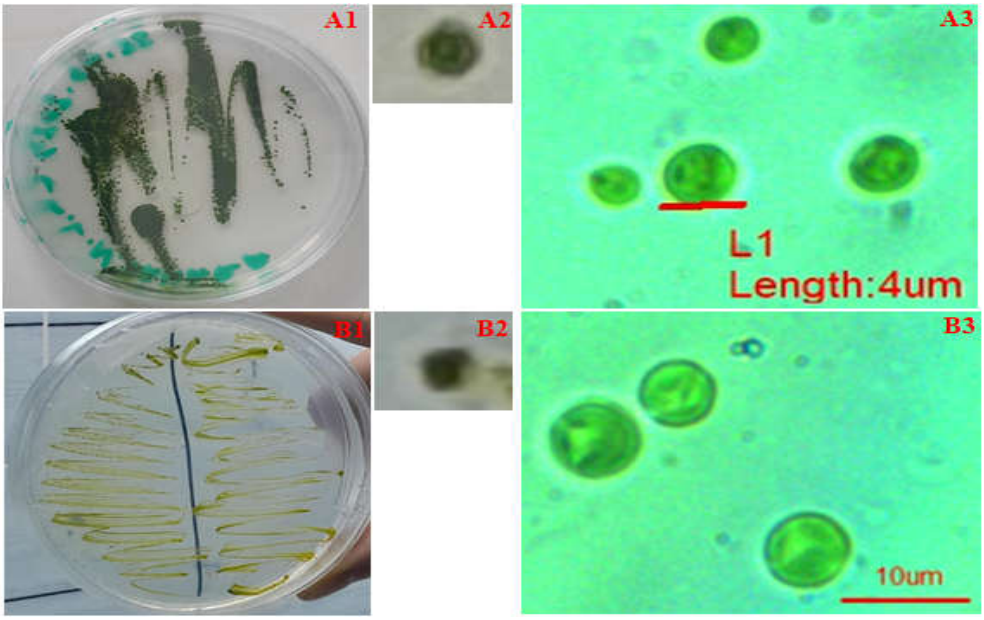

Figure 10. Macroscopic observation (A1-A2) *Chlorella sp.* CHL11 colonies, (B1-B2) *Chlorella sp*. CHL15 colonies and microscopic observation of (A3) *Chlorella sp.* CHL11 and (B3) *Chlorella sp.* CHL15 (1000X).

3.1.2.3. Morphological characteristics of the isolated strain

The microscopy observation of *Chlorella sp*. CHL11 and *Chlorella sp*. CHL15 culture shows an unialgal culture, Cell are green, spherical to oval cell shape(Fig10. 3A, Fig10. 3B), solitary life– form or in aggregates (Fig11. A, Fig11. B).

The cell size of Chlorella sp. CHL11 varies from 2 to 6 µm (Fig 10. 3A), while Chlorella sp. CHL15 ranges from 4 to 12 µm (Fig10. 3B).

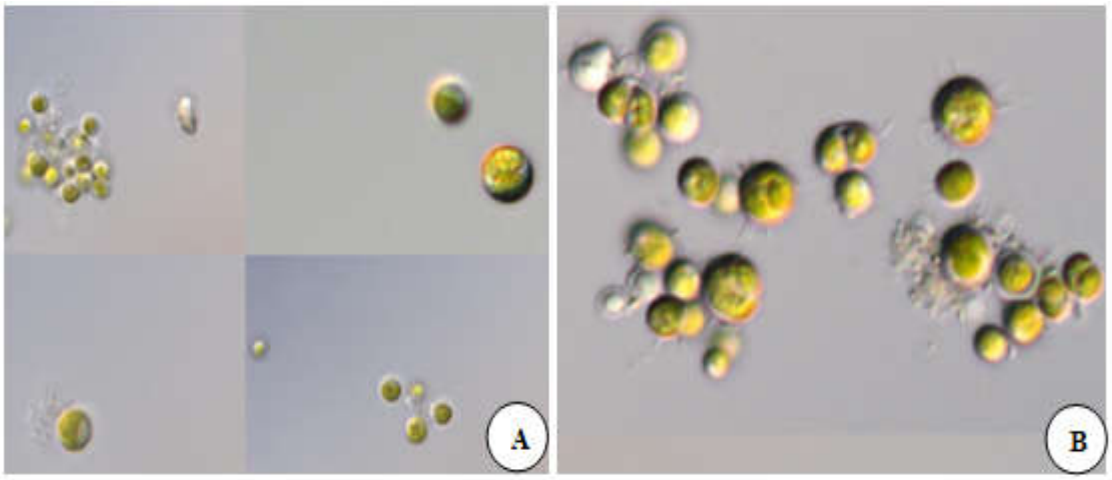

Figure 11. Microscopic observation of *Chlorella sp*. CHL11 (Fig. 3A) and *Chlorella sp*. CHL15 culture (1000 X)

3.2. Molecular characteristics of the isolated strain

3.2.1. *Limnospira fusiformis* TL03

The molecular studies based on the 16S rRNA gene allowed us to identify the newly isolated strain as *Limnospira fusiformis* TL03 with the accession number MZ970329. The partial sequence (1,411 bp length) of the *Limnospira fusiformis* TL03 16S ribosomal RNA gene was compared to all partial sequences presented in NCBI. MegaBlast searches revealed that the isolated strain matched with 100% similarity to *Limnospira fusiformis* SAG 85.79 (Figure 12).

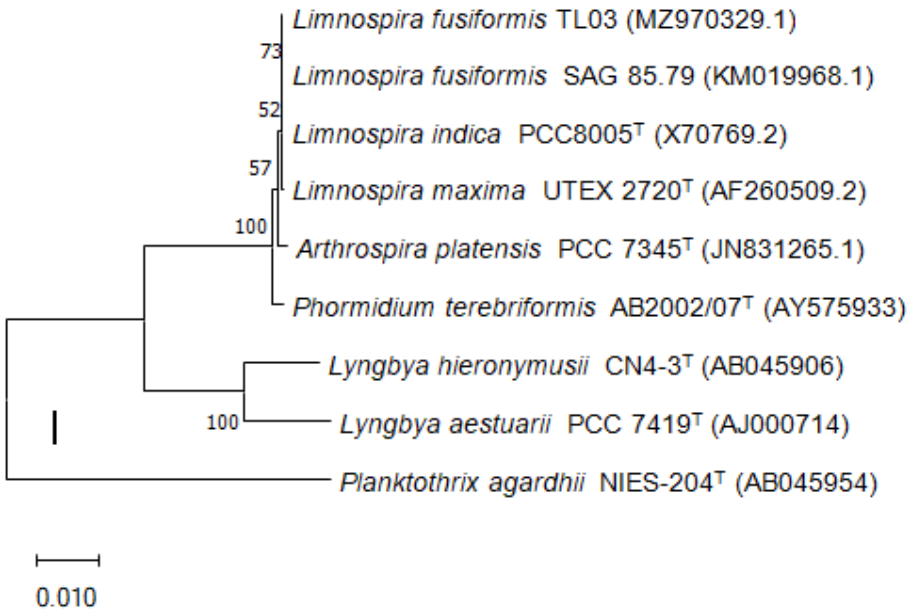

Figure 12. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain TL03 and related type-strains. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site (Bar, 0.01 nt substitution per nt position). The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The percentage of replicate trees (numbers at the nodes) in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. This analysis involved nine nucleotide sequences.

3.2.2. Molecular characteristics *Chlorella sp.* CHL11 and *Chlorella sp.* CHL15

The two selected strains ***C****hlorella sp*. CHL11 and *Chlorella sp*. CHL15 showed a close evolutionary relationship with the Chlorella genus species (Fig. 13), based on their 18S rRNA sequences, with identity % values of 99.26% (CHL11with Chlorella sorokiniana strain SAG 211-8k) and 99.46% (CHL15 with Chlorella vulgaris strain SAG 211-11b)

The two strains of Chlorella were identified as *Chlorella sp.* CLH11 and *Chlorella sp.* CHL15, and were submitted to the NCBI under the accession numbers ON799438.1 and ON799439.1, respectively.

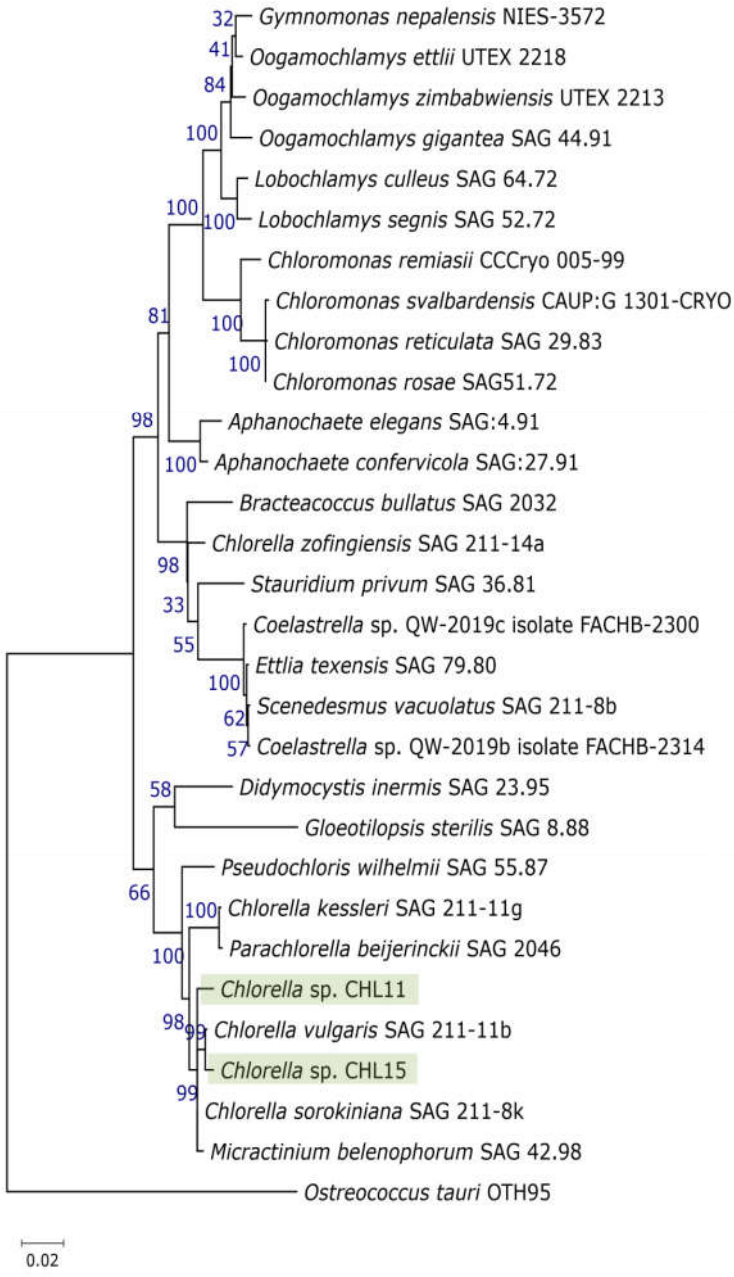

Figure 13. Molecular phylogenetic analysis of the selected strains *Chlorella* sp. CHL11 and *Chlorella* sp. CHL15 and their closest type strains by Maximum Likelihood method based on the 18S rRNA gene sequences

3.3. Preservation of isolate strains

3.3.1. Medium preservation of *L. fusiformis* TL03, *Chlorella sp*. CHL11 and *Chlorella sp*. CHL15 in BG11 solid medium

The preservation of the three pure isolated strains *L. fusiformis* TL03, *Chlorella sp*. CHL11 and *Chlorella* sp. CHL15 preserved in Petri dishes and glass test tubes containing solid BG11 medium under dark and low temperature conditions (4°C) showed their ability to survive, maintain their morphology and subculturing capacity for more than 3 months in Petri dishes and more than 1 year in glass test tubes. The macroscopic and microscopic examination of these preserved cultures using both macroscopic and microscopic techniques showed that they were free from bacterial, fungal or other contamination.

3.3.2. Short preservation of *L. fusiformis* TL03, *Chlorella sp*. CHL11 and *Chlorella sp*. CHL15 in BG11 (Periodic transfer from liquid to liquid medium or serial subculture)

Microscopic examination of isolated microalgae under serial subculture conditions revealed that the cells maintained their shape and pigmentation throughout the subculture period.

L. fusiformis TL03, *Chlorella sp.* CHL11 and *Chlorella sp.* CHL15 preserved in Petri dishes and glass tubes containing solid BG11 medium demonstrated their ability to survive and reculture in fresh liquid BG11 medium. The gradual color change to green of the *L. fusiformis* TL03 culture medium was observed over a period of 4-7 days, while the gradual color change to green of the culture media of two species *Chlorella sp.* CHL11 and *Chlorella sp*. CHL15 occurred after only 3-5 days of incubation under optimal light conditions, demonstrating successful preservation for these species. The examination of these culture showed there are no bacterial or fungi contamination.

4. Discussion

Microscopic examination of the sample taken from Telamine Lake revealed the presence of many cyanobacteria and microalgae, dominated by the strain *L. fusiformis* TL03, which may be a result of the alkalinity and salinity of this lake. This finding is consistent with the findings of Krienitz *et al.* (2005); Sili *et al.* (2012); Cellamare *et al.* (2018), who found that in alkaline saline lakes in tropical and subtropical regions, the alkaliphilic filamentous cyanobacterium *Arthrospira* is frequently the dominant taxon and is responsible for permanent or seasonal blooms.

To reduce the contamination of the sample by removing microorganisms and solid residues in order to isolate *L. fusiformis* TL03, physical techniques were applied in the first stage, such as filtration, centrifugation, and dilution, which are consistent with the techniques applied by many authors, such as filtration (Sena et *al.*, 2011; Lee et *al.*, 2013; Schagerl et *al.*, 2025), centrifugation and dilution (Guillard, 2005; Prasad et *al.*, 2013; Schagerl et *al.*, 2025)

In the same context, Prasad et *al.* (2013) stated that repeated centrifugation at 1000 rpm reduced the percentage of suspended solids and unicellular microorganisms to 90%, with *Spirulina* and other species of blue-green and green algae floating on the surface

The pH treatment of the samples confirmed that *L. fusiformis TL03* has a tolerance to high alkalinity (pH = 12.5); in contrast, *Microcystis sp*. was absent from the sample due to its inability to tolerate this condition. In agreement with our results, Sena et *al.* (2011) reported that treating the sample at pH 12 for 72 h was the most effective, as no protozoa or *Microcystis sp*. were observed in the treated culture, whereas they have been registered the presence of *Arthrospira sp*. On the other hand, the treatment at a lower pH (10 or 11) applied for less than 72 hours did not kill the protozoan, *Microcystis sp*. and *Chroococcus sp*., and when the pH was increased to 13, *Arthrospira sp* was eliminated In another study, Ciferri (1983) also reported that Arthrospira spp. can live in very alkaline environments up to pH 12.

In this study, we used the traditional method based on morphological and molecular characteristics to identify the genus of the isolated strain. The arrangement of its multicellular cylindrical trichomes in an open helix, usually of relatively large diameter, sometimes attenuated at the ends, is the main taxonomic criteria for the differentiation of both genus *Arthrospira* and *Limnospira species* (Sili *et al.*, 2012).

A morphological study of the isolated strain revealed that there are several morphotypes that most likely belong to the same species. During successive cultures in liquid media, this isolated strain maintained a spiral shape with varying degrees of helicity. The results obtained by measuring trichome length, coil diameter, and pitch, as well as cell diameter and length, differ slightly from those reported by Jung *et al.* (2021). Cell diameters of *Arthrospira platensis* grown in a flat-type bioreactor ranged from 5.25 ± 1.26 to 7.5 ± 2.9 µm, and coil diameters ranged from 20 to 60 µm.

Many studies have been conducted to describe the morphological characteristics of *A. fusiformis* and *A. maxima*. The diameter and length of the cells of the latter species range from 7 to 9 µm and 5 to 7 µm, respectively (Sili *et al.*, 2012). They form open regular spirals with coil diameters ranging from 40 to 60 µm and pitches ranging from 70 to 80 µm. Coils have

regular screw-like shapes at the ends that are slightly attenuated (Gardner, 1917; Fott & Karim, 1973; Komárek & Anagnostidis, 2005; Sili *et al.*, 2012). Rout *et al.* (2015) isolated the *Arthrospira* strain NPS-011, which has the same cell trichome length and diameter as previously studied strains. This species has a variable helix pitch ranging from 80 to 150 µm and a helix diameter ranging from 30 to 70 µm. The results for filament length, coil diameter, pitch, cell diameter, and length for our isolate show that they are almost in agreement with those reported in several previous studies (Sánchez *et al.*, 2003; Komárek & Anagnostidis, 2005; Rout *et al.*, 2015; Cellamare *et al.*, 2018; Roussell *et al.*, 2023).

Sili *et al.* (2012) noted that the *Arthrospira* helix architecture (pitch and diameter) is highly dependent on growth and environmental conditions under laboratory and mass cultivation conditions. Dhiab *et al.* (2007) found that increasing the concentration of sodium chloride in the culture medium caused *Arthrospira platensis* to change its trichome shape from spiral to straight, as well as its physiological behavior. The salinity and anion content of the culture medium, according to Kebede (1997), influence the helicity degree of *Arthrospira fusiformis*. The authors observed that higher salinity culture media had the longest pitches, with very lax helices in media containing high concentrations of SO_4^{2-} compared to those containing the same concentrations of HCO_3^- and Cl^-.

In this study, two strains of Chlorella (*Chlorella sp. CLH11* and *Chlorella sp.* CHL15) were successfully isolated and purified using a combination of physical and antibiotic treatments.

There was concordance between the results of the examination of the macroscopic and microscopic morphology of *Chlorella sp. CLH11* and *Chlorella sp.* CHL15 and the results reported by Bock et al. (2011). In agreement with our results, also, van Vuuren et *al.,* (2006) reported that Chlorella consists of spherical or oval small, non-motile, single cells (rarely clustered in small groups). Its reproduction method is asexually by 4 or 8 (rarely 16 or more) autospore formed internally by cell division. The autospores are released by rupture of the parent cell wall.

There are various preservation techniques used for the storage of microalgae (prokaryotic and eukaryotic), such as periodic transfer, freeze drying, and cryopreservation (Arguelles et *al.,* 2020). The most widely used method for preserving isolated microalgal strains is the preservation of microalgae using solid culture media at low temperatures (4°C) and in the dark, because it is easy to apply. In this study, the period of microalgae preservation in Petri dishes containing solid culture medium is consistent with that reported by Prasad et *al.*

(2013). They reported that the preservation period for *Spirulina* is up to two months in liquid and solid (2% bacteriological agar) media at 10°C and 4°C.

Pozzobon et *al*. (2024) reported that regrowth tests of *Chlorella vulgaris* that were maintained at 4 °C for six months showed slightly improved performance compared to those that were subjected to repeated subculture every two weeks.

5. Conclusion

This study reported the identification and isolation of a novel strain of cyanobacteria, *L. fusiformis* TL03, from Telamine Lake in northwest Algeria. Through the examination of morphological characteristics and comparing the 16S rRNA gene sequence of the isolated strain with other *Arthrospira* or *Spirulina* strains, we verified that the strain in question is indeed a member of the *Limnospira* genus. The strain was designated as *Limnospira fusiformis* TL03 and was submitted to the GenBank database with the accession number MZ215991.1.

In result, the high 18S rRNA sequence similarity of *Chlorella sp. CLH11* and *CHL15* with recognized strains of *C. sorokiniana* and *C. vulgaris*, respectively, validates their taxonomic position in the *Chlorella* genus. Strains CHL11 and CHL15 were assigned to the genus *Chlorella*, pending a concise polyphasic taxonomic classification based on additional gene loci (e.g., ITS1 and 5.8S rRNA, and ITS2 and 28S rRNA). The two strains of Chlorella were designated *Chlorella sp*. CLH11 and *Chlorella sp*. CHL15, and submitted to the NCBI with the accession numbers ON799438.1 and ON799439.1, respectively. In addition to contributing to the molecular taxonomy of *Chlorella*, these results will provide a basis for the practical use of these strains in sustainable biotechnology applications.

The results of the preservation of three microalgal strains, *L. fusiformis* TL03, *Chlorella sp*. CLH11 and CHL15, indicate that solid BG11 medium combined with dark refrigeration at 4°C is suitable for medium-term preservation, with storage periods of up to 3 months in Petri dishes and over a year in glass test tubes. This contributes to the possibility of medium-term laboratory preservation of various microalgae in small laboratories where advanced preservation tools are not available.

Chapter 2

Effects of salinity on microalgae growth

1. Introduction

Countries with semi-arid and arid environments face major challenges related to the shortage of fresh water for drinking and other uses in domestic, industrial, agricultural applications. Algeria is one of the countries that suffer from a shortage of water resources despite its great efforts to provide water by applying different strategies to respond to national water demand by constructing several dams and setting up seawater desalination stations along the Algerian coast. On the other hand, Algeria has several unexploited water resources (Lake, Sebkha, Saline groundwater) due to their salinity.

In order to valorize the saline water, it has become necessary to search for agricultural plant or microalgae species that adapt and tolerate different levels of salinity and that can be cultivated using brackishwater, seawater, hypersaline water.

Limnospira (*Spirulina* or *Arthrospira*) is one of the most important microalgae produced in the world (Sativa et *al.*, 2024), as it can live in a range of water bodies and is characterized by its high ability to tolerate different levels of salinity (Kebede, 1997; Zeng & Vonshak, 1998; Sudhir & Murthy, 2004; Thajuddin & Subramanian, 2005; Dadheech *et al.*, 2010; Asulabh *et al.*, 2012; Benavente-Valdés *et al.*, 2016; Costa *et al.*, 2016; Cellamare *et al.*, 2018).

In terms of value, the *Spirulina* market is in first place, followed by *Chlorella*. (Abreu, A.P., et *al*., 2023). *Chlorella* is found in seawater and brackish water, and it is also found in fresh water and has the ability to tolerate varying degrees of salinity (Figler et *al.,* 2019).

The aim of this work is to study the effect of salinity on the growth, chlorophyll a, chlorophyll b and carotenoids of three local microalgae species, *L. fusiformis* TL03, *Chlorella sp*. CHL11, *Chlorella sp*. CHL15.

2. Methods

2.1. Subculture

Several culture media of liquid fresh blue-green medium (BG11) [Allen & Stanier, 1968] were prepared for the culture of the three isolated strains: *L. fusiformis* TL03, *Chlorella sp*. CHL11 and *Chlorella sp*. CHL15. Volumes of 250 ml of a liquid blue-green medium containing different concentrations of sodium chloride were introduced into 500 ml Erlenmeyer flasks. The sodium chloride concentrations of the culture media for *L. fusiformis* TL03 and *Chlorella sp.* CHL15 were 0, 5, 15, 25, 35, 45 and 60 g/l, while for *Chlorella sp.* CHL11 they were 0, 5 and 10 g/l. The pH of the culture medium was adjusted to 9.5 for TL03 and 8 for CHL11 and CHL15 strains. The media were autoclaved at 121ºC for 20 min.

All culture media were uniformly inoculated by adding 25 mL of homogenized cell culture (stock culture). The initial absorbance at 750 nm of the culture concentration for TL03, CHL 11 and CHL15 were 0.220 ± 0.002, 0.452 ± 0.002 and 0.152 ± 0.0002, respectively.

Each treatment was carried out in four replicates. The microalgae incubation was maintained under the above conditions with aeration added using an aeration pump to avoid microalgae settling. The cultures were incubated in a culture chamber at a temperature of 27 ± 2 °C, with continuous illumination provided by light-emitting diodes (LEDs) with an intensity of (2.7 ± 0.2 Klux). A Mini Light Meter UT383 was used to measure the intensity of the light. The culture was continuously agitated with an air pump to keep cells suspended and prevent biomass cell agglomeration. Four replicates were used in the experiments. The cultures were incubated under these conditions for 20 days for TL03 and 12 days for CHL11, CHL15.

2.2. Growth and pigment measurements

Every four days for *L. fusiformis* TL03 and three days for *Chlorella sp*. CHL 11 and *Chlorella sp*. CHL15, a sample of the homogeneous suspensions culture was taken from each Erlenmeyer flask and analyzed for growth, chlorophyll a, chlorophyll b, and total carotenoids. Absorbance at 750 nm was used to calculate cell growth during the incubation period. Other than that, chlorophyll (a, b) and carotenoids were measured in accordance with Wellburn (1994). Volumes of 3 ml of each culture medium's homogenized cell suspension were centrifuged at 3800 rcf for 10 min (Centurion Scientific Ltd, C2 series Benchtop Centrifuges). After centrifugation at the same speed, the pellets were washed twice with 3 ml of distilled water. The supernatant was removed, and the cells were suspended in 3 mL of pure methanol (99.9%) in the dark at 4 °C for 24 h (until colorless pellets). After centrifugation, the aliquots were read at three different wavelengths (470, 653, and 666 nm) using a Jasco V-630 Spectrophotometer.

[Wellburn (1994)] equation

Chlorophyll a (µg/ml) = 15.66x (A666) - 7.34x (A653)

Chlorophyll b (µg/ml) =27.05 x (A653) – 11.21 x (A666)

Total carotenoids (µg/ml) = 1000 × (A470 – 2.86 × Chl a – 129.2 × Chl b)/221

2.3. Statistical analyses

The data from this study were statistically analyzed using IBM SPSS software (version 25) (Corp 2013). To verify if there were statistically significant differences in the growth of different concentrations of sodium chloride, the data of each parameter were subjected to One-Way ANOVA and Tukey's HSD (Honest Significant Difference) tests using SPSS with a 95% confidence interval.

3. Results

3.1. Effects of different NaCl concentrations on the growth of the isolated *L. fusiformis* TL03

Figure 14 depicts the growth of *L. fusiformis* TL03 from the start to the end of the experiment for all treatments. The *L. fusiformis* TL03 strain demonstrated a high level of salt tolerance. The general trend of the curve shows that the highest optical density values for all treatments occurred on the 20th day, whereas the concentration of NaCl had a significant effect on cell growth across all treatment groups ($P < 0.05$). The maximum absorbance (1.580 ± 0.014 and 1.560 ± 0.003) was obtained in both cultures containing 15 g/l and 5 g/l NaCl, respectively, seven times greater than the initial absorbance. In contrast, the lowest absorbance (1.314 ± 0.006 and 1.197 ± 0.003) were recorded in cultures containing 45 and 60 g/l NaCl, respectively.

These results show that when the concentration of sodium chloride increases, the growth of *L. fusiformis* TL03 slows down. We also remarked that _TL03 continued to grow even under NaCl starvation conditions.

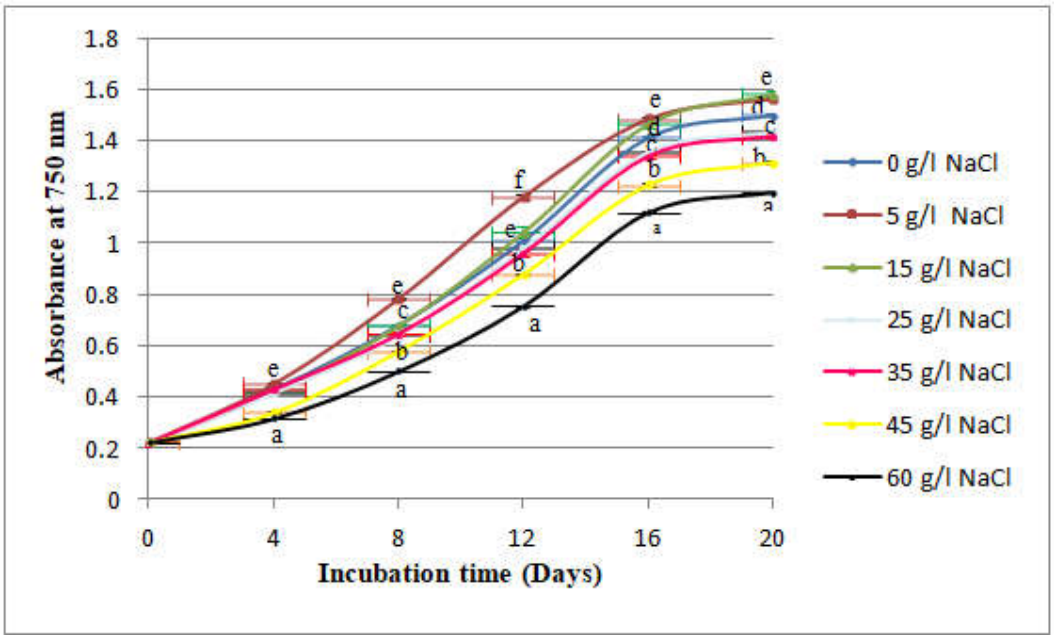

Figure 14. Effect of different NaCl concentrations on the growth of *L. fusiformis* TL03. Data represent mean values ± SEM (n = 4).

The results of chlorophyll a content in the *L. fusiformis* TL03 strain subjected to salt stress for 20 days showed a gradual increase among all NaCl concentrations tested (Fig. 15). By comparing the trend of the curves of the effect of salinity on growth and the concentration of

chlorophyll, it becomes clear that they follow a similar trend. Growth and chlorophylls (a, b) were negatively affected at the highest salt concentrations. Nevertheless, the TL03 strain was still able to grow until the 20th day, which was the end of the experiment. The highest concentrations of chlorophyll a (Fig. 15) and chlorophyll b (Fig. 16) in all tested cultures were observed at the end of the experiment (20th day). The maximum concentrations of chlorophyll a (19.42 ± 0.25 µg/ml) and chlorophyll b (3.52 ± 0.16 µg/ml) were obtained in BG11 containing 15 g/l NaCl. The lowest amounts of Chl *a* (12 ± 0.08 µg/ml) and Chl b (2.22 ± 0.01 µg/ml) were registered in BG11, which contained a high NaCl concentration (60 g/l).

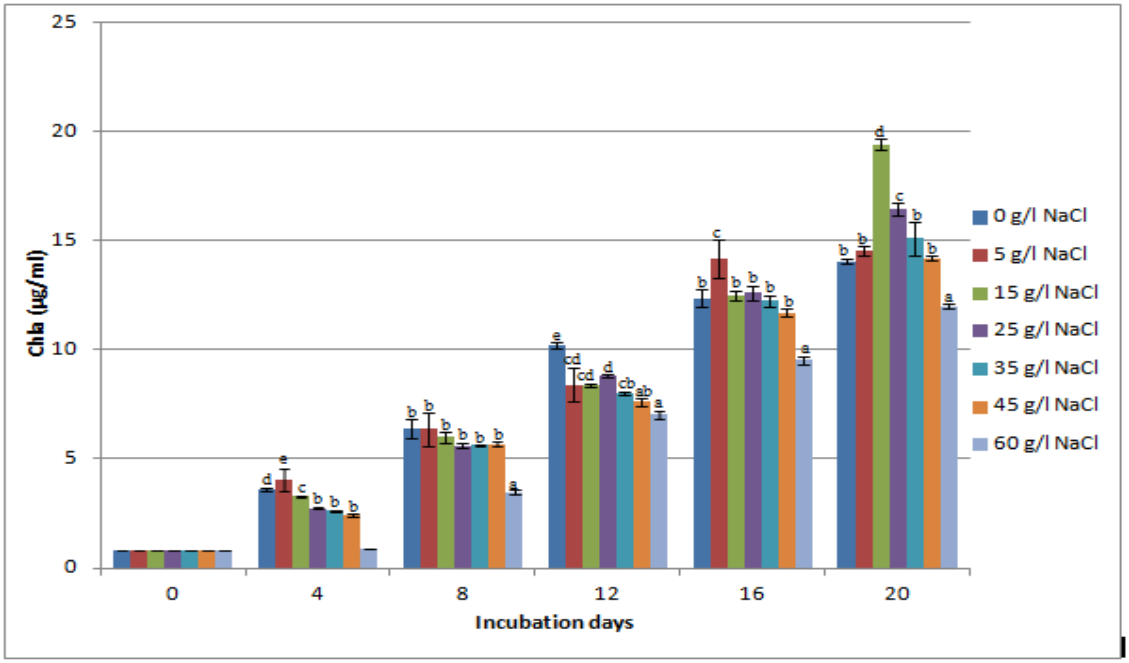

Figure 15. Chlorophyll a amount of *L. fusiformis* TL03 exposed to different NaCl concentrations. Different letters indicate a significant difference (P< 0.5). Error bar represents standard error (n = 4).

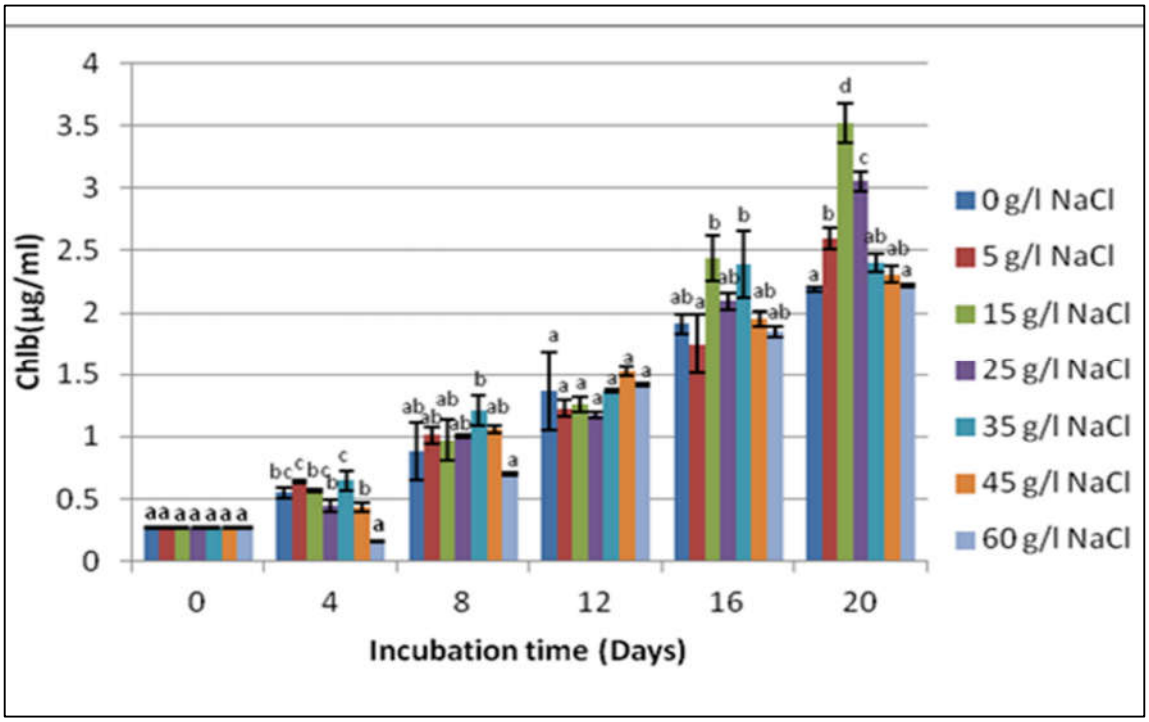

Figure 16. Chlorophyll b amount of *L. fusiformis* TL03 strain exposed to different NaCl concentrations. Data are mean ± SEM (n = 4).

The variation of total carotenoids in the *L. fusiformis* TL03 strain under different NaCl concentrations demonstrated a continuing increase in all treatment groups, reaching a maximum on the 20th day of the experiment, with a significant difference for all treatment media ($P < 0.05$) (Fig. 17). The medium without NaCl yielded the highest number of carotenoids, a 23-fold increase over the initial value. At the same time, carotenoid concentration ranged from the highest value of 1.88 ± 0.02 µg/ml in BG11 without NaCl to the lowest value of 1.10 ± 0.01 µg/ml under high salt stress (60 g/l). The total carotenoid concentration was lower in the TL03 strain cultured at 15 g/l NaCl (control) compared to those cultured at 25 and 35 g/l and was higher compared to those cultured in higher salinity culture media (45 and 60 g/l). On the fourth day of the experiment, the maximum concentrations of carotenoids (0.71 ± 0.02 and 0.71 ± 0.08 µg/ml) were observed in BG11 and BG11 containing 5 g/l of NaCl, respectively.

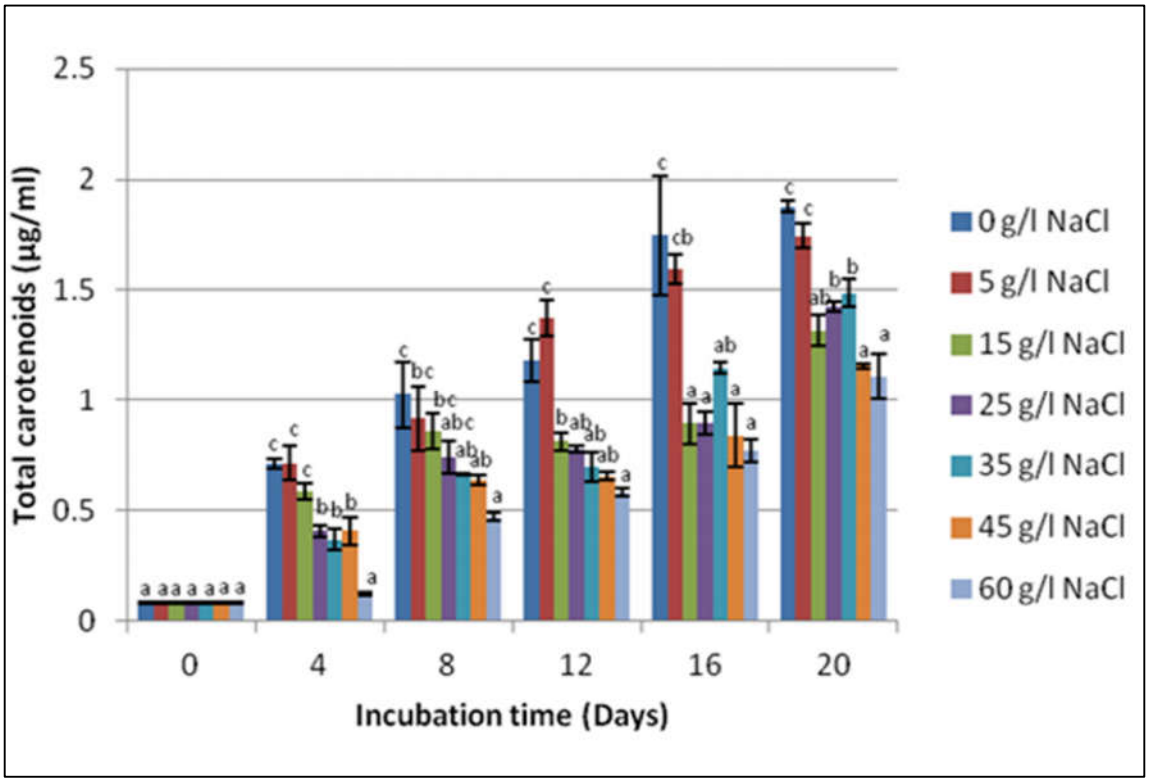

Figure 17. Total carotenoids amount of *L. fusiformis* TL03 strain exposed to different NaCl concentrations. Data are mean ± SEM (n = 4).

3.2.Effects of different NaCl concentrations on the growth of the isolated *Chlorella sp*. CH11

In order to determine the optimal salinity for the growth of two studied *chlorella* (*Chlorella sp.* CHL11 and *Chlorella sp*. CHL15), they were cultivated in BG-11 culture medium with different concentrations of sodium chloride.

The assessment of growth, chlorophyll, and carotenoids content of *Chlorella. sp* CHL11 cultured in BG11 containing different NaCl concentrations showed different growth patterns, although all cultures started with equal initial concentration inoculums.

Figure. 18 show the effect of NaCl concentration on the growth of *Chlorella sp.* CHL11 in BG11 medium containing different NaCl concentrations. The growth curves show an increasing trend in the optical density value of the microalgae suspension in all the tested media, with the highest values recorded during all the experiments for the algae grown in the culture medium without NaCl addition. After 12 days of incubation, the maximum absorbance (2.737±0.01) was recorded in BG11 without NaCl addition, while the lowest value (1.77±0.001) was found in BG11 with 10 g/l NaCl.

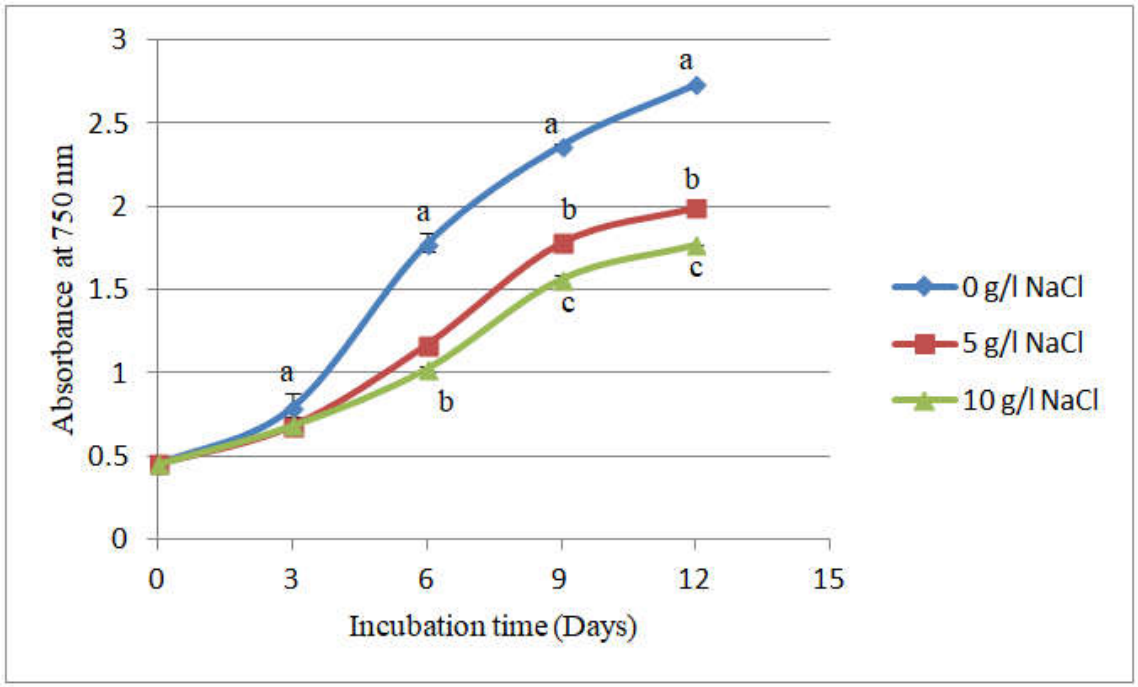

Figure 18. Effect of different NaCl concentration on *Chlorella sp.* CHL11 growth. Data are mean ± SEM (n = 4).

The histogram trends of chlorophyll a, chlorophyll b and carotenoid content in *Chlorella* sp. CHL11 are shown in the figure 19, 20 and 21.

The chlorophyll a and chlorophyll b levels showed significant differences between all treatments throughout the experiment ($p<0.05$), where the highest values were recorded on day 12. The maximum concentrations of chlorophyll a (25.579±0.08 µg/ml) and chlorophyll b(16.321±0.057) were found in *Chlorella sp* CHL11 cultured in BG11, and the lowest concentrations of chlorophyll a (17.286±0.047 µg/ml) and chlorophyll b(8.146±0.105 µg/ml) were recorded in that grown in BG11 containing 10 g/l of NaCl.

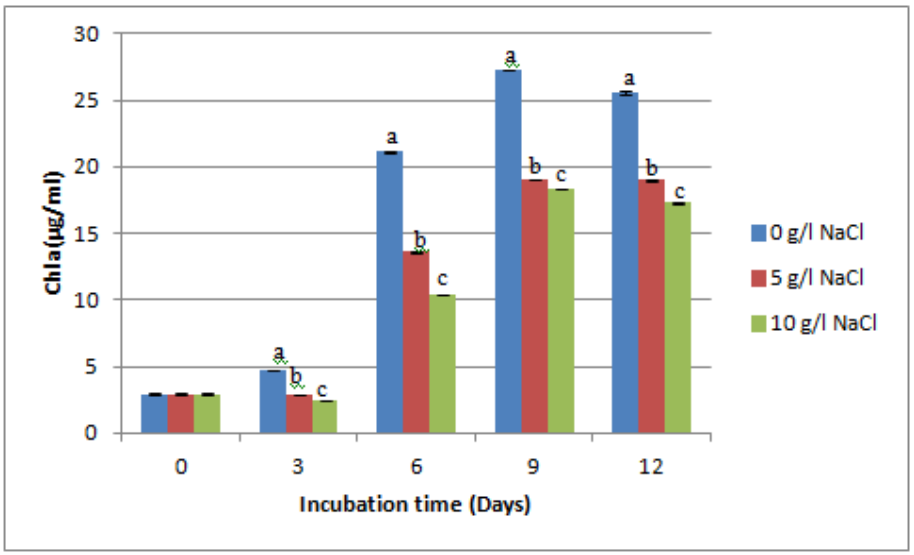

Figure 19. Effect of different NaCl concentration on chlorophyll a content in *Chlorella sp.* CHL11. Data are mean ± SEM (n = 4).

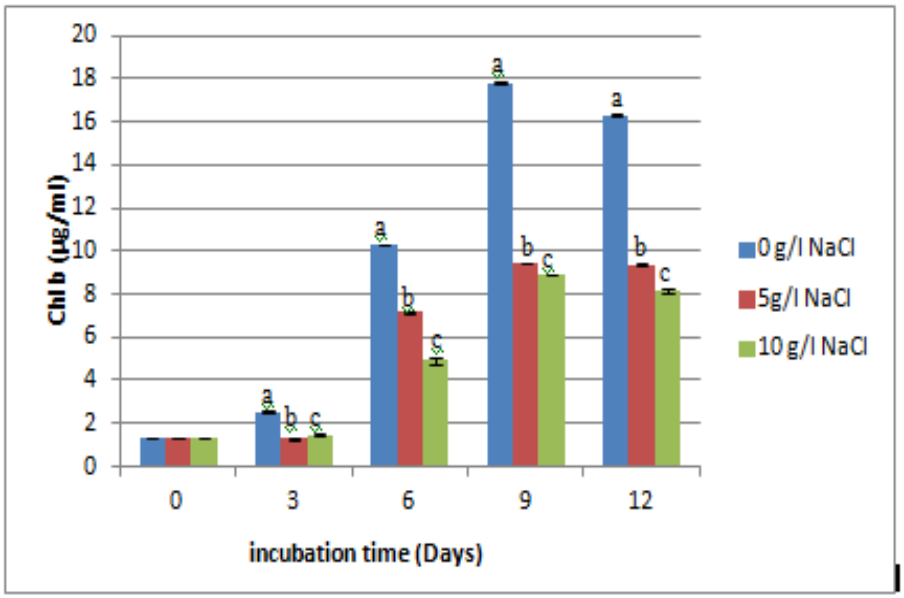

Figure 20. Effect of different NaCl concentration on chlorophyll b content in *Chlorella sp.* CHL11. Data are mean ± SEM (n = 4).

Figure 21. shows that the concentration of carotenoids produced was proportional to the biomass productivity of *Chlorella sp*. CHL11, with trends tending to increase with increasing biomass. The highest concentration of carotenoids (3.395±0.030 µg/ml) was found in *Chlorella sp*. CHL11 cultivated in BG11, while the lowest (2.635±0.051 µg/ml) was found in those cultivated in BG11 containing 10 g/l NaCl.

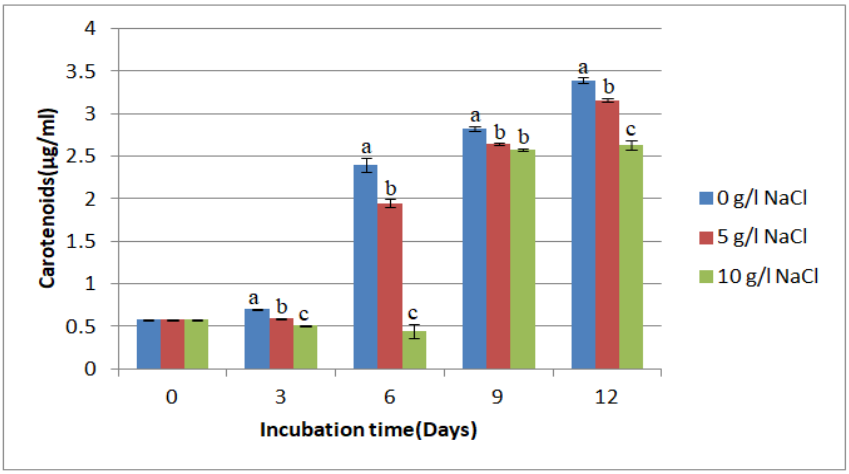

Figure 21. Effect of different NaCl concentration on carotenoids content in *Chlorella sp.* CHL11. Data are mean ± SEM (n = 4).

3.3. Effects of different NaCl concentrations on the growth of the isolated *Chlorella sp*. CHL15

Figure 22. Shows that the growth of *Chlorella sp.* CHL15 increased throughout the experimental period in all treatments.

The growth of *Chlorella sp.* CHL15 was directly proportional to culture time at all NaCl concentrations, with continuous and increasing growth throughout the culture time, while growth was inversely proportional to NaCl concentrations in the culture media, with faster growth in BG11 media containing less sodium chloride.

The growth curve (Fig. 22) showed a rapid adaptation of *Chlorella sp.* CHL15 to different sodium chloride concentrations in the culture media. There are no lag phase was observed in the treatments, except for the one grown in media culture containing high salinity (60 g NaCl/L), where a lag phase was recorded during the first three days after inoculation, followed by an exponential growth phase, reaching a maximum absorbance of 0.747 ± 0.033 on the 12th day of the experiment.

The results presented in Figure 22 show that on day 12 of the experiment, the BG11 medium had the highest growth of *Chlorella sp*. CHL15 with an absorbance of 1.884±0.036, while the lowest absorbance was recorded in BG11 containing 60 g NaCl/L.

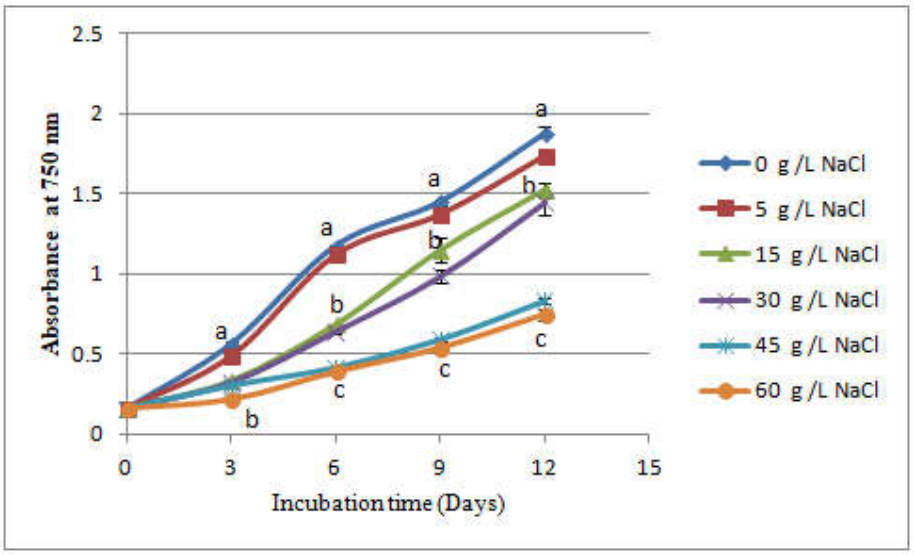

Figure 22. Effect of different NaCl concentration on *Chlorella sp.* CHL15 growth. Data are mean ± SEM (n = 4).

The tendency towards an increase in the amount of chlorophyll a, b, and carotenoids in *Chlorella sp*. CHL15 was maintained in all the treatments during all the experiments.

The highest chlorophyll a values were obtained on days 3, 6, 9 and 12 for *Chlorella sp*. CHL15 grown in BG11 without NaCl, followed by those grown in BG11 containing 5 g L-1, 15 g L-1, 30 g L-1, 45 g L-1 and 60 g L-1 NaCl, respectively.

Figure 23 and 24 show that, on the 12th day of the experiment, significant differences were observed in the chlorophyll a and chlorophyll b contents of *Chlorella sp*. CHL15 between all treatments, as the maximum concentration of chlorophyll a (16.158±0.021 µg/ml) and chlorophyll b (3.225±0.013 µg/ml) was recorded in the one cultivated in BG11 without sodium chloride, whereas the lowest levels of chlorophyll a (6.402±0.036 µg/ml) and chlorophyll b (1.268±0.059 µg/ml) were found in the one cultivated in BG11 containing 60 g/l NaCl.

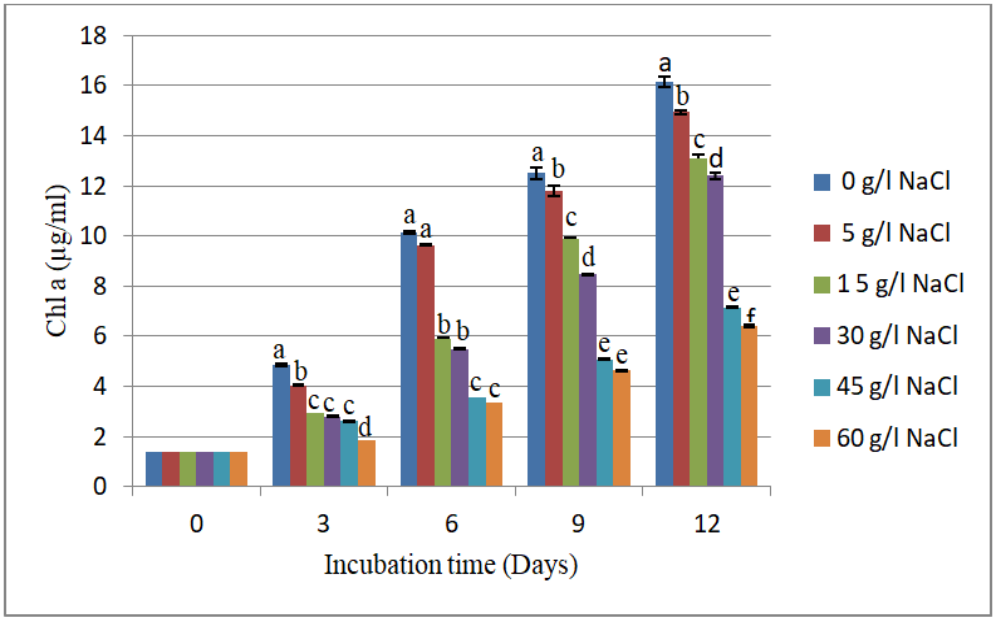

Figure 23. Effect of different NaCl concentrations on chlorophyll a content in *Chlorella sp.* CHL15. Data are mean ± SEM (n = 4).

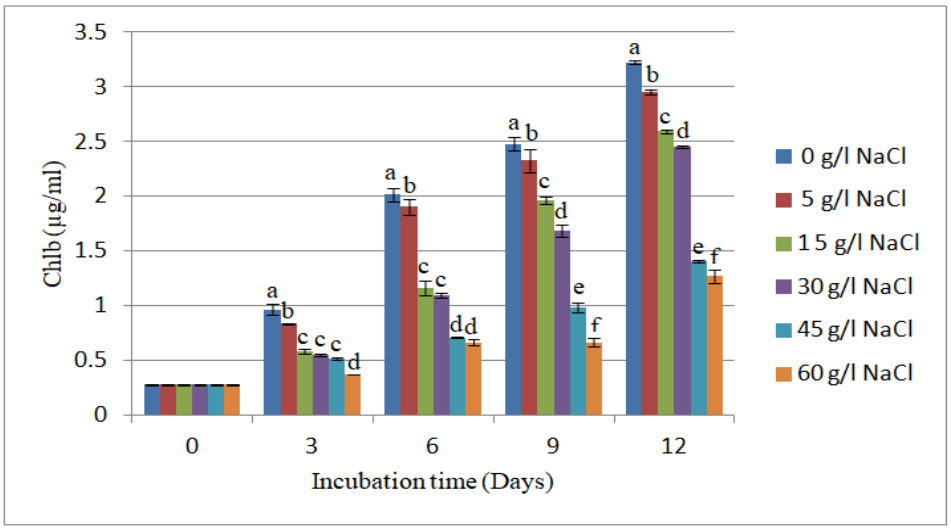

Figure 24. Effect of different NaCl concentration on chlorophyll b content in *Chlorella sp.* CHL15. Data are mean ± SEM (n = 4).

During the experiment, a gradual increase in the concentration of carotenoids was observed in all treatments (Fig.25). On the 9th and 12th day, a significant difference was observed between all treatments ($p<0.05$).

On the 12th day, the maximum level of carotenoids (3.216±0.064 µg/ml) was observed in *Chlorella sp.* CHL15 cultivated in BG11 without NaCl, followed by those cultivated in 5g/l

NaCl (2.921±0.016 µg/ml), 15 g L-1(2.583±0.064 µg/ml), 30 g L-1(2.408±0.062 µg/ml), 45 g L-1(1.390±0.075 µg/ml) and the last in BG11 containing 60 g L-1 NaCl with a concentration of (1.244±0.032 µg/ml).

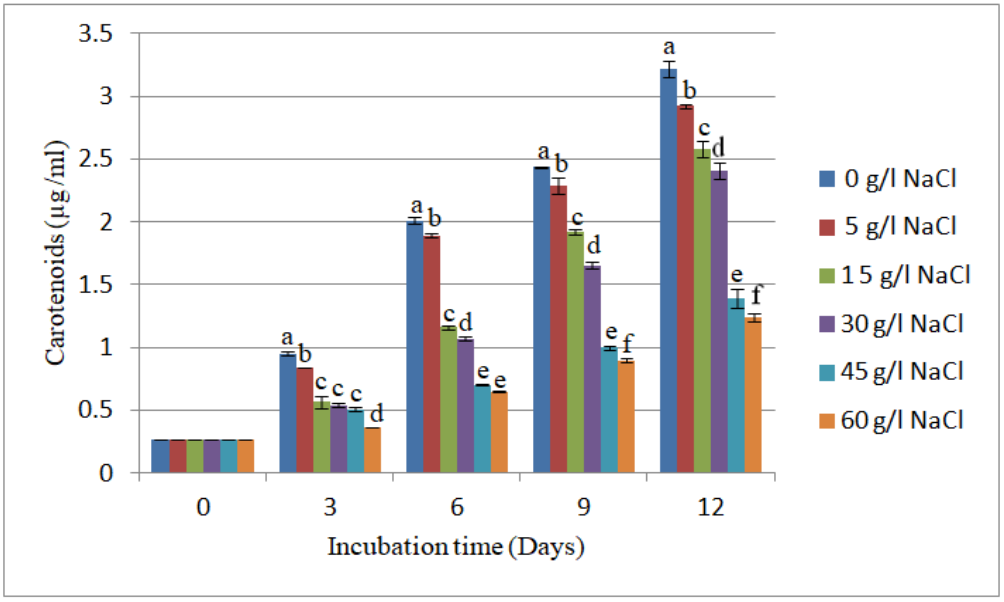

Figure 25. Effect of different NaCl concentrations on the carotenoid content of *Chlorella sp.* CHL15. Data are mean ± SEM (n = 4).

4. Discussion

Telamine Lake is characterized by high alkalinity and middle to high salinity, depending on season. This lake contains high plant biodiversity and phytoplankton. From the end of March to the end of April 2018, phytoplankton blooms were observed.

Despite the high salinity of the culture medium (60 g/l), the *L. fusiformis* TL03 strain showed tolerance and continued growth in culture media with varying salinity rates. These findings are consistent with those reported by Reed & Stewart (1985), Kebede (1997), and Moisander *et al.* (2002), who found that *A. platensis* did not stop growing even at 88 g/l salinity. According to Dadheech *et al.* (2010), *Arthrospira* lives in alkaline waters with pH ranging from 8 to 11 and salinity ranging from 1.1 to 300 g/l.

When compared to other media, the *L. fusiformis* TL03 isolates grew the best in both media containing 5 and 15 g/l NaCl. The salinity of the BG11, which contains 15 g/l NaCl, is thought to be similar to that of our strain's original habitat (Telamine Lake). BG11 with 5 g/l NaCl has the same salinity concentration as when the strain was grown and adapted before

being used in this experiment. In terms of absorbance, chlorophylls (a, b) levels increased slowly in BG11 containing up to 15 g/l NaCl (control) during incubation compared to a lower salinity medium. These findings differ from those of several previous studies. According to Ayachi *et al.* (2007), *Spirulina platensis* (*A. platensis*) grew the fastest at a NaCl concentration of 1 g/l. Meanwhile, at 60 g/l NaCl, the first five days are latent, followed by a decreasing growth phase until the end of the experiment.

Phang (2002), on the other hand, described an immediate cessation of *A. platensis* growth when exposed to high NaCl concentrations. Slower growth usually follows this stage. Sudhir *et al.* (2005) discovered that a 47 g/l NaCl concentration significantly reduced *A. platensis*'s photosystem II (PS II)-mediated oxygen evolution activity. Sharma *et al.* (2014) reported that increasing the NaCl concentration reduced growth and completely inhibited *A. platensis* chlorophyll a biosynthesis. The total carotenoid content of *L. fusiformis* TL03 grown in a low-salinity medium was significantly higher than in the control medium but lower in higher-salinity media (45 and 60 g/l). This fact could be explained as follows: exposing *L. fusiformis* TL03 to hypersalinity and a slightly higher salinity compared to the *Arthrospira* origin environment induced carotenoids biosynthesis, but the biosynthesis was lower at a higher salinity rate.

The growth pattern of *Chlorella sp.* CHL11 and *Chlorella sp.* CHL15 cultured in different NaCl concentrations was evaluated during the 12 days of the experiment using the optical density at D750 nm. The *Chlorella sp.* CHL11 strain grown in BG11 (0 g/L NaCl) showed a high level of growth, with absorbance values increasing sharply from day 3 to day 12, reaching approximately 2.73, with a multiplication of 3.43 times compared to that of the third day of the experiment.

At 5 g/L NaCl, *Chlorella sp.* CHL11 showed slower growth than those grown in BG11, and growth was further reduced at 10 g/L, indicating a clear response to salt stress. These results are in line with those of Kumar et *al*. (2010), who found that freshwater microalgae exhibit decreased cell division and photosynthesis when exposed to high salinity.

Our results showed that the two *Chlorella* species studied tolerated different levels of salinity while maintaining their continuous growth throughout the 12 days of the experiment. However, the growth rate was inversely proportional to the increase in salinity concentration in the culture media. These results are in agreement with those reported by El-Adl et *al*. (2024), who showed that the growth of *Chlorella sorokiniana* decreased progressively with increasing salinity of the culture medium.

According to Talebi et *al*. (2013), increased salt concentrations negatively affected the growth of some freshwater microalgae species such as *Chlorella vulgaris*, *Chlorella salina*, and *Chlorella emersonii*. On contrast, Farkas et *al*. (2023) stated that *Chlorella sp*. showed the highest tolerance to increased salinity, and its growth was not affected up to a concentration of 0.6 M NaCl.

Consistent with our findings, several studies have reported that many species of *chlorella* possess the halotolerance (NaCl) capacity (Wang et *al*., 2016; Li et *al*., 2018; Sahle-Demessie et *al*., 2019).

Several algal species are adaptable to variations in salinity through biochemical responses such as the production and accumulation of osmolytes or use of efficient Na+/K+ pump system (Brown, 1982; Kirst, 1989; Von Alvensleben et *al*., 2013; Talebi, et *al*., 2013; Slama, et *al*., 2015)

Slower algal growth and reduced photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) under salt stress may be due to increased uptake of Na+ and Cl-, causing ionic imbalance, cell dehydration, and excessive production of reactive oxygen species (ROS), which is leading to induction of oxidative stress, enzyme deactivation, and inhibition of photosynthetic activity (Erdmann & Hagemann, 2001; Sudhir & Murthy, 2004; Mahajan & Tuteja, 2005)

5. Conclusion

L. fusiformis TL03 has the ability to adapt and grow in a wide range of environmental salinity variation (from 0 to 60 g/l of NaCl) with maximum growth and chlorophyll a at 15g/l of salinity, making it a strong candidate to occupy an important position in algae cultivation investment projects in Algeria. Both NaCl concentration and exposure time had a significant effect on the growth of *L. fusiformis* TL03 and the amounts of chlorophyll (a, b) and carotenoids. This study is very important to contribute to the development of the cultivation of this local strain in different regions of Algeria using unexploited salt groundwater, as well as the use of brackish and saline water to reduce the cost of microalgae cultivation and provide and conserve potable water. The results of *L. fusiformis* TL03 grown in the laboratory under controlled conditions and different salt concentrations allow us to produce it outdoors on a large scale, especially since it is a local strain adapted to local climatic conditions.

It was concluded that these two strains (*Chlorella sp*. CHL11, *Chlorella sp*. CHL15) grow rapidly in BG11 culture media; however, their growth decreases with increasing salinity, and

they have the ability to tolerate high concentrations of salinity, especially *Chlorella sp*. CHL15, which tolerates up to 60 g/L of sodium chloride.

These halotolerant *chlorella* strains can be used for mass production in production in unexploited brackish and saline waters and in wastewater. These criteria could make this green algae strain a key element in future bio-desalination strategies.

Chapter 3

Antioxidant activity

1. Introduction

The treatment of oxidative stress through the long-term use of synthetic antioxidants may cause some health problems, such as skin allergies, digestive problems, and, in some cases, an increased risk of cancer (Botterweck et *al.*, 2000; Jeong et *al.*, 2005; Randhawa& Bahna, 2009; Engin et *al.*, 2011). In light of this, the search for effective natural antioxidants represents an important area of research in the present. Microalgae have recently attracted considerable attention worldwide due to their wide range of biotechnological applications such as biopharmaceutical, nutraceutical and cosmetic industries (Khan et *al.,* 2018; Abdel-Karim et *al.,* 2019; Rani et *al*., 2021; Shaima et *al*., 2022).

Microalgae are considered to be a promising source of natural antioxidant compounds (Goodarzi et *al*., 2014; Khan et *al.,* 2018; Saide et *al*., 2021; Silva et *al.,* 2022; Yang et *al*., 2023; Pereira et *al.,* 2024). They contain a variety of bioactive molecules, such as alkaloids, polysaccharides, vitamins , lipids, pigments, carotenoids, phycocyanin, phenolics, phycocyanin, terpenes and aromatic organic acids (Shannon and Abu-Ghannam, 2016; Marrez et *al.*, 2019 ; Sansone & Brunet, 2019; Vikneshan et *al.*, 2020; Pereira et *al.,* 2024).

Antioxidants were defined as any substance which, when existing at low concentrations compared with the concentrations of an oxidizable substrate, significantly retards or inhibits the oxidation of that substrate (Halliwell & Gutteridge, 1995).

The present study aimed to determine the total phenolic and flavonoid content and characterize the phenolic compounds in the hydromethanolic extract of three isolated local microalgae (*L. fusiformis* TL-3, *Chlorella sp*. CHl11, *Chlorella sp*. CHl15). In addition to evaluating the antioxidant activity of the extracts using DPPH free radical scavenging activity and beta-carotene bleaching methods.

2. Materials and methods

2.1.Material

The used materials are presented in Annex 2

2.2.Methods

2.2.1. Extract preparation:

A crude hydromethanolic extract of freez dried biomass of *L. fusiformis* TL03 and two *chlorella* (*Chlorella sp*. CHL11, *Chlorella sp*. CHL15) was prepared separately by macerating 2 g of freeze-dried algae in 100 mL of methanol/water (7 v: 3 v). The mixture was kept at room temperature in the dark for 48 hours and then filtered through Wathman No. 1 paper.

These procedures were repeated three times to extract the maximum amount of compounds. The three filtrates of each strain were collected in an opaque glass vial and then dried at 40 °C using a rotary vacuum evaporator (Buchi R-210). The dried extracts were stored at 4°C in the dark until use.

The extraction yield was calculated according to the following formula (Bouslamti, M et *al.*, 2022):

$$Y\ (\%) = (EW/WP) \times 100$$

Y: yield of extract in %; EW: extract weight obtained; WP: weight of the powder.

2.2.2. Total phenolic assay

The total phenolic content of the extract was assessed using spectrophotometric methods based on the Folin-Ciocalteu reagent (Singleton & Rossi, 1965). 3.9 ml of distilled water was combined with 0.1 ml of diluted hydromethanolic crude extracts (10 mg/mL), and the mixture was thoroughly mixed with 0.25 mL of Folin-Ciocalteu reagent for 3 min. Then, 0.75 mL of 20% (w/v) sodium carbonate (Na2CO3) was added to the mixture. After thorough mixing, samples were incubated in a water bath at 40 °C for 40 min. The absorbance was measured at 765 nm against a blank sample consisting of distilled water instead of the diluted sample. The total phenolic content of the extracts was determined and expressed as mg of Gallic acid equivalents (GAE) per gram of sample in dry weight (mg GEA/g) using the regression equation of the calibration curve. The analysis was conducted three times.

2.2.3. Total flavonoid content

The total content of flavonoids in the crude extract was determined by the Aluminum chloride colorimetric method (Lamaison & Carnat, 1990).

Briefly, 0.75 mL of hydromethanolic crude extracts (10 mg/mL) was made up to 1.5 mL with 2% AlCl3 solution methanol. The mixture was well mixed before incubation at room temperature in the dark for 10 min. The absorbance of the mixture was measured at 440 nm against the standard containing a diluted solution of AlCl3.

A calibration curve for quercetin was used to determine the total flavonoid concentration and the result was expressed as milligram equivalents of quercetin per gram of dry microalgae (mg QE/g). The analysis was also repeated three times

2.2.4. Polyphenol analysis HPLC

The HPLC analysis of crude hydromethanolic extracts was performed using a Young Lin YL 9100 HPLC chromatograph with a UV-Vis detector (YL9120). The separation was achieved by a reversed-phase Agilent XDB Eclipse C8 column (250 mm/4.6 mm/5 m). The mobile phase was bi-distilled water enriched with 0.1 % acetic acid (Eluent A) and gradient methanol

(Eluent B). The flow rate was adjusted at 1 mL/min, the temperature was regulated at 30 °C, and the injection volume was 20 µL. The HPLC gradient elution were as follows: 0–50 min, 95% elution A and 5% elution B; 50–60 min, 5% A and 95% elution B. The chromatograms were recorded at 254 and 280 nm.

Each compound of phenolic acids and flavonoids contained in hydromethanolic extract was identified by comparing its retention time (Rt) and the UV spectra to those of 35 standards (Galic acid, Rutin, Vanillic acid, Ascorbic acid, Galangin, Cafeic acid, Tannic acid, Protocatechuic acid, Robinin ,Myricetin, Morin, Apegin, Diosmin, Catechin, Tangeretin**,** 5-Hydroxy-flavon, Chrysin, 3-Hydro-flavon, Acacetin, Hesperetin, Quercetin, Xanthotoxine, Luteotin, Kaempferol, Luteolin 7,3'-diglucoside, Apigenin 7-glucoside, isorhamnetin-3-glucoside, Hesperidin, Rhamnetin, penta-hydroxyflavone, Naringenin-7-glucoside, Naringenin, Isorhamnetin-3-rutinoside, Kaempferide)[appendix 2]

2.2.5. Antioxidant Activity

2.2.5.1.DPPH radical scavenging activity:

The DPPH assay was performed according to Tep et *al.* (2006), the Crude hydromethanolic extracts (10 mg/ml) were prepared in a series of dilutions (0.125, 0.250, 0.5, 1, 2, 3, 4, and 5 mg/ml) by adding methanol.

An equal volume of DPPH solution dissolved in methanol (0.004%) was added to 1.5 ml of each diluted test extract (0.125 to 5 mg/ml). After vigorous mixing for 10 seconds, the solution was incubated at room temperature in the dark for 30 minutes. The absorbance was read at a wavelength of 517 nm. Reference standards of BHT, Ascorbic acid, Trolox, Vitamin E were used as benchmarks. All samples were performed in triplicate.

The following formula was used to determine the percentage of free radical DPPH inhibition

$$(I\ \%) = [(Ac - As)/ (Ac)] \times 100$$

Where As is the sample absorbance and Ac represents the absorbance of the control reaction, which uses all the reagents except the test substance.

The IC50 value was used to determine the antiradical activity of the samples; this value is the concentration of the sample required for 50% inhibition of DPPH radicals.

2.2.5.2.Beta-carotene determination

Two mg of beta-carotene was dissolved in 10 ml of chloroform, and 2 ml of this solution was added to a vial previously containing 40 mg of linoleic acid and 400 mg of Tween 80. The mixture was agitated, and then the chloroform was completely evaporated using a rotary vacuum evaporator. Afterward, 100 ml of distilled water was added to the residue. After thoroughly mixing, a volume of 4.8 mL of the obtained emulsion was transferred to test tubes,

each one containing 0.2 mL of the extract (10 mg/mL) or the same volume of control antioxidants (BHT).

The tubes were placed in a water bath at 50 °C and the absorbance was measured at 470 nm at intervals of 30, 60, 90 and 120 minutes. Samples were performed in triplicate

The bleaching rate of beta-carotene (R) is calculated according to Al-Shaikhan et *al.* (1995) as follows:

$$Rt = In (Abst0/Abst)/t$$

Rt: the bleaching rate of beta-carotene at times (30, 60, 90, and 120 minutes);

In: natural logarithm; Abst0 corresponds to the initial absorbance of the emulsion immediately after sample preparation (t = 0 minutes); Abst: the absorbance of the emulsion at times (30, 60, 90, and 120 minutes). The percentage of antioxidant activity is calculated using the following equation:

$$\text{Antioxidant activity \%} = [(R \text{ control} - R \text{ sample})/R \text{ control}] \times 100.$$

R control and R sample are the average rates of control and sample (extract) bleaching, respectively.

3. Results

3.1.Extraction Yields for Microalgae and Cyanobacteria:

Methanol is the most suitable solvent for the extraction of bioactive metabolites due to its higher polarity and cell disintegrating ability (Safafar et *al.*, 2015, Fardous et *al.* 2023).

The results of the extraction yields showed that the highest percentage was recorded for by *Chlorella* sp CH11 (38.89%) followed *Chlorella* sp CH15 (35.16%), and *L. fusiformis* TL03 (32.22%).

3.2.Total phenolic contents

Using a spectral assay, we calculated the polyphenol concentrations in our study. The results were expressed in milligrams equal to the standard used per gram of dry extract, derived from the linear regression equation of a calibration curve created with gallic acid.

$$\text{Equation: } y = 0.0795X - 0.1033$$

The total phenolic content of the hydromethanolic extract of the three locale studied microalgae ranged from 13.46±0.56 to 18.41±0.32 mg/g GAE, with statistically significant differences between the species (Table 15). The highest value was obtained in *Limnospira fusiformis* TL03.

3.3.Total contents of flavonoids

The total flavonoid content (TFC) was performed using the Aluminium chloride method (AlCl3), quercetin was used as a standard and the flavonoid contents corresponding are reported in milligrams equivalent of the standard used per gram of dry extract. The results are presented in Table 15.

Equation: Y = 0.5658X - 0.1155

The results of the total flavonoid content in the hydromethanolic extract of three microalgae species showed a significant difference between *Chlorella sp*. CHL11 (3.59±0.06 mg QE/g DW) and the three(two)studied microalgae species ($p<0.05$). No significant difference was found between *Limnospira fusiformis* TL03, *Chlorella sp*. CHL15 (Table 14).

Table 14. Total phenolic and flavonoid content in the hydromethanolic extract of *Limnospira fusiformis* TL03*, Chlorella sp*. CHL11 and*, Chlorella sp*. CHL15.

Hydromethanolic extracts	***Limnospira fusiformis TL03***	***Chlorella sp. CHL11***	***Chlorella sp. CHL15***
Total Phenolic Contents(mg GEA/g)	18.41± 0.32(a)	15.57±0.42(b)	13.56± 0.50©
Total Flavonoid Content(mg QE/g DW).	2.11± 0.12(a)	3.59±0.06(b)	1.99± 0.06(a)

3.4. Phenolic Compounds (HPLC)

Phenolic compounds are secondary metabolites essential used for plant physiological processes, stress responses, and survival under harsh conditions (Cichoński& Chrzanowski, 2022). Phenolic compounds such as phenolic acids, flavonoids, coumarins, lignans, and tannins are the major group of secondary metabolites in plants (Nezafatian et *al.,* 2024).

The results obtained from the HPLC analysis of the crude hydromethanolic extract of the microalgae studied were 55 peaks for *L. fusiformis* TL03, 69 peaks for *Chlorella sp*. CHL11 and 47 peaks for *Chlorella sp*. CHL15(Fig.26, 27, 28). A number of phenolic compounds were identified by comparing the retention times and UV spectra of the obtained peaks with standards and algal extracts in which, five compounds (Ascorbic acid, Gallic acid, Luteolin, Quercetin, Kaempferol)were identified in *L. fusiformis* TL03, seven compounds (tannic acid, robinin, luteolin 7,3'-diglucoside, vannilic acid, apigenin 7-glucoside, quercetin, and galangin) in *Chlorella sp*. CHL11 and four compounds(protocatechuic acid, isorhamnetin-3-glucoside, hesperidin, quercetin) in *Chlorella sp*. CHL15 (Table 15).

HPLC-DAD analysis of the polyphenolic compounds of the hydromethanolic extract of two studied species of C*hlorella* revealed the presence of seven compounds (tannic acid, robinin, luteolin 7,3'-diglucoside, vannilic acid, apigenin 7-glucoside, quercetin, and galangin) in *Chlorella sp*. CHL11, four compounds (protocatechuic acid, isorhamnetin-3-glucoside, hesperidin, quercetin) in *Chlorella sp*. CHL15.

Table 15. Phenolic compounds identified in hydromethanolic extracts of three studied microalgae species: *L. fusiformis* TL03, *Chlorella sp*. CHL11, and, *Chlorella sp*. CHL15.

Species	Peak number	Compound	Area	Retention time	Compounds class
L. fusiformis TL03	1	Ascorbic acid	0.5	3.66	Vitamine C
	2	Gallic acid	1	10.34	phenolic acid.
	3	Luteolin	1.6	33.53	Flavone
	4	Quercetin	1	35.703	Flavonol
	5	Kaempferol	0.1	49.303	Flavonol
Chlorella sp. CHL11	1	Tannic acid	0.5	6.62	Phenolic acid
	2	Robinin	0.1	17.3	Flavanol
	3	Luteolin 7,3'-diglucoside	0.01	24.253	Flavone
	4	Vanillic acid	0.01	24.710	Phenolic acid
	5	Apigenin 7-glucoside	0.1	29.207	Flavone
	6	Quercetin	0.1	35.703	Flavonol
	7	Galangin	0.3	47.293	Flavonol
Chlorella sp. CHL15	1	Protocatechuic acid	0.9	8.547	Phenolic acid
	2	isorhamnetin-3-glucoside	0.1	28.120	Hydroxy-flavone
	3	Hesperidin	0.01	28.284	flavanone
	4	Quercetin	0.01	35.703	Flavonol

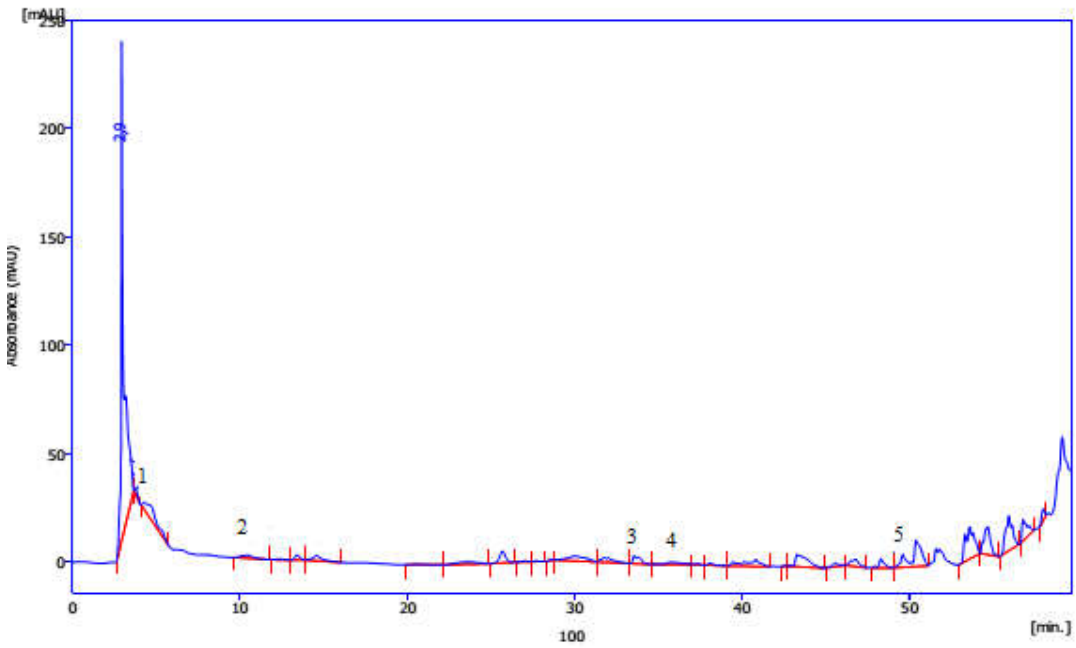

Figure 26. Chromatogram of the hydromethanolic extract of *L. fusiformis* TL03. Phenolic compounds identified: (1): Ascorbic acid, (2): Gallic acid, (3): Luteotin, (4): Quercetine, (5): Kaempferol.

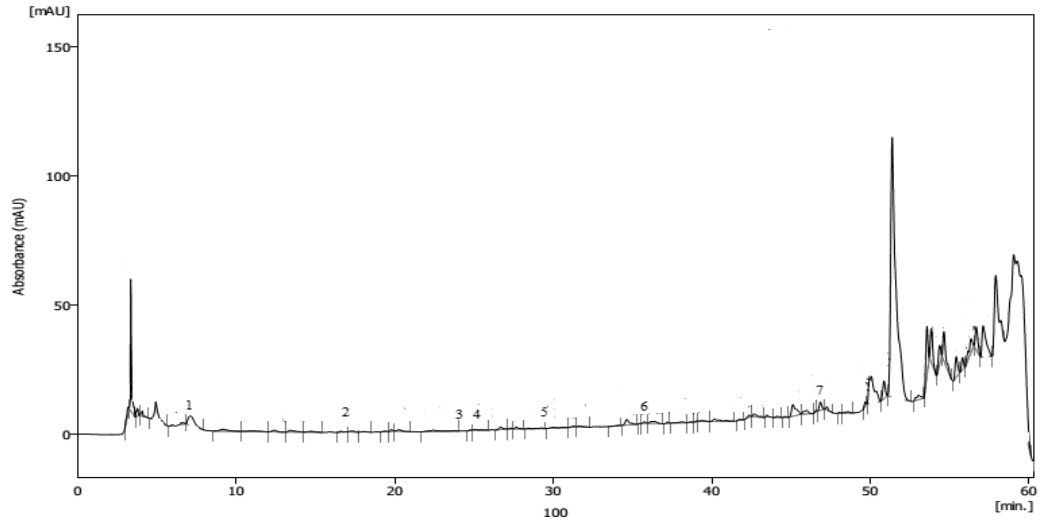

Figure 27. Chromatogram of the hydromethanolic extract of *Chlorella sp*. CHL11. Phenolic compounds identified: (1): Tannic acid, (2): Robinin, (3): Luteolin 7,3'-diglucoside, (4): Vanillic acid, (5): Apigenin 7-glucoside, (6): Quercetin, (7): Galangin.

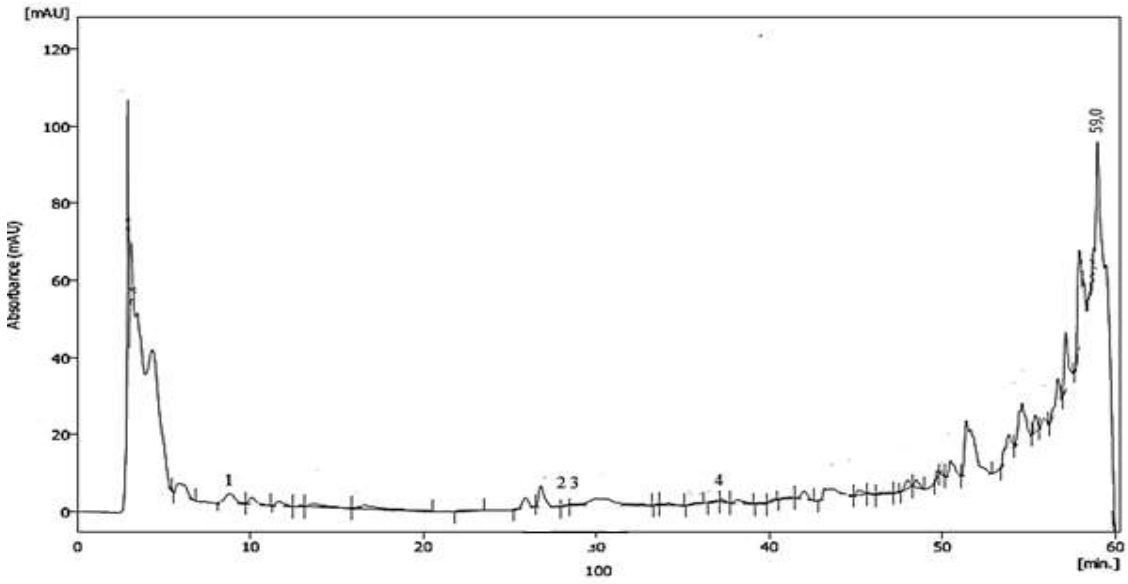

Figure 28. Chromatogram of the hydromethanolic extract of *Chlorella sp*. CHL15. Phenolic compounds identified: (1): Protocatechuic acid, (2): isorhamnetin-3-glucoside, (3): Hesperidin, (4): Quercetin.

3.5.Antioxidant activity

3.5.1. DPPH radical scavenging activity:

The DPPH (2,2-diphenyl-1-picrylhydrazyl) assay is a simple, inexpensive, and fast method widely used to evaluate antioxidant capacity; handling the DPPH radical is much more stable and easier than oxygen free radicals. The anti-radical activity profile against the DPPH radical of different extracts as well as those of standard antioxidants (ascorbic acid, α-tocopherol and BHT) are represented in the Figure 29.

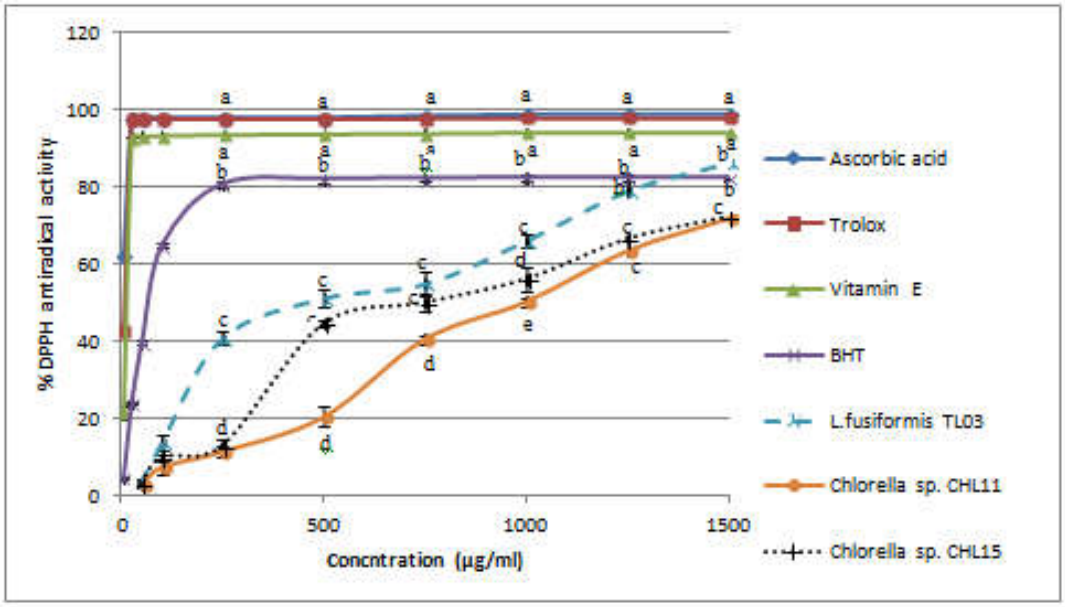

Figure. 29 DPPH radical scavenging activity of hydromethanolic extract of *L.fusiformis* TL03, *Chlorella sp*. CHL11, *Chlorella sp*. CHL15 and standards (BHT ,Ascorbic acid, Trolox, and Vitamine E).

The results of the percentage inhibition of the DPPH radical scavenging activity of the extract of three microalgae have shown that there is a high significant difference between *L. fusiformis* TL03 and the two *Chlorella* species; *Chlorella sp*. CHL15 and *Chlorella sp*. CHL11.(Table 16).

Table 16. Antioxidant activity (DPPH assay) and IC_{50} value in hydromethanolic extract of *L.fusiformis* TL03, *Chlorella sp*. CHL11, *Chlorella sp*. CHL15.

	Species	IC50 (µg ml $^{-1}$) DPPH	% inhibition of DPPH
Hydromethanolic extracts	*L. fusiformis* TL03	470	86.19 ± 1.61 (a)
	Chlorella sp. CHL11	994	71.69±1.44(b)
	Chlorella sp. CHL15	745	72.30± 3.35(b)
standards	BHT	72,16 ± 0,1	82,36 ± 0,94 (a)
	Ascorbic acid	4 ± 0,1	98,54 ± 0,25©
	Trolox	6,86 ± 0,05	98,03 ± 0,09©
	vitamine E	9,55 ± 0,07	94,08 ± 0,1©

The perentage inhibition of the DPPH of microalgae extract varied from 71.69±1.44 to 86.19 ± 1.61%. *L.fusiformis* TL03 provided the highest antioxidant activity, followed by *Chlorella sp.* CHL15 (72.30± 3.35%), and *Chlorella sp*. CHL11(71.69±1.44 %).

On the other hand, the comparison of the percentage inhibition of the DPPH radical scavenging activity of the three microalgae extracts with the standards showed that the DPPH of the standards (ascorbic acid, torolox, and vitamin E) was significantly higher than that of the three microalgae extracts($p<0.05$), whereas there was no significant difference between BHT and the extracts of *L. fusiformis* TL03.

The inhibition concentration (IC50) is widely used to determine the antioxidant activity of test samples. It is defined as the concentration of antioxidant required to reduce the initial concentration of DPPH by 50%. Therefore, the lowest IC50 value would correspond to the strongest antioxidant activity of the substance.

The extracts of *L. fusiformis* TL03 showed the highest antioxidant activity (IC50 = 470 µg/mL), followed by *Chlorella sp*. CHL15 (IC50 = 745 µg/mL), and the lowest value (IC50 = 994 µg/mL) was found in the extract of *Chlorella sp*. CHL11.

The IC50 values of the extracts of three microalgae were significantly lower than those of the references (BHT, ascorbic acid, Trolox, and vitamin E).

3.5.2. Beta-carotene determination

Natural carotenoids (lutein, β-carotene, and lycopene) have beneficial effects on both human and animal health (Von Lintig et *al.*, 2005; Amengual, 2011). B-carotene remains their essential source of vitamin A, which is necessary for the functions of the retina.

The beta-carotene bleaching test is based on the preservation of the yellow color of the beta-carotenoid acid system when the tested material contains antioxidants (Velioglu et *al.* 1998; Demirel. et *al.*, 2009).

The bleaching kinetics of β-carotene in the absence and presence of *L. fusiformis* TL03, *Chlorella sp*. CHL11 and, *Chlorella sp*. CHL15 extracts and antioxidants are shown in the Figure 30.

After 120 minutes of bleaching kinetics of β-carotene, the absorbance of the control and Chlorella sp. CHL11 at 470 nm decreased towards a lower value, while this decrease remained slower in the extracts of two microalgae species *L. fusiformis* TL03, *Chlorella sp*. CHL15 and in the tested standard (BHT).

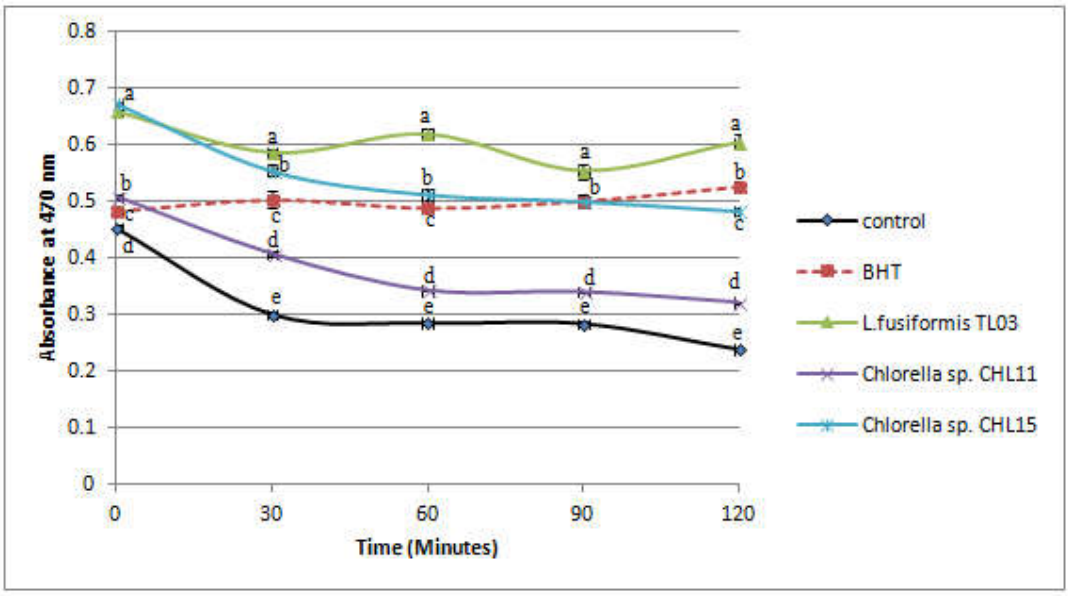

Figure.30. Bleaching kinektics of β-carotene at 470 nm of the three microalgal hydromethanolic extract (*L.fusiformis* TL03, *Chlorella sp*. CHL11, *Chlorella sp*. CHL15) and standard antioxidant (BHT).

The results percentage of beta-carotene bleaching revealed a significant difference among the hydromethanolic extracts of three studied microalgae and BHT ($P<0.05$). The highest percentage of beta-carotene bleaching was found in *L. fusiformis* TL03 (60.35±1.10) followed by that of BHT (52.5±0.7%), *Chlorella sp*. CHL15 (48.3±0.70%), and the *Chlorella sp*. CHL11 (32.05±0.27%), respectively.

Table 17. % Antibleaching of Beta carotene of hydromethanolic extract of *L.fusiformis* TL03, *Chlorella sp*. CHL11, *Chlorella sp*. CHL15BHT, time 120 min.

Species	*TL03*	*Chl11*	*Chl15*	*BHT*
%	60.35±1.10(a)	32.05±0.27(b)	48.3±0.70(c)	52.5±0.7(d)

4. Discussion

Methanol is recommended and frequently used for the extraction of natural compounds (Falleh et *al*., 2008). 70% aqueous methanol is twice as effective as pure methanol (Vuorela, 2005).

In same context Jerez-Martel et *al*. (2017) reported that the extract of *Arthrospira platensis* using methanol solvent yielded 28.1%, which is consistent with the data obtained for *L.*

fusiformis TL03. In another study, Ferdous et *al.* (2023) found that the yield of crude extract of *Chlorella sp*. using different solvents (methanol, ethanol, ethyl acetate, DCM, acetone, chloroform, and hexane) ranged from 12 to 41%, with the highest yield recorded when methanol was used for extraction.

The total phenol content in the hydromethanolic extract of *L. fusiformis* TL03 agrees well with the results of other previously reported *spirulina* species. Zainoddin et *al.* (2018) reported that the value of TPC in *A. platensis* was 19.64 ± 0.52 mg GAE/g.

Comparing the results of the total flavonoid content in *L. fusiformis* TL03 (2.11 ± 0.12 QE mg/g DW) with those reported by several authors on the same species, we noticed that the level of flavonoids was higher than that recorded in other studies. Bellahcen et *al.* (2020) found the total contents of flavonoids in ethanolic and aqueous extracts (0.21 ± 0.01 and 0.15 ± 0.01 mg quercetin/g dw, respectively) of Moroccan *Spirulina*. (Thangaraj et *al.,* 2022) reported that the total flavonoids in the methanolic extract of *Arthospira platensis* (*spirulina*) were 1.42 ± 0.05 mg/g dry weight.

On the other hand, some studies have reported higher levels of total flavonoids in *spirulina* compared to *L. fusiformis* TL03. Zainoddin et *al.* (2018) reported that the total flavonoid content in *spirulina* was 11.19 ± 0.07 mg QE/g DW. Seghiri et *al.* (2019) found the content in the methanolic extract of Moroccan *spirulina* to be 15.60 ± 2.74 mg RE/g dw.

Other studies reported that the amount of flavonoids present in a *spirulina* extract was 142.23 mg quercetin/kg extract (A El-Chaghaby et al., 2019) and 176.3 ± 7.65 mg QE/100 g in the aqueous extract of *spirulina* powder (Elbaz et *al.,* 2022). Papalia et *al.* (2019),found that the TFC in *A. platensis* varied from 11.91 ± 0.28 to 22.65 ± 0.46 (mg g D.W.−1); Elbaz et *al.* (2022) reported that, the TPCcontent in *spirulina platensis* ranged from 321.3 ± 18.4 to 1580 ± 63.2 (mg GAE/100 g), while being highest than those documented in several research papers (Bellahcen et *al.,* 2020; Martelli et *al.,* 2020; Seghiri et *al.,* 2019; Thangaraj et *al.,* 2022; A El-Chaghaby et *al.,* 2019; Salamatullah, 2014; Kumar et *al.,* 2022).

The results of the total flavonoid content (TFC) in the extracts of *Chlorella sp*. CHL11 and *Chlorella sp*. CHL15, were similar to those reported for *C. vulgaris* (Dinev et *al.*, 2021) but lower than those found in the methanolic extract of marine *Chlorella sp*. (Ferdous et *al.*, 2023) and the ethanolic extract of *Chlorella sp*. (Fristiohady et *al.*, 2024) and *Chlorella vulgaris* (Tiong et *al*., 2020).

The values of total phenolic compounds in the two species of the genus Chlorella (*Chlorella sp*. CHL11, *Chlorella sp*. CHL15) were similar to those reported for *Chlorella sp*. (Fardous, et *al.,* 2023), *Chlorella saccharophila* (RNY), *Chlorella vulgaris* (LNY), and *Chlorella*

sorokiniana (21-21) [León-Vaz et *al.,* 2023], while they were higher than those of *Chlorella vulgaris* (13-1) [León-Vaz et *al.,* 2023] and *Chlorella vulgaris* (Fihri et *al.,* 2024), *Chlorella sorokinia* (Safafar et *al.*, 2015),(*Chlorella* UMACC 051 , *Chlorella* UMACC 038, *Chlorella* UMACC 250 and *Chlorella* UMACC 234)[Wong et *al.,* 2023], *C. vulgaris* (Dinev et *al.,* 2021) . On the other hand, Choochote, W et *al.* (2014).reported that the content of TCP in *Chlorella sp*. E53 and *Chlorella sp*.ED53 were higher compared to those of the investigated strains of this study.

Fihri et *al.* (2024) reported that the content of phenolic compounds in the extract of *Chlorella vulgaris* obtained using hydroethanol, methanol, and water ranged from 3,800 to 6,020 mg EAG/g DM.

In agreement with the result of polyphenolic compounds identified by HPLC in *L. fusiformis* TL03, several authors confirmed the presence of these compounds in *Arthrospira* and *Spirulina* (El-Baky et *al*., 2009; Sahin, 2018; Seghiri et *al*., 2019; Papalia et *al*., 2019; Akbarizareh et *al*., 2019; Guldas et *al*., 2020; Bellahcen et *al*., 2020; Farg et *al*., 2021).

Few research papers have been published on the identification of phenolic compounds in *Chlorella* species. Katırcıoğlu et *al*. (2020) reported the presence of vanillic acid, protocatechuic acid and other phenolic compounds in Chlorella vulgaris C1. Other studies,;confirmed the presence of protocatechuic acid in Chlorella pyrenoidosa(Yadavalli et *al*., 2018), vanillic acid in *Chlorella vulgaris* (Grácio et *al.,* 2024). tannic acid in the extract of *Anabeanae oryzae* as well as in *Nostoc sp*(Ijaz & Hasnain, 2016, Rashad et *al.,* 2020), Hesperidin in 23 species of seawoods (Yoshie-Stark et *al.,* 2003). The isorhamnetin 3-O-glucoside has been detected in the aquatic plant *Cymodocea nodosa* (Grignon-Dubois & Rezzonico, 2013), *Ruppia cirrhosa*, and in *Ruppia maritima* (Enerstvedt, 2018.).

Microalgae are considered to be a promising source of natural antioxidant compounds (Goodarzi et *al*., 2014, Khan et *al.,* 2018; Saide et *al*., 2021; Silva et *al* 2022; Yang et *al*., 2023; Pereira et *al.,* 2024). They contain a variety of bioactive molecules, such as alkaloids, polysaccharides, vitamins , lipids, pigments, carotenoids, phycocyanin, phenolics, phycocyanin, terpenes and aromatic organic acids (Shannon and Abu-Ghannam, 2016; Marrez et *al.*, 2019 ; Sansone & Brunet, 2019; Vikneshan et *al.*, 2020; Pereira et *al.,* 2024).

These results of percentage inhibition of DPPH were higher than those reported by Wong et *al*. (2023) in two tropical *Chlorella* strains (*Chlorella* UMACC 051 and *Chlorella* UMACC 038) and two polar *Chlorella* strains (*Chlorella* UMACC 250 and *Chlorella* UMACC 234), ranging from 15.42 ± 0.30 to 30.15 ± 3.22 %, and also higher than that of *Chlorella vulgaris*

(Abdel-Karim et *al*., 2020), anticancer, antiviral, and antibacterial properties (Gloria et *al.,* 2014).

Carotenoids are important groups of pigments (red, orange, or yellow colors) that are present in plants, fungi, bacteria, and algae and cyanobacteria (Hermanns et *al.,* 2020; Bhatt & Patel, 2020). They are also key metabolites for human nutrition and health (Sharma et *al.,* 2024). The foundation of the beta-carotene bleaching test is the retention of the beta-carotenoid acid system's yellow color when the item being evaluated includes antioxidants. (Velioglu et *al.,* 1998; Demirel et *al.*, 2009).

Numerous studies have documented the pharmacological and biological properties related to nearly all secondary metabolites synthesized by microalgale(Hernández-Urcera et *al.,* 2024). Those properties encompass a range of activities, such as anti-cholesterol (Ibrahim et *al.,* 2021),antitumor(Silva et *al* 2022; Eze et *al.*, 2023; Sruthy & Baiju 2024), anti-inflammatory(Wan Afifudeen et *al*., 2022; Sruthy & Baiju, 2024; Siddhnath et *al.,* 2024), anticoagulant (Siddhnath et *al.,* 2024), antifungal, antiviral and antibacterial, activities (Ribeiro et *al.,* 2022., Sruthy & Baiju, 2024; Ahirwar et *al.,* 2022; Eze et *al.*, 2023), and antioxidant activity(Siddhnath et *al.,* 2024; Eze et *al.*, 2023).

5. Conclusion

In conclusion, the results of this study showed that the hydromethanolic extract of the three microalgae species tested in this study contained different amounts of phenolic acid, with the highest amount found in *Limnospira fusiformis* TL03, compared to the two *Chlorella* species, in which the highest flavonoid content was found in the *Chlorella* sp. CHL11 extract.

HPLC analysis of the microalgae extracts revealed a large number of phenolic compounds, some of which were identified; five compounds (Ascorbic acid, Gallic acid, Luteolin, Quercetin, Kaempferol)were identified in *L. fusiformis* TL03, seven compounds (tannic acid, robinin, luteolin 7,3'-diglucoside, vannilic acid, apigenin 7-glucoside, quercetin, and galangin) in *Chlorella sp*. CHL11 and four compounds(protocatechuic acid, isorhamnetin-3-glucoside, hesperidin, quercetin) in *Chlorella sp*. CHL15.

The results of antioxidant activity tests using the DPPH and beta-carotene methods showed that, all extracts possessed antioxidant activities, with the highest percentage inhibition of DPPH and percentage of beta-carotene bleaching in *Limnospira fusiformis* TL03.

This large variety of phenolic compounds possesses strong antioxidant activity. Therefore, further research is needed to identify, isolate and characterise the molecules responsible for these activities, which could be used as alternatives to antibiotics and other traditional chemical drugs for the treatment of various diseases. Further studies are useful to investigate their benefits in various pharmaceutical and food industry applications.

Chapter 4

Antibacterial activity

1. Introduction

The use of antibiotics has saved millions of lives from bacterial infections (Salam et *al.,* 2023). They are not only used to treat humans but are also used for a variety of agriculture activities such as aquaculture and animal husbandry and animal production as preventive measures in many countries (Williams-Nguyen et *al.,* 2016; Salam et *al.,* 2023). On the other hand, the misuse of antibiotics has led to an increase in drug-resistant micro-organisms. 700,000 people die every year from antibiotic-resistant bacteria, and the death rate is expected to reach 10 million by 2050 (O'Neill, 2016; Mc Gee et *al.,* 2020).

Cyanobacteria and microalgae have been reported as a rich source of antimicrobial secondary metabolites in several studies (Cannell et *al.,* 1988; Ördög et *al.,* 2004; Soltani et *al.,* 2005; Mudimu et *al.,* 2014; Mc Gee et *al.,* 2020; Bhuyar et *al.,* 2020; Shaima et *al.,* 2022; Ilieva et *al.,* 2024;).

Microalgae have potential antimicrobial activity due to their bioactive compounds, such as proteins (Nur Fadillah et *al.,* 2023), lipids and fatty acids(Wang et *al.,* 2021), polysaccharides (Wan et *al.,* 2021; Laroche, 2022; Mansour et *al.,* 2024),phycobiliprotein (Ismail et *al.,* 2023), phenolic compounds(De Morais et *al.,* 2015; Pratita et *al.,* 2019; Abidizadegan et *al.,* 2024)**.**

The aims of this study are to explore the antibacteri*al* activity of three hydromethanol extracts from the lyophilized biomass of local microalgae: *L. fusiformis* TL-3, *Chlorella sp*. CHL11, and *Chlorella sp*. CHL15 against six pathogenic bacterial strains.

2. Materials and methods

2.1.Bacterial strains

The tested bacteria were provided by the Microbial Systems Biology Laboratory, Kouba Higher Normal School of Kouba, Algeria. Hydromethanolic extracts were tested for antibacterial activity against six pathogenic strains (Table 18).

Table 18 : Tested bacterial strains

Bacteria	Strains	Origin
Bacteria gram positive	*Bacillus subtilis* (*B. subtilis*)	ATCC 6633
	Staphylococcus aureus (*S. aureus*)	CIP 7625
	Listeria monocytogenes (*L. monocytogenes*)	CIP 82110
Bacteria gram negative	*Escherichia coli* (*E. coli*)	ATCC 10536
	Pseudomonas aeruginosa(*P. aeruginosa*)	CIP A22
	Klebseilla pneumoniae (*K. pneumoniae*)	CIP 82.91

2.2.Antibacterial activity test

The antibacterial activity of crude hydromethanolic extracts of local microalgae, *L. fusiformis* TL03, *Chlorella sp*. CHl11, and *Chlorella sp*. CHl15, was assessed in this work using the disc diffusion and the agar dilution methods.

The test of the antibacterial activity was carried out in the Microbial Systems Biology Laboratory, Kouba Higher Normal School of Kouba, Algeria.

2.2.1 Disc diffusion method

The disc diffusion technique was used to evaluate the antibacterial activity of the hydromethanolic extract. Sterile Whatman No. 1 discs (5.5 mm diameter) were impregnated with 10 µL of the hydromethanolic extracts (50 mg/mL), and other discs were impregnated with the same volume of ampicillin (1 mg/mL) and used as a control.

The tested bacterial strains were plated individually on nutrient agar. After 18 hours of incubation at 35°C, the colonies were transferred to saline (0.9% NaCl) to obtain a suspension turbidity equivalent to 1.5 x 10^8 CFU/mL (Alagawany et *al.,* 2021).

One hundred microliters of bacterial aliquots were spread on the surface of Mueller-Hinton agar (MHA) Petri dishes and then, three sterilized dried paper discs containing crude hydromethanolic extract or control (ampicillin) were placed equidistantly on the surface of agar Petri dishes (90 mm diameter) for each bacterial strain tested. Prior to incubation, the Petri dishes were placed in the dark at 4°C for 1 hour to allow the hydromethanolic extract and the ampicillin to spread. Then the plates were incubated at 37 °C for 24 h. The transparent ruler measured the inhibition zones diameters (mm) around the discs.

2.2.2 Agar dilution method (Minimum inhibition concentration: MIC).

From the initial concentration of crude hydromethanolic extract (50 mg/ml), a series of double dilutions (25, 12.5, 6.25, 3.125, 1.56, 0.78 mg/ml) were prepared by addition to Mueller-Hinton broth. The control was prepared using the same method as described above with double dilution of ampicillin (0.5, 0.25, 0.125, 0.0625, 0.032, and 0.016 mg/mL).

One hundred µL of each tested bacterial cell suspension (1.5 108 CFU/ml) was placed separately on the Petri agar surface. All plates were incubated at 37 °C for 24 hours.

The MIC values were defined as the lowest concentration of antibacterial agents that inhibited the growth of bacteria (Ashour et *al*., 2020).

2.3.Statistical analysis

The statistical analysis was conducted using IBM SPSS 25.0 software. The data were presented as means ± standard error (S.D.). The differences between groups were analyzed using a one-way analysis of variance (ANOVA) test, followed by the Tukey test.

3. Results

3.1. Antibacterial activities of *L. fusiformis* TL03 extract

The antibacterial activities of crude hydromethanolic extract of *L. fusiformis* TL03 were evaluated against six strains of bacteria by measuring the diameter of the inhibition zones, and the results were registered in Table 19 and Figure 31.

The tested bacterial strains revealed different results, in which the diameters of the inhibition zone (ZID) ranged from 16.33±0.58 to 33.33±1.53 mm at a concentration of 50 mg/ml. The highest mean of zone inhibition diameter was registered against the Gram-positive bacterium *S. aureus (33.33±1.53 mm),* followed by *B. subtilis* (16.67±0.58 mm) and *L. monocytogenes* (16.33±0.58 mm). For Gram-negative bacteria, the highest value of zone inhibition diameter (16.33±1.15mm) against *K. pneumoniae was* followed by *P. aeruginosa* (14.67±0.58 mm) and the lowest *E. coli* (11±1mm).

The results showed that the MICs (minimum inhibitory concentrations) of the hydromethanolic extract of *L. fusiformis* TL03 (Table 19) ranged from 0.78 mg/ml to 12.5 mg/ml. The lowest MIC value (0.78 mg/ml) was registered against *K. pneumoniae,* followed by *P. aeruginosa* (1.56 mg/ml. whereas the highest value (12.5 mg/ml) was against *E. coli.* However, the MIC of this extract was 6.25 mg/ml against three bacteria gram+, B. *subtilis , S. aureus* and *L. monocytogenes*. The MIC of ampicillin ranged from 0.5 mg/ml to less than 0.016 mg/ml, where the highest value was registered against *S. aureus* and *L. monocytogenes,* followed by 0.25 mg/ml against *K. pneumoniae*, while the lowest value (less than >0.016 mg/ml) was obtained against *B. subtilis*, *P. aeruginosa*, and *E. coli.*

Table 19: Antibacterial activity of crude hydromethanolic extract of *L. fusiformis* TL03

	Bacterial strains	***L. fusiformis TL03 extract***		**Ampicillin**	
		ZID(mm)	MIC(mg/ml)	ZID(mm)	MIC(mg/ml)
Bacteria	B. subtilis	16.67±0.58(a)	6.25	19.67± 0.58(d)	< 0.016
gram	*S. aureus*	33.33±1.53(b)	6.25	40±00(e)	0.5
positive	*L. monocytogenes*	16.33±0.58(a)	6.25	35±00(b)	0.5
Bacteria	*E. coli*	11±1(c)	12.5	24.33±1.15(f)	<0.016
gram	*P. aeruginosa*	14.67±0.58(a)	1.56	9.67±0.58(c)	< 0.016
negative	*K. pneumoniae*	16.33±1.15(a)	0.78	40.33±0.58(e)	0.25

3.2.Antibacterial activities of *Chlorella sp.* CHL11 extract

The hydromethanolic extract of *Chlorella sp*. CHL11 (Table 20, Figure31) at a concentration of 50 mg/ml showed antibacterial activity against all studied bacteria. The zone inhibition diameters were ranged from 11.67 mm - 24.33±1.15 mm. The antibacterial activity of the extract against *S. aureus* was significantly higher compared to its effect against other tested bacteria, whereas the lowest activity was obtained against *L. monocytogenes*. The minimum inhibitory concentration (MIC) of the hydromethanolic extract of *Chlorella sp*. CHL11 (Table 20) ranged from 1.56 to 12.5 mg/ml. The lowest concentration of the extract was recorded against *P. aeruginosa* while the highest were against *E. coli*.

Table 20: Antibacterial activity of crude hydromethanolic extract of *Chlorella sp*. CHL11

	Bacterial strains	***Chlorella sp.CHL11 extract***		**Ampicillin**	
		ZID(mm)	MIC(mg/ml)	ZID(mm)	MIC(mg/ml)
Bacteria	B. subtilis	19.67±0.58 (a)	6.25	19.67± 0.58(a)	< 0.016
gram	*S. aureus*	24.33±1.15 (b)	3.125	40±00(g)	0.5
positive	*L. monocytogenes*	11.67±0.58(d)	3.125	35±00(f)	0.5
Bacteria	*E. coli*	13.67±0.58 (c)	12.5	24.33±1.15(b)	<0.016
gram	*P. aeruginosa*	14.67±0.58 (c)	1.56	9.67±0.58(e)	< 0.016
negative	*K. pneumoniae*	14.33±0.58 (c)	6.25	40.33±0.58(g)	0.25

3.3.Antibacterial activities of *Chlorella sp.* CHL15 extract

The results obtained for the antibacterial effect of the hydromethanol extract of the microalga *Chlorella sp*. CHL15 (Table 21; Figure 31) against the tested bacteria showed that *S. aureus* was more sensitive to the extract with the zone inhibition diameter (21±1 mm), followed by *B. subtilis* (17.33±0.58 mm), *L. monocytogenes* (15.67±0.58 mm), *P. aeruginosa* (15.67±1.15), and *K. pneumoniae* (15.33±0.33 mm), and the less activity was against *E. coli* (10.67±0.58 mm).

The minimum inhibitory concentrations (MIC) of the hydromethanolic extract of Chlorella sp. CHL15 against 6 tested bacteria were varied from 1.56 to 6.25 mg/ml. The lowest MIC concentrations were registered against *P. aeruginosa* and *K. pneumoniae*, whereas the highest values were registered against *L. monocytogenes* and *E. coli.*

Table 21: Antibacterial activity of crude hydromethanolic extract of *Chlorella sp*. CHL15

	Bacterial strains	***Chlorella sp.*CHL15 extract**		**Ampicillin**	
		ZID(mm)	MIC(mg/ml)	ZID(mm)	MIC(mg/ml)
Bacteria gram positive	B. subtilis	17.33±0.58 (b)	3.125	19.67± 0.58(c)	< 0.016
	S. aureus	21±1 (c)	3.125	40±00(f)	0.5
	L. monocytogenes	15.67±0.58 (ab)	6.25	35±00(e)	0.5
Bacteria gram negative	*E. coli*	10.67±0.58 (h)	6.25	24.33±1.15(d)	<0.016
	P. aeruginosa	15.67±1.15 (ab)	1.56	9.67±0.58(h)	< 0.016
	K. pneumoniae	15.33±0.33 (a)	1.56	40.33±0.58(f)	0.25

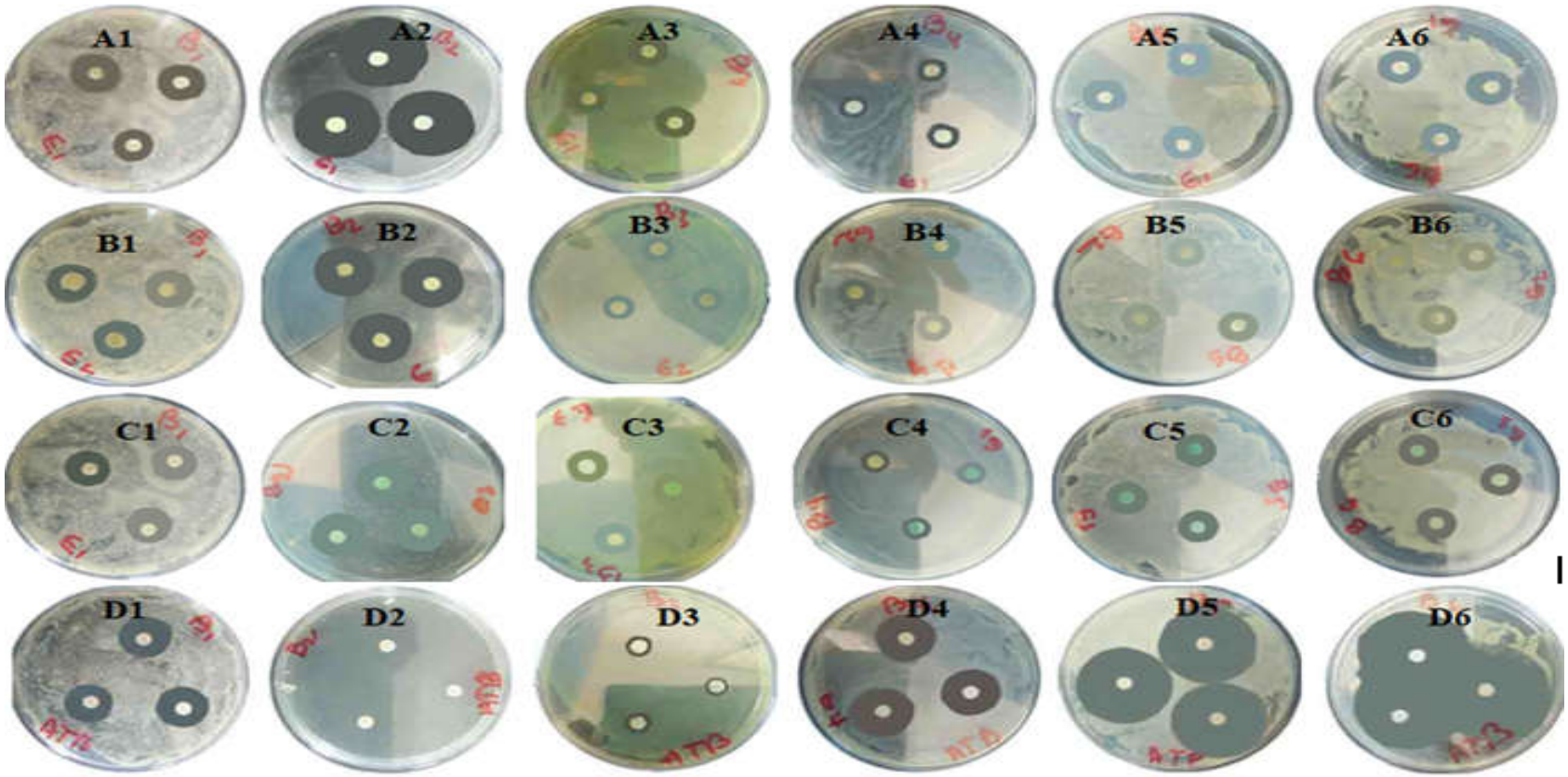

Figure 31 . Zone inhibition diameters (ZID) of the crude hydromethanolic extract of *L. fusiformis TL03* (A) , *Chlorella sp*. CHL11(B), and *Chlorella sp*. CHL15(C) and the antibiotic Ampicillin (D) against six bacterial strains. 1: *B. subtilis*; 2: *S. aureus*; 3: *L. monocytogenes*; 4: *E. coli*; 5: *P. aeruginosa*; 6: *K. pneumoniae*

4. Discussion

In line with the results of this investigation, several previous studies have confirmed the antibacterial activity of Limnospira extracts against the bacteria tested such as .*B. subtilis* (Neelam Arun et *al.*, 2012 ; El-Sheekh et *al.,* 2014) and *S. aureus* (Kaushik & Chauhan, 2008 ; Sarada et *al.,* 2011; Santoyo et *al.,* 2011; Al-ghanayem, 2017; Martelli et *al.,* 2020; Karray et *al.,* 2021; Alshuniaber et *al.* 2021; Sankarapandian et *al.,* 2022; Al.ghamdi et *al.*, 2024), *L. monocytogenes*, *E. coli* (Mendiola et *al.*, 2007; Kaushik & Chauhan, 2008 ; Sarada et *al.,* 2011; Martelli et *al.,* 2020 ; Karray et *al.,* 2021; Alshuniaber et *al.,* 2021; Sankarapandian et *al.,* 2022), *P. aeruginosa* (Kaushik & Chauhan, 2008 ; Sarada et *al.,* 2011; El-Sheekh et *al.,* 2014 ; Sankarapandian et *al.,* 2022) and *K. pneumoniae* (Kaushik & Chauhan, 2008 ; Sarada et *al.,* 2011; Sankarapandian et *al.,* 2022).

Our findings (Table 19) showed that *L. fusiformis* TL03 extracts had strong antibacterial activity against *S. aureus, B. cereus, K. pneumoniae*, *P. aeruginosa*, and *L. monocytogenes*, whereas they had less activity against *E. coli*. These results are also in agreement with previous studies (Kaushik & Chauhan, 2008; Usharani et *al.,* 2015; Gheda & Ismail, 2020), which confirmed that the methanol extract of *Limnospira platensis* showed high activity against several positive and negative gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae, Streptococcus pyogenes, Streptococcus epidermidis, Proteus mirabilis, Salmonella typhi,* and *Shigella flexneri*). According to Abdel-Moneim et *al.* (2022), The methanolic extract of *Spirulina platensis* exhibited greater total phenolic content, antioxidant activity, and antibacterial activity in comparison to other extracts (hexane and acetone extracts). Furthermore, it has been noted that the methanolic extract of *Spirulina platensis* has potent antimicrobial and antifungal activities against some pathogenic microbial strains (Hetta et *al.,* 2022).

Martelli et *al.,* 2020, demonstrated similar results as our findings, where the hydroethanolic extracts of *A. platensis* showed the highest antimicrobial activity (ZID = 15 mm) against gram-positive bacteria (*L. monocytogenes, S. aureus, and B. cereus*) and interesting inhibition results for gram-negative bacteria (*E. coli, Salmonella* spp.).

Kaushik & Chauhan (2008), reported that the ZID of methanolic extract of *Limnospira platensis against* three bacteria (*S. typhi*, *P. aeruginosa*, *E. coli*, *S. aureus*) ranged from 11.52±.18 to 15.21±.1mm but no activity was observed against *K. pneumoniae.*

Abdel-Moneim et *al.* (2022) stated Reported that the strong antimicrobial activity of the methanolic extract may be due to its high phenolic content.

According to some published papers (Bartolomeu et *al.,* 2022; Afzal et *al.,* 2023), the antimicrobial activities of algae against a variety of bacteria and fungus are linked to polyphenols, terpenes, flavonoids, alkaloids, pigments,amino acids, polysaccharides (such as depolymerized fucoidans), lipids, and other lipid-soluble substances.

Spirulina bioactive compounds can damage bacterial cell integrity by increasing membrane permeability, which leads to loss of cytoplasmic content and then cell death (Abdel-Moneim et *al.,* 2022). On the other hand, De Mule et *al.* (1996) reported that the antibacterial activity of the methanolic extract of *S. platensis* is caused by the presence of a significant amount of linolenic acid.

The minimum inhibitory concentration (MIC) of hydromethanolic extract of *L fusiformis TL03* (Table 19) *ranging* from 0.78 to 12.56 mg/ ml. These results agree in part with previous studies by Abdel-Moneim et *al.* (2022), which recorded that the MIC values of *Spirulina* extracts (methanol, hexane, and acetone) against tested bacteria ranged from 1.2 to 10 mg/ml, with the lowest level MIC in methanol extracts (1.2 to 2 mg/ml).

These data are also in agreement with the previous study by Usharani et *al.* (2015)., who reported that the MIC value of methanolic extract of *Spirulina platensis against eleven bacterial strains (Staphylococcus aureus, Streptococcus epidermidis, Streptococcus pyogenes, Bacillus cereus, Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholerae, Salmonella typhi, Klebsiella pneumoniae, and Shigella flexneri*) ranged from 1.25 mg/ml to 5 mg/ml.

Kaushik& Chauhan (2008) reported that .The MIC of methanol extract of *Spirulina platensis* against *S. aureus* and *E. coli* were 128 µg /ml and 256 µg /ml, respectively.

Numerous research papers have reported that extracts of unicellular green algae have remarkable antibacterial activity against a wide range of opportunistic and pathogenic bacteria (Sukhikh et *al.,* 2022; Vornoli et *al.,* 2023).

Consistent with the result of the antibacterial activity of the extract of two species of Chlorella (*Chlorella sp*. CHL11, and *Chlorella sp*. CHL15) against the tested bacteria, it has been published in several scientific papers that extracts of different *Chlorella spp*. have antibacterial activity against both gram-negative (*E. coli*) and gram-positive (*S. aureus*) bacteria [Zielinski et *al*., 2020; Sukhikh et *al*., 2022]. In the same context, other studies have reported that chlorelin (a mixture of fatty acids) from *Chlorella* sp. (Pratt et *al*., 1944) and *Chlorella vulgaris* (Ru et *al*., 2020) has antibacterial activity against Gram-positive (*B. subtilis, S. aureus and S. epidermidis*) and Gram-negative (*E. coli and P. aeruginosa*) bacteria.

Microalgae contain many bioactive compounds with antibacterial properties. One of the most potent antibacterial compounds found in microalgae is fatty acids, particularly polyunsaturated fatty acids (PUFAs), which have been shown to have antimicrobial therapeutic potential(Alsenani et *al.,* 2020; Firoozabad & Nasr, 2022).

Bhagavathy et *al.* (2011) found that purified carotenoids and chlorophyll extracted from the green microalga *Chlorococcum humicola*, obtained using different organic solvents, inhibited the growth of *B. subtilis*, *S. aureus*, *P. aeruginosa*, *Klebsiella pneumoniae*, *E. coli*, *S. typhimurium*, and *Vibrio cholera*.

Makarewicz et *al.* (2021) reported that, the polyphenol antimicrobial activity mechanisms against bacteria can differ and are also related to the type of phenolic compounds and the bacterial species.

The phenolics compounds can denature proteins in bacteri*al.* cell walls and membranes by forming hydrogen bonds, thus causing their lysis (De Morais et *al.,* 2015; Li et *al.,* 2016; Pratita et *al.,* 2019; Abidizadegan et *al.*, 2024). Additionally, the phenolic compounds extracted from microalgae could cause the bacterial lysis by altering the permeability and integrity of the phospholipid layer in the membrane (Daglia, 2012).

5. Conclusion

The antimicrobial activities of *L. fusiformis* TL03*, Chlorella sp.* CHL11 and *Chlorella sp*. CHL15 have demonstrated their potential as natural antibacterial agents against several pathogenic bacteria.

The inhibition zone diameters and minimum inhibitory concentrations (MICs) highlight the bioactive potential of these local microalgae, likely due to their richness in secondary metabolites such as polyphenols, fatty acids, pigments, and polysaccharides. Among the extracts, *L. fusiformis* TL03 demonstrated the most potent antibacterial activity, notably against *S. aureus* and *K. pneumoniae*, suggesting a synergistic effect of its bioactive compounds.

Based on the preliminary results obtained, further advanced research is needed to identify, isolate and characterize the molecules responsible for these activities, which could be used as alternatives to antibiotics and other traditional chemical drugs for the treatment of various diseases. Further studies are warranted to verify their benefits in various pharmaceutical and food industry applications.

Chapter 5

Artemia feeding

1. Introduction

In recent years, the world has seen a significant increase in the production of aquaculture products (Madkour et *al.,* 2022) such as fish, crustaceans, mollusks, and algae (FAO, 2020).
Feeding the larvae with good quality of food and in sufficient quantities plays an important role in increasing growth, reducing mortality and producing healthy larvae, which is considered one of the most important steps for successful production in the hatchery (Hashemi et *al.,* 2023). Most fish and crustaceans feed on microalgae and zooplankton in their early stages, which are the main food sources, especially for larvae feeding on live food.
Microalgae are the primary food source for all aquatic trophic chains, providing energy for all subsequent trophic levels in the aquatic ecosystem. Microalgae are used directly as live feed in aquaculture for all growth stages of bivalve molluscs (e.g., oysters, clams, and mussels), for the larval stages (Pratiwy et *al.,* 2021) of some marine molluscs (abalone, oysters), crustaceans, and some fish species, sea cucumber and crab hatcheries (Zmora et *al.,* 2013) and indirectly, in which the energy are transferred from microalgae to higher trophic levels via intermediate zooplankton (Rotifers, Daphnia, and Artemia).
Artemia is an essential link in the production of modern aquaculture hatcheries as it is the primary feed source for some freshwater fish larvae (largemouth bass, pike perch), seawater (sea bass and sea bream), and for shrimp. Using Artemia *nauplii* to feed fish larvae improved fish growth and development and reduced mortality (Łączyńska *et al.,* 2016; Prusińska *et al.,* 2020).
In Algeria, many "private" aquaculture farms have been set up to produce marine and freshwater fish and molluscs (mussels and oysters). In addition, several experimental stations have been set up throughout the country, including the Experimental Station for Marine Fish Farming, the Experimental Station for Continental Fish Farming, the Experimental Station for Shrimp Production, and a centre for the production of molluscs such as oysters and mussels. However, the provision of artificial and live feed such as Artemia and microalgae is considered one of the main challenges for the development of aquaculture in Algeria
In view of the significant growth of aquaculture activities in Algeria, it has become necessary to carry out studies on the suitability of isolated local microalgae for feeding Artemia. In this context, this study was conducted to feed *Artemia franciscana* with three local microalgae strains in addition to yeast. The present study aimed to investigate the effect of three local microalgae species and yeast on the growth and survival rate of *Artemia franciscana* and to determine the most suitable algae for its growth.

2. Materials and Methods

2.1.Preparation of concentrated solutions stock

Three algal species (*L.fusiformis* Tl03, *Chlorella sp.* CHL11and *Chlorella sp.* CHL15) and Yeast (*Saccharomyces cerevisiae*) were tested as feed to study their effect on growth and survival rate of *Artemia franciscana*

During the exponential growth phase of the three microalgae (2 weeks), biomass was collected and lyophilized.

2.1.1. *L. fusiformis* Tl03 solution

One gram of freeze-dried biomass was crushed with a mortar and pestle to obtain small filaments. It was then lightly crushed and filtered through a 50 µm mesh with the gradual addition of fresh water to obtain a final concentrate of 1 g/l

2.1.2. *Chlorella* solution

A microalgae solution of both *Chlorella sp.* CHL11 and *Chlorella sp.* CHL15 was prepared by adding 1 g of freeze-dried microalgae from each strain to 1 liter of fresh water

2.1.3. Yeast solution

One gram of baker's yeast (*Saccharomyces cerevisiae*) was added to 1 liter of freshwater

Stock solutions were stored in a refrigerator at 4°C immediately after their preparation.

2.2.Incubation of *Artemia* cysts

A quantity of one gram of *Artemia franciscana* cyst was added to bottles containing one liter of filtered, sterile seawater at 37‰ salinity. These bottles were placed in a glass tank containing 20 liters of water. The cysts were incubated for 24 hours under a constant temperature of 28 °C using a thermostat and continuous white light of 2500 lux. The cysts were kept in suspension (Sorgeloos et *al.,* 1996) by using an air pump.

The newly hatched nauplii were starved for 24 hours to allow the yolk reserve to be depleted before starting exogenous feeding.

2.3. Feeding experiment

Two hundred starved nauplii for 24h were introduced in a conical flasks containing 500 milliliter of filtered seawater (salinity 37‰, pH was previously adjusted to 8±0.2) with a 1 ml of stocks microalgal solution (*L.fusiformis* Tl03, *Chlorella sp.* CHL11 and *Chlorella sp.* CHL15) and Yeast with a concentration of 5µg/individual of Artemia (Seixas et *al.*,2009). The quantity of feed (*L. fusiformis* Tl03, *Chlorella sp*. CHL11, and *Chlorella sp*. CHL15 and Yeast) was gradually increased as the Artemia individuals body length grew, when the culture

media were observed transparent, so that almost all of the provided feed was eaten (Sorgeloos et *al.*, 1986).

The flasks were placed in an aquarium of 10 liter of water. The flasks were supplied with dissolved oxygen (5 ± 0.2 mg/L) using an air pump. The incubation was carried out at 28 ± 2 °C with continuous lighting at 2.5 Klux. The experiment was performed in four replicates.

The water of the Artemia culture was completely changed every 2 days to remove the faeces of the Artemia and any old microalgae cells that might have remained in the water.

2.4.Body length measurement

The initial body length was measured on ten individuals after 24 hours of cyst hatching which corresponds to the yolk reserve depletion. The same number of Artemia nauplii were randomly sampled every two days from each treatments to measure their body length over the 12 days of the experimental period.

Measurements of the body length of Artemia larvae were taken using an Optika dissecting microscope equipped with a calibrated ocular micrometer (Optika, Ponteranica, Italy) and photographed to measure the length between the eye and the end of the tail (Fig 32).

The body length was identified as the distance from the eye and the end of the tail. [Ekonomou et *al.*, 2019].

Figure 32. Body length measurement of *Artemia franciscana* X 40 (Original)

2.5. Survival rate

Artemia survival rate was determined by dividing the final number by the initial number of Artemia in each treatment every two days during the experiment. The survival rate has been calculated according to the following formula (Amin et *al.*, 2022):

$$SR = Nt/N0 \times 100$$

Where SR represents survival rate, Nt represents final nauplius numbers, and N0 represents initial nauplius numbers.

3. Results

The microscopic observation under the lens showed that the colour of 00Artemia fed on Yeast, *L.fusiformis* TL03, *Chlorella sp.* CHL11 *and Chlorella sp.* CHL15 was yellow to green during all the experiment, (Fig.33 A, B, C and D).

Figure 33. Observation of *Artemia franciscana* under an Optika dissecting microscope. A, B, C, D Artemia fed on Yeast, *L.fusiformis* TL03, *Chlorella sp*. CHL11 and *Chlorella sp.* CHL15 respectively on day 12(X 40)

3.1.Body length measurement

The initial body length of the nauplii was 422 ± 12.62 µm. After 12 days of rearing, their body lengths ranged from 2666.4 ± 46.22 to 5030.13 ± 189.82 µm depending on the feed used (Table 22). At the end of the experiment, the highest body length was recorded in Artemia fed on *L. fusiformis* Tl03 (5030.13 ± 189.82 µm), followed by those fed on yeast (4457.63 ± 241.09 µm), *Chlorella sp*. CHL11 (4396.23 ± 78.17 µm), and *Chlorella sp*. CHL15 (2666.4 ± 46.22 µm).

As shown in Figure 34, Artemia fed on *Chlorella sp*. CHL11 and *Chlorella sp*. CHL15 showed similar slow growth patterns during the first six days of the experiment, while those fed on yeast, *L. fusiformis* Tl03, showed fast growth patterns throughout the first eight days of the experiment.

During the last six days of rearing, Artemia fed with *Chlorella sp*. CHL11 showed significantly higher growth compared to those fed with *Chlorella sp*. CHL15.

A comparaison of the mean length of the larvae at the beginning of the experiment with that at the end, it was found that the body length of Artemia fed on *L. fusiformis* Tl03 increased

11.92 folds, followed by those fed on yeast (10.56 folds), on *Chlorella sp*. CHL11 (10.42 folds), and the lowest for those fed on *Chlorella sp*. CHL15 (6.32 folds).

The comparison of Artemia body length results on day 12 showed a significant difference ($P<0.05$) between the three different treatments, whereas no difference was registered among those fed yeast and *Chlorella* sp. CHL11.

Table.22: Growth expressed as mean total body length of *Artemia franciscana* fed on *L.fusiformis* Tl03, *Chlorella sp*. CHL11, and *Chlorella sp*. CHL15, Yeast.

Days	*L. fusiformis* TL03	*Chlorella sp.* CHL11	*Chlorella sp.* CHl15	Yeast
0	422 ± 12.62(a)	422 ± 12.62(a)	422 ± 12.62(a)	422 ±12.62(a)
2	768.93 ± 35.46(a)	798.13± 25.85(a)	807.47± 46.69 (a)	821.67 ±24.28(a)
4	1637.80 ± 80.65(a)	1052.67± 48.49 (b)	1029.67± 50.95 (b)	1592.8 ±92.57(a)
6	2340.23 ±102.62(a)	1184 ±60.77(b)	1247. 3 ±65.02(b)	2396.47±109.78(a)
8	2978.80 ±305.65(a)	1893 ±252.05(b)	1439.97 ±132.47(c)	2958.47±452.73(a)
10	4467.93 ±328.79(a)	2801.47 ±135.39(c)	2411.2 ±80.06(d)	4071.17±417.58(b)
12	5030.13 ±189.82(a)	4396.23 ±78.17(b)	2666.4 ±46.22(c)	4457.63±241.09(b)

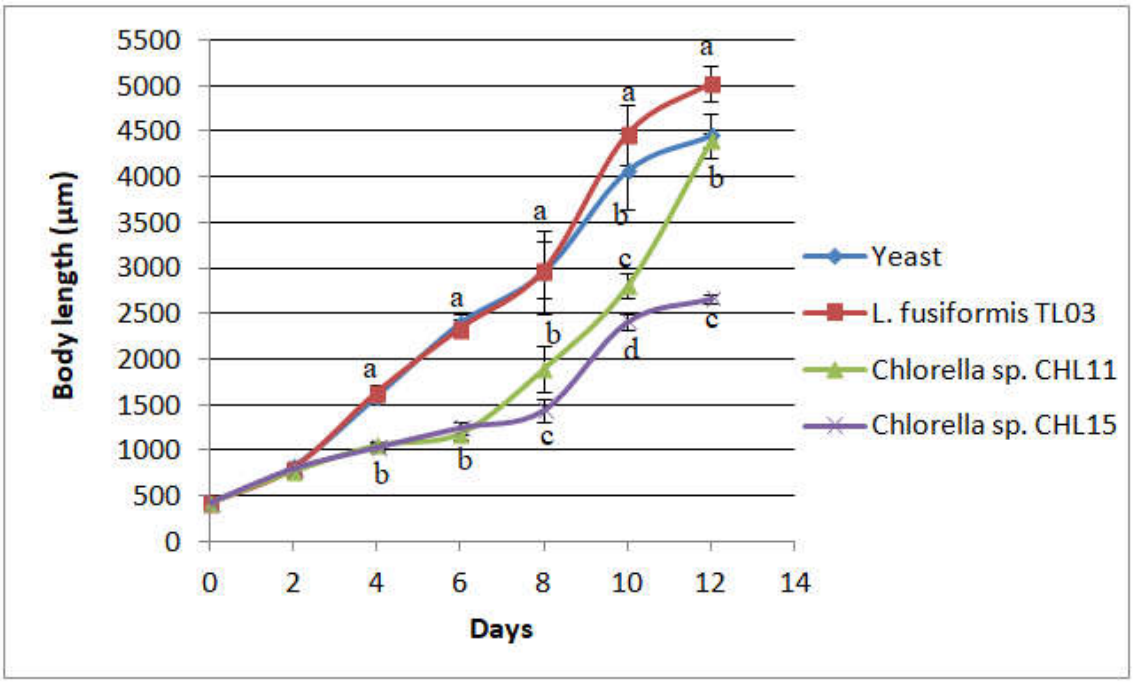

Figure 34. Mean total body length of *Artemia franciscana* fed with *L.fusiformis* Tl03, *Chlorella sp*. CHL11, and *Chlorella sp*. CHL15, and Yeast.

3.2.Survival rate

Figure 35 and Table 23 show the survival rate of Artemia fed on different species of microalgae.

The initial density of Artemia nauplii in the experimental containers was 200 nauplii per 0.5 liters of seawater. Counting of the Artemia every two days during the experiment revealed that the survival rate decreased in all treatment groups. At the end of the experiment, the highest survival rate was recorded in Artemia fed with *L. fusiformis* Tl03 (78.33 ± 0.88%), followed by those fed with *Chlorella sp.* CHL11 (75.33 ± 0.88%), yeast (74.33 ± 2.91%), and the lowest was found in those fed with *Chlorella sp*. CHL15 (36.66 ± 0.88%).. The survival rate of Artemia in the last treatment was significantly lower ($p<0.05$) than in the other treatments (Yeast, *L. fusiformis* Tl03, and *Chlorella sp*. CHL11), whereas there was no significant difference between the three different treatment groups (Fig. 35).

Table.23: Survival rate of *Artemia franciscana* fed on *L.fusiformis* Tl03, *Chlorella sp.* CHL11, *Chlorella sp.* CHL15 and Yeast.

Days	*L fusiformis* Tl03	*Chlorella sp.* CHL11	*Chlorella sp.* CHl15	Yeast
0	100	100	100	100
2	98 ± 0.58 (a)	95.67± 0.88 (ab)	92± 1.15 (b)	95.33 ±1.45 (ab)
4	96.33 ± 0.88 (a)	90.33± 0.88 (ab)	82± 0.58 (b)	92.33±1.67 (ab)
6	95.33 ±0.88 (a)	87 ±0.58 (ab)	76.33 ±1.20 (b)	90 ±1.53 (ab)
8	91.67 ±0.88 (a)	85 ±1.15 (ab)	71 ±1.15 (b)	88.33 ±0.33 (ab)
10	87.67±1.45 (a)	82.33 ±0.88 (a)	55 ±1.15 (b)	85.33 ±0.88 (a)
12	78.33 ±0.88 (a)	75.33 ±0.88 (a)	36.66 ±0.88 (b)	74.33 ±2.91 (a)

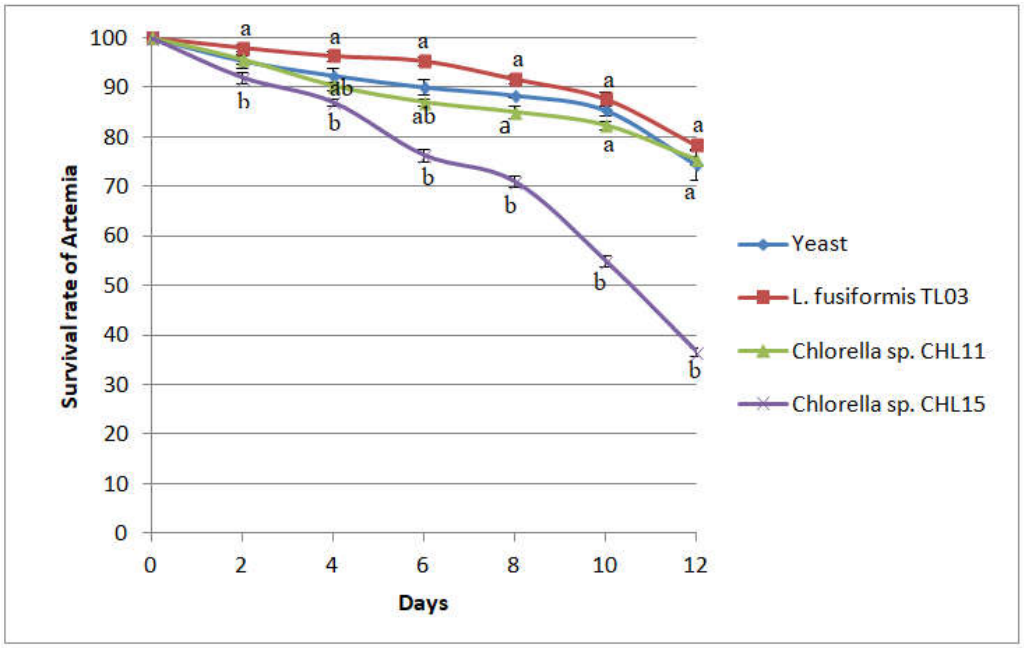

Figure.35: Survival rate of *Artemia* fransciscana fed on *L.fusiformis* Tl03, *Chlorella sp.* CHL11, *Chlorella sp.* CHL15 and Yeast.

4. Discussion

Growth and survival rates are the most important parameters for assessing the success of *Artemia* biomass production. These parameters are highly dependent on the quality and quantity of the food provided the quality of the water, and the culture conditions such as water salinity, temperature, light intensity, and oxygen concentration.

The pigmentation of the naupllii may be due to the pigments stored in the microalgae cell. The coloration of Artemia nauplii in green to yellow may be due to the carotenoids content in microalgae; however, the green color is due to the chlorophyll content in *Chlorella sp.* CHL11 and *Chlorella sp.* CHL15. According to Gui et *al.* (2022), the carotenoids initially stored in the algal cells can be delivered to Artemia through the feeding. In addition, Nelis et *al.* (1984) and Maan & Sefc (2013) reported that the Artemia feeding may influence body color and the appearance of specific carotenoid pigments may be related to reproduction and/or embryonic development.

At the end of the experiment, the mean body lengths of *A. franciscana* fed on yeast, *L. fusiformis* TL03, and *Chlorella* sp. CHL11 were approximately equal to those reported by Amin et *al.* (2022), who found that the mean body lengths of *A. franciscana* fed on *C. calcitrans* for the same period (12 days) were 4.7 mm. and also consistent with those fed on *Schizochytrium sp*., *Rhabdonema sp*., and the mixed algae (*Navicula sp*. + *Schizochytrium sp*.) [Pacheco Vega et *al.,* 2015]. These results were in agreement with those obtained with three local strains of *Artemia* (Bethioua, El Melah, Timimoun) fed on *Tetraselmis suecica* (Chabet dis et *al.,* 2023).

The mean total body length of Artemia fed on *Chlorella sp. CHL15* was similar to that of Artemia fed on monoalgal culture (*Nitzschia sp*., *Chaetoceros muelleri*., *Navicula sp*., and *Grammatophora sp*.) during a period of 12 days, in which their body length ranged from 2.69 to 3.43 mm (Pacheco Vega et *al.,* 2015). Turcihan et *al.* (2021) have studied the effect of five microalgae (*Amphora viridis*, *Chlamydomonas reinhardtii*, *Chlorella vulgaris*, and *Dunaliella salina*, and a combination of four microalgae) on the total body length of *Artemia franciscana*. Their results showed that the total length after 15 days of the experiment was lower than that of Artemia fed on yeast ,*L. fusiformis* TL03, and *Chlorella* sp. after 12 days of feeding.

A number of studies have shown that feeding Artemia on *Spirulina* has the best growth performance of Artemia compared to some feeds. Sulistiyarto and Bakrie (2024) found that, on day 21 of the experiment, the body length of Artemia fed on *Spirulina* powder was slightly longer than that fed on soybean flower powder. In a study conducted by Qaranjıkı & Kırkağaç (2022), *Artemia parthenogenetica* were fed on four different types of powdered and ground dried feed (*Spirulina*, yeast, rice bran, and oats) as well as their combinations of these

feeds, in ten experimental groups for 20 days. Their results showed that the Artemia fed on spirulina had the highest average length and weight.

In contrast to our findings, Arumugam et *al.* (2013) reported in their study that the body length of *Artemia* salina fed with *Spirulina* powder for 20 days was smaller (1.1 ± 0.6 cm) than that obtained in our experiments.

The best growth of Artemia fed on *L. fusiformis* Tl03 can be explained by the fact that this alga was crushed to small cells in addition it has contains a wide range of nutrients and has a higher nutritional value compared to yeast and two *Chlorella* species (*Chlorella* sp. CHL11 and *Chlorella* sp. CHL15). There are several factors related to the nature of microalgae that influence the growth of Artemia, such as cell size, shape, digestibility, and nutritional value. According to Bernards et *al.* (2018) and Dan et *al.* (2022), juvenile stages of *Artemia* sp. are often unable to ingest some microalgae due to their size, chemical composition, and cell wall rigidity, which affect growth and surviv*al.* Makridis & Vadstein (1999) reported that the *Artemia franciscana* prefers particles with a diameter of 4-8 micrometers in the three growth stages of the metanauplii.

The results of the survival rate (Fig 36) showed a significant difference ($p<0.05$) between Artemia fed with *Chlorella sp*. CHL15 and those fed with *Yeast*, *L.fusiformis* Tl03, and *Chlorella sp*. CHL11. The lowest survival rate was recorded in *Chlorella sp*. CHL15. The lowest survival rate was recorded in those fed on Chlorella sp. CHL15 during the experimental period. The results of the survival rate of *A. franciscana* fed on yeast, *L. fusiformis* Tl03, and *Chlorella* sp. CHL11 were similar to those reported by Pacheco Vega et *al.* (2015), where they found that the survival rate of *Artemia franciscana* fed on different microalgae species *(Grammatophora sp*., *Navicula sp*., *Rhabdonema sp*., *Schizochytrium sp*., *Navicula sp*. + *Schizochytrium sp*., and *Chaetoceros muelleri*) for 12 days ranged from 71.2 to 78.9%.

Qaranjıkı & Kırkağaç. (2022) , found that *Artemia parthenogenetica* was fed on *Spirulina* powder for 20 days had the highest survival rate (90.64 0.06 %) compared to those fed on different types of ground dried and combined feeds (*Spirulina*, yeast, rice bran, and oat) during the same experimental period, where their survival rate ranged from 78.22 ± 1.78 to 89.25 ± 0.14 %

In another study, Gui et *al.* (2022) reported that the survival rate of Artemia fed with different microalgae (*Haematococcus pluvialis*, *Spirulina* sp., *Myrmecia incica*, *Isochrysis galbana*,

and *Dunaliella salina*) ranged from 57.8 ± 1.9 to 87.8 ± 1.9% on the 5th day of the experiments, with the survival rate of those fed with *Spirulina* being 67.8 ± 1.9%.

5. Conclusion

The crushed *L. fusiformis* Tl03, *Chlorella* sp. CHL11 and yeast were found to be the best live feeds for the *Artemia franciscana* in this experiment, with high growth and survival rates, whereas those fed on *Chlorella* sp. CHL15 had low survival rate and growth rates during the experiment.

Based on the preliminary results of this study, it was concluded that local ground *L. fusiformis* Tl03 and *Chlorella* sp. CHL11 could be used as feed for the production of *A. franciscana* in Algeria. However, this research should be extended to other aspects, such as testing a others local microalgal strains, analyzing the quality of algae and *Artemia*, as well as the eggs produced, with a technical and economic study of the production of this species or local species of Artemia using local microalgae.

The results of this study could be useful in developing effective strategies for culturing this species of Artemia in controlled aquatic systems and could serve as an incentive to study the quality and valorization of local Artemia and the possibility of establishing productive farms of local Artemia fed on local microalgae, thus contributing to the achievement of sustainable aquaculture in Algeria.

Chapter 6

Daphnia feeding

1. Introduction

Daphnia magna (water flea) is used as live prey for freshwater and marine aquatic species (Zeybek et *al.*, 2023). May also be utilised as alternative fish feed in a different form, such as fresh, freeze-dried, or dried *Daphnia* meal in microdiets for several fish species, barramundi(Chiu et *al*., 2015). It has a high nutritional value, containing 45-72% protein (Sangeetha et *al.*,2024)and 11-27% fat (Jorge et *al.*, 2016) and it small size make it an excellent live feed that enhances the growth and survival rates of larvae fish.

Successful mass-produced *Daphnia magna* requires sustainable, high-quality feed. Microalgae are an essential source of nutrition for *Daphnia* due to their high nutritional value. The selection and supply of a suitable microalgae helps to master the production of *Daphnia* to satisfy the nutritional needs of various aquatic animals, particullary in hatcheries at the larval stage of some fish species such as in case of sander and largemouth bass fish.

With the expansion of aquaculture in Algeria, it is important to search and to identify microalgae strains, particularly indigenous ones that are suitable for *Daphnia* growth and reproduction.

This study investigated the growth and survival rates of *Daphnia magna* fed with three different strains of local microalgae (*L.fusiformis* Tl03, *Chlorella sp.* CHL11 and *Chlorella sp.* CHL15) and yeast (*Saccharomyces cerevisiae*).

2. Materials and Methods

2.1.Feed experiment:

Freeze dried biomass of three algal species (*L.fusiformis* Tl03, *Chlorella sp.* CHL11and *Chlorella sp.* CHL15) and Yeast (*Saccharomyces cerevisiae*) were tested as feed to study their effect on growth and survival rate of *Daphnia magna.*

2.2.Preparation of concentrated microalgae and yeast solutions:

The same feeding protocol for Artemia was used to prepare concentrated solutions for feeding *D. magna*

2.3. *Daphnia* Sampling:

Samples of *D. magna*. were collected from basins at the National Centre for the Development and Research of Fisheries and Aquaculture (CNRDPA). Sampling was carried out using a landing net with a mesh of 1 mm. Two grams of collected biomass were placed in a container containing 20 liters of freshwater, then transferred immediately to the laboratory for treatment.

Two thousand adult Daphnia magna were separated from the other zooplankton, particulate matter and insects. The adult *Daphnia* were introduced in a small aquarium containing 5 l of filtred freshwater and incubated for 48 hours at 25°C and continuous lighting (2.7 Klux), the aquarium was supplied with air pumps for water oxygenation (5-6 mg/l). Then the Daphnia were filtered using a 600 µm mesh sieve to separate the neonates (less than 600 µm) from the larger individuals.

Two hundred newly hatched *D. magna*, which had been starved for 24 hours, were placed in one liter Erlenmeyer flasks containing 500 ml of fresh, filtered freshwater (pH 7.5–8). In order to investigate the effect of four diets, one millilitre from each diet concentrated solution (*L. fusiformis* Tl03, *Chlorella* sp. CHL15, *Chlorella* sp. CHL11 and yeast solution) was added to the Erlenmeyer flasks containing the starved neonates. The feed rate for *L. fusiformis* Tl03, CHL15, CHL11 and yeast was gradually increased over time as the D. magna individuals grew. Once the feed was completely consumed (clear culture medium), 1 ml of concentrated diet solution was added to the Daphnia culture. The conditions for the incubation were the same as those described above. The water of the *Daphnia magna* culture was completely changed after each measurement. All treatment was conducted in four replicates. In this experimental part, the mean total body length was measured and the survival rate of each treatment group of Daphnia was calculated.

2.4. Body length measurement

The mean total body length of *Daphnia magna* larvae was measured every three days by taking 10 individuals from each bottle using an Optika dissecting microscope (Optika, Ponteranica, Italy) linked with a camera. The total body length was measured from the eye to the end of the tail and then photographed. (Figure. 36).

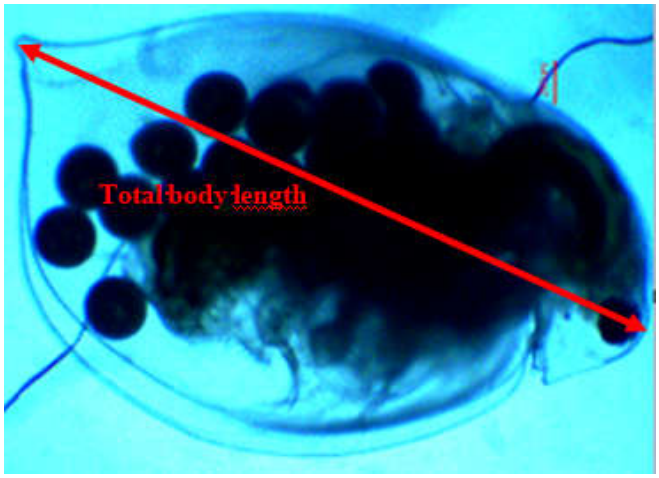

Figure 36. Total body length measurement of *Daphnia magna* X40 (Original).

2.5.Survival rate

The survival rate has been calculated every three days according to the following formula (Amin et *al.*, 2022):

$$SR = Nt/N0 \times 100\%$$

2.6.Statistics Analysis

The data of this study were analyzed statistically using IBM SPSS software (version 25) (Corp 2013). The data of each parameter were subjected to One-way ANOVA and Tukey's HSD (Honest Significant Difference) test using SPSS a 95% confidence interval to verify the statistically significant differences in the growth of *Daphnia magna* fed on different diets *L.fusiformis* Tl03, *Chlorella sp.* CHL11and *Chlorella sp.* CHL15 and Yeast(*Saccharomyces cerevisiae*). Statistical values of $P < 0.05$ were considered significant.

3. Results

3.1.Total body length measurement

The total body length of the *Daphnia magna* was used to assess growth performance. Figure 37 and Table 24 show a gradual increase in the body length of Daphnia magna during the experiment in all of the studied groups.

The initial body length of *D. magna* was 512.6±18.53 µm at the beginning of the experiment. During the first three days of the experiments, *Daphnia magna* total body length showed exponential growth in all treatment groups with the highest total body length was recorded in those fed on yeast (2070.7±71.66 µm) followed by *L. fusiformis* TL03 (1962.03±66.71 µm), *Chlorella* sp. CHL11 (1848.6±85.56 µm) and the lowest in those fed on *Chlorella* sp. CHL15 (1772.6±71.85µm).

Comparing the initial body length with that after three days of the experiment, it was recorded that it doubled with a greater value in the one fed with yeast (4.04 folds), followed by *L. fusiformis* TL03 (3.83), then *Chlorella* sp. CHL11 (3.61), and the latter was *Chlorella* sp. CHL15 (3.46-folds)

The first six days of the *Daphnia* culture indicated highest total body length for those fed on yeast (2330.3±93.59 µm) and *L. fusiformis* TL03 (2324±54.04 µm), while significantly lower total length result ($p<0.005$) were recorded for those fed on *Chlorella* sp. CHL11 (2044.3±96.01 µm) and *Chlorella sp*. CHL15 (2003.2±104.73 µm). During the last three days of the experiment (12-15 days), rapid growth was observed in *Daphnia* fed on *Chlorella* sp. CHL15, which on day 15 slightly exceeded that of *Daphnia* fed on yeast and *L. fusiformis*

TL03, with no significant difference, while slower growth was observed in *Daphnia* fed on *Chlorella sp*. CHL11.

Table 24. Growth of *Daphnia magna* (µm) fed with *L.fusiformis* Tl03, *Chlorella sp*. CHL11, *Chlorella* sp. CHL15 and, yeast.

Days	*L. fusiformis* TL03	*Chlorella sp.* CHL11	*Chlorella sp.* CHL15	Yeast
0	512.6±18.53	512.6±18.53	512.6±18.53	512.6±18.53
3	1962.03±66.71	1848.6±85.56	1772.6±71.85	2070.7±71.66
6	2324±54.04	2044.3±96.01	2003.2± 104.73	2330.3±93.59
9	2480.07±106.36	2382.26±80.77	2406.57±62	2575.53±98
12	2898.8±89.05	2504.87±105.50	2542.63±104	2898.8±137.78
15	2932.43±51.41	2793.53±60.91	2997.63±60.04	2919.97±79.55

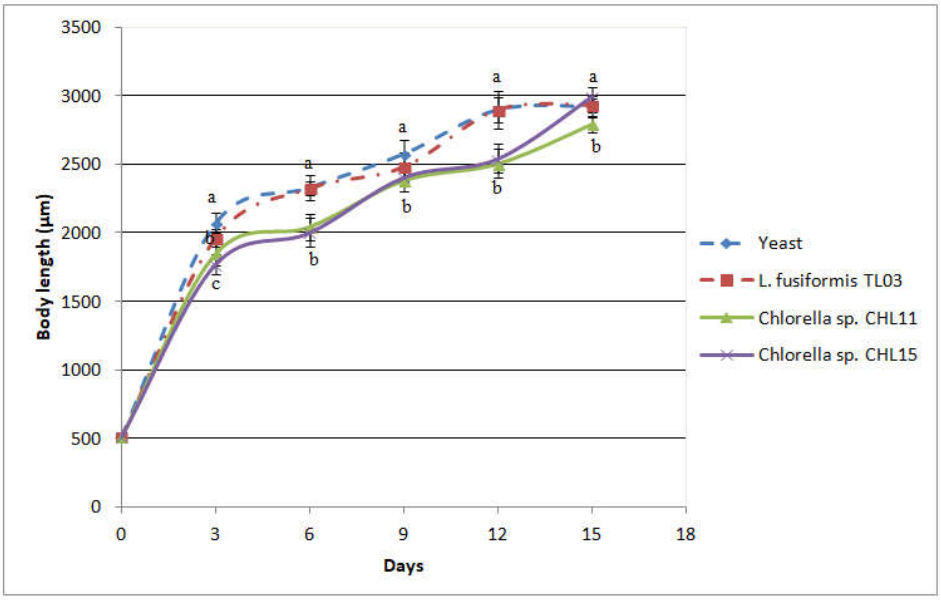

Figure 37. Mean total body length of *Daphnia magna* fed with *L.fusiformis* Tl03, *Chlorella* sp. CHL11, and *Chlorella* sp. CHL15, yeast.

3.2. Survival rate

On the 15th day of the experiment, the survival rate of the Daphnia showed that the survival values for the different applied diets ranged from 25±1.15% to 67±1.15% (see Figure 38 and Table 25). Figure 38 shows that there was a significant difference ($p < 0.05$) between all treatment groups from day 6 to day 15 of the experiment. The highest survival rate at the end of the experiment was recorded in *Daphnia magna* fed with *Chlorella* sp. CHL11, followed

by those fed with *L. fusiformis* TL03 and *Chlorella* sp. CHL15, while the lowest in those fed with baker's yeast.

Table 25 shows that on the 15th day of the experiment, *Daphnia* fed with *Chlorella* sp. CHL11 showed a significantly higher survival rate (67±1.15%) compared to the other treatments, followed by those fed with *L. fusiformis* TL03 (51±0.57%), *Chlorella* sp. CHL15 (42.33±1.20%), and the lowest was observed in those fed with yeast (25±1.15%).

Table 25. Survival of *Daphnia magna* (%) fed with yeast, *L.fusiformis* Tl03, *Chlorella* sp. CHL11, and *Chlorella* sp. CHL15

Days	Yeast	*L. fusiformis TL03*	*Chlorella sp. CHL11*	*Chlorella sp. CHL15*
0	100±00	100±00	100±00	100±00
3	90±1.15	94±0.58	99±0.58	80±2.87
6	59±0.58	89.33±1.33	96±0.58	68.33±1.2
9	54±0.58	75±2.52	95±0.58	63.67±1.2
12	41±0.58	69±0.57	85±1.15	58.67±0.88
15	25±1.15	51±0.57	67±1.15	42.33±1.20

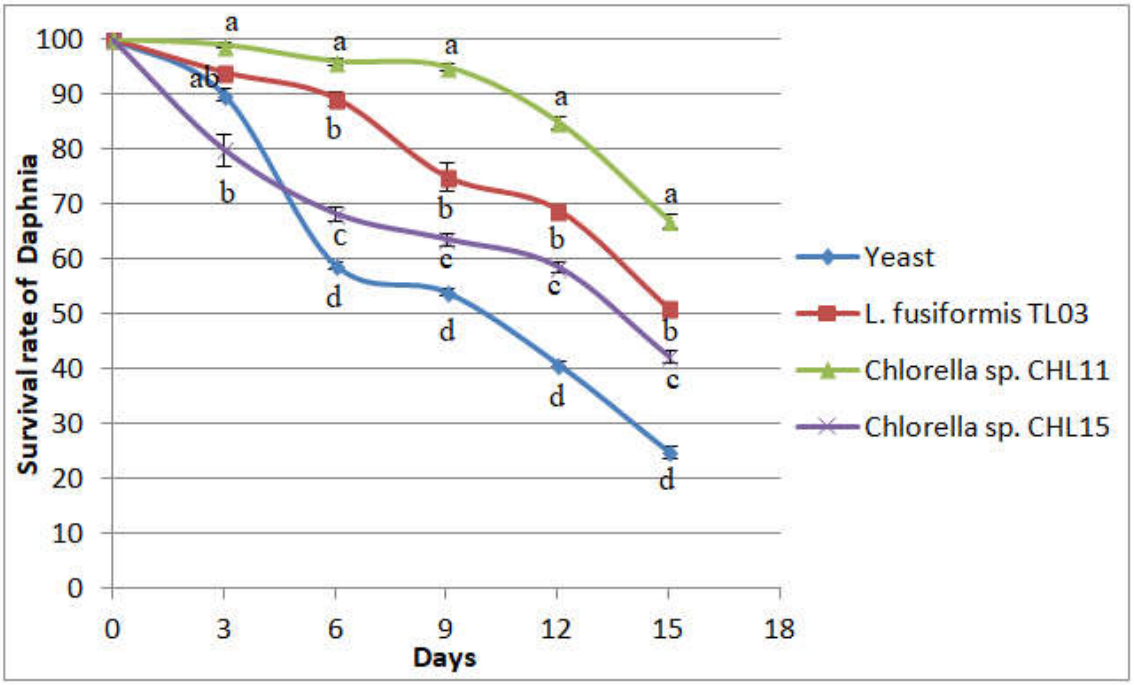

Figure.38: Survival rate (%) of *Daphnia magna* fed on *Yeast*, *L.fusiformis* Tl03, *Chlorella sp.* CHL11, and *Chlorella sp*. CHL15.

3.3. Reproduction

Four days after the start of the experiment, microscopic examination of various *Daphnia* culture media revealed that those fed on *Chlorella* sp. CHL11 possessed a clutch of eggs in the brood chamber located dorsal to the females (Fig. 39A**).** The number of eggs in each female ranged from 4-14 and the eggs diameter ranged from 250 to 350 µm **(**Fig. 39 B**)**

During the experiment, the neonates of Daphnia were observed in different treatments. The first neonates (Fig. 39C) were seen after five days of the experiment in the group fed on *Chlorella* sp. CHL11, followed by those fed on *Chlorella* sp. CHL15 and *L. fusiformis* after seven days and in those fed with yeast after 10 days of the experiment.

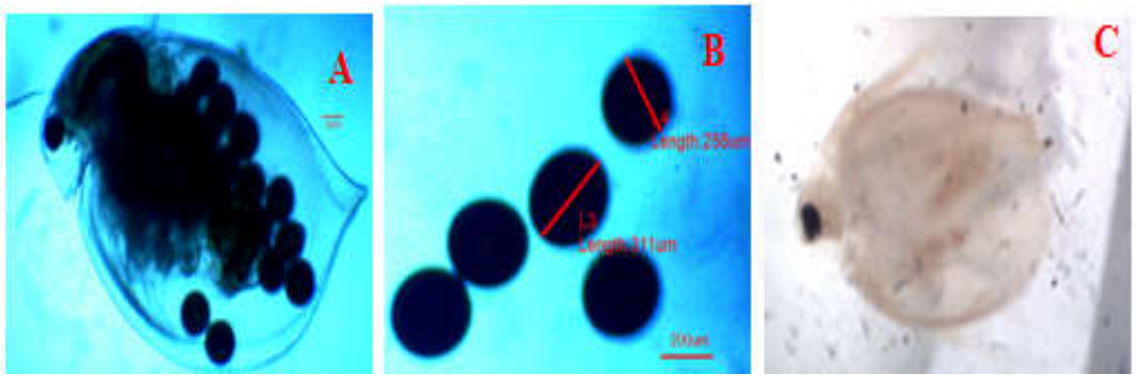

Figure 39. Different steps of *Daphnia magna* reproduction (A) Adult female of *Daphnia magna* with a clutch of eggs in its brood chamber , (B) Eggs, C(neonate of *Daphnia* magna)(40X).

4. Discussion

The success of *Daphnia* production has been linked to environmental factors such as temperature, food quality and availability (Giebelhausen & Lampert, 2001).

The growth and survival of *Daphnia magna* are largely related on their diet. The ideal foods for growing Daphnia are algae, yeast and bacteria, or a combination (Driesen, K. 2015)

The results of this study showed that growth trajectories varied over time depending on the type of feed used. *Daphnia magna* fed *Chlorella* sp.CHL15 exhibited significant growth in the second week of the experiment, whereas those fed yeast demonstrated relatively smaller growth compared to those fed *L. fusiformis* TL03 and *Chlorella* sp. CHL11.

In same context, Castro Mejía et *al.* (2016) investigated the laboratory production of D. *magna* by examining the effects of different diets, including *Chlorella sp*., *Haematococcus sp*. *Sphaerocystis sp.* and a combination of previous microalgae. They revealed that the highest growth rate, and population density was found in *Chlorella* sp.

Mezgebua et *al.* (2025) reported that, although *Spirulina* (*Limnospira*) is rich in protein, it lacks essential sterols and long-chain polyunsaturated fatty acids (PUFAs), both of which are essential for the growth and reproduction of *D.magna*. It was also confirmed that supplementing *Spirulina* with cholesterol and eicosapentaenoic acid (EPA) improved survival and reproduction of *Daphnia magna*. Furthermore, Gulati and Demuth (1997) reported that, while baker's yeast is a poor food source for zooplankton on its own, it can increase their survival rate when mixed with other food sources, such as microalgae. The diet of *Daphnia* plays a key role in its maturation, egg production and egg quality (Turcihan et *al.*, 2022; Karakaş et *al.*, 2023).

5. Conclusion

After two weeks of the experiment, it was concluded that all the diets tested had a positive effect on growth, with greater growth recorded in those fed *Chlorella sp.* CHL15 and lower growth in those fed baker's yeast. These results are preliminary and should be followed by further studies to search the ideal microalgae essential for improving *Daphnia* production.

In conclusion, the high demand and prices of imported *Artemia* bags, as well as the time and potential investment required for pending studies on the geographic distribution, biomass and quality of local *Artemia*, make the development of *Daphnia* production a key element in the future of sustainable aquaculture. This is particularly true in terms of providing food for larvae and juvenile fish, either directly or as micro-feed additives. This is particularly pertinent given the affordable production costs, rapid growth, and high reproductive rate. This study paves the way for further research into selecting microalgae and other feeding patterns to enhance the quality, growth and reproduction of *D. magna*.

General Conclusion

General Conclusion

Microalgae are of great interest due to their many applications in various fields, which contribute to economic growth and environmental sustainability. They are used in wastewater treatment, energy production, pharmaceuticals, cosmetics, biotechnology, human and animal nutrition, and aquaculture. In aquatic ecosystems, microalgae are considered the first link in the food chain.

In this study three strains of microalgae were isolated cyanobacteria, *L. fusiformis* TL03, *Chlorella sp*. CHL15 from Telamine Lake and Chlorella *sp*. CHL11 from Gharabas Lake locatd in northwest Algeria. Three strains of microalgae were successfully isolated, purified, identified, and preserved for a short to medium period by transferring them from a culture medium to another.

The study of the effect of the salinity on three strains (*L. fusiformis* TL03, *Chlorella sp*. CHL11, and *Chlorella sp*. CHL15) revealed that all these strains grew rapidly in BG11 culture media; however, their growth decreases with increasing salinity, with a tolerance up to 60 g/L of sodium chloride for *L. fusiformis* TL03 and *Chlorella sp*. CHL15.

The three microalgae contain a remarkable amount of phenolic compounds, with the highest value of total phenolic content registered in *Limnospira fusiformis* TL03, whereas the highest amount of total flavonoid content is found in *Chlorella sp*. CHL11.

HPLC analysis of the crude hydromethanolic extracts of the studied microalgae revealed 55 peaks for *L. fusiformis* TL03, 69 peaks for *Chlorella sp.* CHL11 and 47 peaks for *Chlorella sp*. CHL15. However, only five compounds were identified in *L. fusiformis* TL03 and seven *Chlorella sp*. CHL11, and four in *Chlorella sp.* CHL15.

The resultat of the antioxidant activity by using two methods DPPH radical scavenging activity and the beta-carotene bleaching test revealed that the highest activity was registred in *L. fusiformis* TL03.

The antimicrobial properties of *L. fusiformis* and the two *Chlorella* species (CHL11 and CHL15) indicate their potential as natural antibacterial agents against several pathogenic bacteria(*S. aureus* , *B. subtilis*, *L. monocytogenes*, *P. aeruginosa*, and *K. pneumoniae*). Whereas the *E.coli* revealed its relative resistance against the thee microalgae extract.

In this experiment, the crushed *L. fusiformis* Tl03, *Chlorella* sp. CHL11 and yeast were found to be the most effective live feeds for the *Artemia*, resulting in high growth and survival rates. .In the same context, after two weeks of feeding *Daphnia magna* different diets, it was

concluded that all the diets tested had a positive effect on growth with the best growth was recorded in those fed *Chlorella sp*. CHL15.

The results of this study showed that these microalgae are a valuable source of active natural compounds that can be used in the pharmaceutical and food industries, in addition to the possibility of using them as food for zooplankton such as Artemia, Daphnia, and fish larvae.

This work is recommended to be complemented by further studies

Perspective:

Mastering the isolation, purification, identification and preservation of microalgae opens up the prospect of establishing a cultural collection (microalgae bank) of locally sourced species with economic value.

The studied microalgae have proven their ability to tolerate varying concentrations of salinity, making them candidates for production using waters that are underutilized in Algeria due to salinity and can be used in various biotechnological applications .

These microalgae contain a wide range of phenolic compounds, only a few of which have been identified. Therefore, further research is needed to identify, isolate, and characterize the molecules responsible for these activities, which could be used as alternatives to antibiotics and other traditional chemical drugs for treating various diseases. Further studies are also useful to verify their benefits in various pharmaceutical and food industry applications.

The results of the study on the diets of *Artemia* and *Daphnia* magna ranged from good to acceptable. Therefore, it is recommended that the study be expanded to include the isolation and testing of other local microalgae, in order to select the most suitable diets for *Artemia* and *Daphnia* growth, survival and reproduction.

References

1. A El-Chaghaby, G., Rashad, S., F Abdel-Kader, S., A Rawash, E. S., & Abdul Moneem, M. (2019). Assessment of phytochemical components, proximate composition and antioxidant properties of *Scenedesmus obliquus*, *Chlorella vulgaris* and *Spirulina platensis* algae extracts. *Egyptian Journal of Aquatic Biology and Fisheries*, *23*(4), 521-526

2. Abd El-Baky, H. H., El-Baz, F. K., & El Baroty, G. S. (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.

3. Abdel-Karim, O. H., Gheda, S. F., Ismail, G. A., & Abo-Shady, A. M. (2019). Phytochemical Screening and antioxidant activity of Chlorella vulgaris. *Delta Journal of Science*, *41*(1), 79-91.

4. Abdel-Karim, O.H., Gheda, S.F., Ismail, G.A. & AboShady, A.M. 2020. Phytochemical Screening and antioxidant activity of *Chlorella vulgaris*. Delta J. Sci., 41(1): 79-91.

5. Abdel-Moneim, A. M. E., El-Saadony, M. T., Shehata, A. M., Saad, A. M., Aldhumri, S. A., Ouda, S. M., & Mesalam, N. M. (2022). Antioxidant and antimicrobial activities of Spirulina platensis extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi. *Saudi Journal of Biological Sciences*, *29*(2), 1197-1209.

6. Abidizadegan, M., Peltomaa, E., Ilina, P., Tammela, P., & Blomster, J. (2024). Cryptophytes as potential source of natural antimicrobials for food preservation. *Frontiers in Microbiology*, *15*, 1462696.

7. Abreu, A. P., Martins, R., & Nunes, J. (2023). Emerging applications of *Chlorella sp*. and *Spirulina (Arthrospira) sp*. *Bioengineering*, *10*(8), 955.

8. Abuhasheesh, Y., Ghazal, A., Tang, D. Y. Y., Banat, F., Hasan, S. W., & Show, P. L. (2025). Advances in *Chlorella* Microalgae for Sustainable Wastewater Treatment and Bioproduction. *Chemical Engineering Journal Advances*, 100715.

9. Aburai, N., Ohkubo, S., Miyashita, H., & Abe, K. (2013). Composition of carotenoids and identification of aerial microalgae isolated from the surface of rocks in mountainous districts of Japan. *Algal Research*, *2*(3), 237-243.

10. Acién, F. G., Molina, E., Reis, A., Torzillo, G., Zittelli, G. C., Sepúlveda, C., & Masojídek, J. (2017). Photobioreactors for the production of microalgae. In *Microalgae-based biofuels and bioproducts* (pp. 1-44). Woodhead Publishing.

11. Acuner, E., & Dilek, F. B. (2004). Treatment of tectilon yellow 2G by *Chlorella vulgaris*. *Process Biochemistry*, *39*(5), 623-631.

12. Afzal, S., Yadav, A. K., Poonia, A. K., Choure, K., Yadav, A. N., & Pandey, A. (2023). Antimicrobial therapeutics isolated from algal source: Retrospect and prospect. *Biologia*, *78*(2), 291-305.

13. Ahirwar, A., Kesharwani, K., Deka, R., Muthukumar, S., Khan, M. J., Rai, A., & Morjaria, S. (2022). Microalgal drugs: A promising therapeutic reserve for the future. *Journal of Biotechnology*, *349*, 32-46.

14. Aigner, S., Glaser, K., Arc, E., Holzinger, A., Schletter, M., Karsten, U., & Kranner, I. (2020). Adaptation to aquatic and terrestrial environments in *Chlorella vulgaris* (Chlorophyta). *Frontiers in microbiology*, *11*, 585836.

15. Akbarizareh, M., Ofoghi, H., & Hadizadeh, M. (2019). Assessment of Phenolic Components of the microalgae *Spirulina platensis* using two methods of Chromatography, TLC and HPLC.

16. Al Hattab, M., Ghaly, A., & Hammouda, A. (2015). Microalgae harvesting methods for industrial production of biodiesel: critical review and comparative analysis. *J Fundam Renew Energy Appl*, *5*(2), 1000154.

17. Alagawany, M., Madkour, M., El-Saadony, M. T., & Reda, F. M. (2021). *Paenibacillus polymyxa* (LM31) as a new feed additive: Antioxidant and antimicrobial activity and its effects on growth, blood biochemistry, and intestinal bacterial populations of growing Japanese quail. *Animal feed science and technology*, *276*, 114920.

18. Albuquerque, R. C. V., Silva, C. E. D. F., Carneiro, W. D. S., Andreola, K., da Gama, B. M. V., & Silva, A. E. D. (2024). Incorporation of Cyanobacteria and Microalgae in Yogurt: Formulation Challenges and Nutritional, Rheological, Sensory, and Functional Implications. *Applied Microbiology*, *4*(4), 1493-1514.

19. AlFadhly, N. K., Alhelfi, N., Altemimi, A. B., Verma, D. K., & Cacciola, F. (2022). Tendencies affecting the growth and cultivation of genus Spirulina: An investigative review on current trends. *Plants*, *11*(22), 3063.

20. Alghamdi, M. A., Reda, F. M., Mahmoud, H. K., Bahshwan, S. M., Salem, H. M., Alhazmi, W. A., ... & Abdelgeliel, A. S. (2024). The potential of Spirulina platensis to substitute antibiotics in Japanese quail diets: impacts on growth, carcass traits, antioxidant status, blood biochemical parameters, and cecal microorganisms. *Poultry science*, *103*(3), 103350.

21. Al-ghanayem, A. A. (2017). Antimicrobial activity of *Spirulina platensis* extracts against certain pathogenic bacteria and fungi. *Advances in Bioresearch*, *8*(6), 96-101.

22. Allen MM, & Stanier RY .(1968). Growth and division of some unicellular blue-green algae. Microbiology 51(2): 199-202.

23. Al-Saikhan, M. S., Howard, L. R., & Miller Jr, J. C. (1995). Antioxidant activity and total phenolics in different genotypes of potato (*Solanum tuberosum*). *Journal of food science*, *60*(2), 341-343.

24. Alsenani, F., Tupally, K. R., Chua, E. T., Eltanahy, E., Alsufyani, H., Parekh, H. S., & Schenk, P. M. (2020). Evaluation of microalgae and cyanobacteria as potential sources of antimicrobial compounds. *Saudi Pharmaceutical Journal*, *28*(12), 1834-1841

25. Alshuniaber, M. A., Krishnamoorthy, R., & AlQhtani, W. H. (2021). Antimicrobial activity of polyphenolic compounds from Spirulina against food-borne bacterial pathogens. *Saudi Journal of Biological Sciences*, *28*(1), 459-464.

26. Amaral, M. S., Loures, C. C., Naves, F. L., Samanamud, G. L., Silva, M. B., & Prata, A. M. (2020). Microalgae Cultivation in Photobioreactors Aiming at Biodiesel Production. In *Biomass*. IntechOpen.

27. Amaro, H.M., Guedes, A.C., Malcata, F.X., 2011. Advances and perspectives in using microalgae to produce biodiesel. Appl. Energy 88 (10), 3402e3410.

28. Amengual, J., Gouranton, E., van Helden, Y. G., Hessel, S., Ribot, J., Kramer, E., ... & von Lintig, J. (2011). Beta-carotene reduces body adiposity of mice via BCMO1. *PloS one*, *6*(6), e20644.

29. Amin, M., Erwinda, M., Nissa, M., Nindarwi, D. D., Satyantini, W. H., Mubarak, A. S., ... & Alamsjah, M. A. (2022). Fatty acids profiles and growth performances of Artemia franciscana fed with different types of microalgae. *Sains Malaysiana*, *51*(8).

30. Anand, U., Dey, S., Parial, D., Federici, S., Ducoli, S., Bolan, N. S., ... & Bontempi, E. (2023). Algae and bacteria consortia for wastewater decontamination and transformation into biodiesel, bioethanol, biohydrogen, biofertilizers and animal feed: a review. *Environmental Chemistry Letters*, *21*(3), 1585-1609.

31. Ang, W. S., Kee, P. E., Lan, J. C. W., Chen, W. H., Chang, J. S., & Khoo, K. S. (2024). Unveiling the rise of microalgae-based foods in the global market: perspective views and way forward. *Food Bioscience*, 105390.

32. Arguelles, E. D. L. R., NH, T. G., & Monsalud, R. G. (2020). Maintenance and preservation of microalgal cultures. *Methods in microalgal studies*, *1*, 53-60.

33. Ariede, M.B., Candido, T.M., Jacome, A.L.M., Velasco, M.V.R., de Carvalho, J.C.M., Baby, A.R. (2017). Cosmetic attributes of algae - a review. Algal. Res. 25, 483–487.

34. Arsad, S., Mulasari, Y. W., Sari, N. Y., Lusiana, E. D., Risjani, Y., Musa, M., ... & Sari, L. A. (2022). Microalgae diversity in several different sub-habitats. *Global Journal of Environmental Science and Management*, *8*(4), 561-574.

35. Arumugam, P., Inbakandan, D., Ramasamy, M. S., & Murugan, M. (2013). Encapsulated *Spirulina* powder feed for the nutritional enrichment of adult brine shrimp (*Artemia salina*). *Journal of Applied Aquaculture*, *25*(3), 265-270.

36. Asgharpour, M., Rodgers, B., & Hestekin, J. A. (2015). Eicosapentaenoic acid from Porphyridium cruentum: Increasing growth and productivity of microalgae for pharmaceutical products. *Energies*, *8*(9), 10487-10503.

37. Ashour, E. A., El-Hack, M. E. A., Shafi, M. E., Alghamdi, W. Y., Taha, A. E., Swelum, A. A., ... & El-Saadony, M. T. (2020). Impacts of green coffee powder supplementation on growth performance, carcass characteristics, blood indices, meat quality and gut microbial load in broilers. *Agriculture*, *10*(10), 457

38. Asulabh KS, Supriya G, Ramachandra TV. (2012) . Effect of salinity concentrations on growth rate and lipid concentration in *Microcystis* sp., *Chlorococcum* sp., and *Chaetoceros* sp. In National Conference on Conservation and Management of Wetland Ecosystems. School of Environmental Sciences, Mahatma Gandhi University, Kottayam, Kerala: Pp 1-7.

39. Ayachi S, El Abed A, Dhifi W, Marzouk B. (2007). Chlorophylls, proteins, and fatty acids amounts of *Arthrospira platensis* growing under saline conditions. Pakistan Journal of Biological Sciences: PJBS 10(14): 2286-2291.

40. Barkia, I., Saari, N., & Manning, S. R. (2019). Microalgae for high-value products towards human health and nutrition. *Marine drugs*, *17*(5), 304.

41. Barone, V., Baglieri, A., Stevanato, P., Broccanello, C., Bertoldo, G., Bertaggia, M., Cagnin, M., Pizzeghello, D., Moliterni, V. M. C., Mandolino, G., Fornasier , F., Squartini, A., Nardi ,S., & Concheri,

G. (2018). Root morphological and molecular responses induced by microalgae extracts in sugar beet (*Beta vulgaris* L.). *Journal of Applied Phycology*, 30, 1061–1071

42. Barros, A. I., Gonçalves, A. L., Simões, M., & Pires, J. C. (2015). Harvesting techniques applied to microalgae: a review. *Renewable and sustainable energy reviews*, *41*, 1489-1500.

43. Bartolomeu, M., Vieira, C., Dias, M., Conde, T., Couto, D., Lopes, D., ... & Domingues, M. R. (2022). Bioprospecting antibiotic properties in photodynamic therapy of lipids from Codium tomemtosum and Chlorella vulgaris. *Biochimie*, *203*, 32-39.

44. Baweja, P., & Sahoo, D. (2015). Classification of algae. *The algae world*, 31-55.

45. Beijerinck, M. W. (1890). Culturversuche mit Zoochlorellen, Lichenengonidien und anderen niederen. *Algen. Bot. Ztg.*, *48*, 725-772.

46. Bellahcen, T. O., AAmiri, A., Touam, I., Hmimid, F., Amrani, A. E., Cherif, A., & Cherki, M. (2020). Evaluation of Moroccan microalgae: *Spirulina platensis* as a potential source of natural antioxidants. *Journal of Complementary and Integrative Medicine*, *17*(3), 20190036.

47. Benavente-Valdés JR, Aguilar C, Contreras-Esquivel JC, Méndez-Zavala A, Montañez J. (2016). Strategies to enhance the production of photosynthetic pigments and lipids in Chorophyceae species. Biotechnology Reports 10: 117-125.

48. Benemann, J. R., Woertz, I., & Lundquist, T. (2018). Autotrophic microalgae biomass production: from niche markets to commodities. *Industrial Biotechnology*, *14*(1), 3-10.

49. Bernaerts, T. M., Gheysen, L., Kyomugasho, C., Kermani, Z. J., Vandionant, S., Foubert, I., & Van Loey, A. M. (2018). Comparison of microalgal biomasses as functional food ingredients: Focus on the composition of cell wall related polysaccharides. *Algal Research*, *32*, 150-161.

50. Bhagavathy, S., Sumathi, P., & Bell, I. J. S. (2011). Green algae *Chlorococcum humicola*-a new source of bioactive compounds with antimicrobial activity. *Asian Pacific Journal of Tropical Biomedicine*, *1*(1), S1-S7.

51. Bhatt, T., & Patel, K. (2020). Carotenoids: potent to prevent diseases review. *Natural Products and Bioprospecting*, *10*, 109-117.

52. Bhuyar, P., Rahim, M. H. A., Maniam, G. P., Ramaraj, R., & Govindan, N. (2020). Exploration of bioactive compounds and antibacterial activity of marine blue-green microalgae (Oscillatoria sp.) isolated from coastal region of west Malaysia. *SN Applied Sciences*, *2*, 1-10.

53. Bock, C., Krienitz, L., and Pröschold, T. (2011). Taxonomic reassessment of the genus Chlorella (Trebouxiophyceae) using molecular signatures (barcodes), including description of seven new species. Fottea 11, 293–312.

54. Bora, A., Rajan, A. S. T., Kumar, P., Muthusamy, G., & Alagarsamy, A. (2024). Microalgae to bioenergy production: Recent advances, influencing parameters, utilization of wastewater–A critical review. *Science of The Total Environment*, 174230.

55. Borics, G., Várbíró, G., Falucskai, J., Végvári, Z., T-Krasznai, E., Görgényi, J., ... & Lerf, V. (2023). A two-dimensional morphospace for cyanobacteria and microalgae: Morphological diversity, evolutionary relatedness, and size constraints. *Freshwater Biology*, *68*(1), 115-126.

56. Borowitzka, M. A. (1995). Microalgae as sources of pharmaceuticals and other biologically active compounds. *Journal of applied phycology*, *7*, 3-15.

57. Borowitzka, M. A. (2013). High-value products from microalgae—their development and commercialisation. *Journal of applied phycology*, *25*, 743-756.

58. Borowitzka, M. A. (2018). Biology of microalgae. In *Microalgae in health and disease prevention* (pp. 23-72). Academic Press.

59. Borowitzka, M. A., & Moheimani, N. R. (2012). Open pond culture systems. In *Algae for biofuels and energy* (pp. 133-152). Dordrecht: Springer Netherlands.

60. Botterweck, A. A., Verhagen, H., Goldbohm, R. A., Kleinjans, J., & Van den Brandt, P. A. (2000). Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: results from analyses in the Netherlands cohort study. *Food and Chemical Toxicology*, *38*(7), 599-605.

61. Boukhari, N., Doumandji, A., Sabrine Ait chaouche, F., & Ferradji, A. (2018). Effect of ultrasound treatment on protein content and functional properties of Spirulina powder grown in Algeria. *Mediterranean Journal of Nutrition and Metabolism*, *11*(3), 235-249.

62. Bouslamti, M., El Barnossi, A., Kara, M., Alotaibi, B. S., Al Kamaly, O., Assouguem, A., ... & Benjelloun, A. S. (2022). Total polyphenols content, antioxidant and antimicrobial activities of leaves of *Solanum elaeagnifolium* Cav. from Morocco. *Molecules*, *27*(13), 4322.

63. Brasil, B. D. S. A. F., de Siqueira, F. G., Salum, T. F. C., Zanette, C. M., & Spier, M. R. (2017). Microalgae and cyanobacteria as enzyme biofactories. *Algal research*, *25*, 76-89.

64. Brennan, L., & Owende, P. (2010). Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable and sustainable energy reviews*, *14*(2), 557-577.

65. Brown, L. M. (1982). Photosynthetic and Growth Responses to Salinity in a Marine Isolate of *Nannochloris Bacillaris* (Chlorophyceae) 1. *Journal of Phycology*, *18*(4), 483-488.

66. Čabarkapa, I., Rakita, S., Popović, S., Tomičić, Z., Spasevski, N., Vulić, J., & Đuragić, O. (2022). Characterization of organic *Spirulina spp*. and *Chlorella vulgaris* as one of the most nutrient-dense food.

67. Cabrera-Capetillo, C. A., Castillo-Baltazar, O. S., Petriz-Prieto, M. A., Guzmán-López, A., Valdovinos-García, E. M., & Bravo-Sánchez, M. G. (2023). Simulation and economic analysis of the biotechnological potential of biomass production from a microalgal consortium. *Marine Drugs*, *21*(6), 321.

68. Cannell, R. J., Owsianka, A. M., & Walker, J. M. (1988). Results of a large-scale screening programme to detect antibacterial activity from freshwater algae. *British Phycological Journal*, *23*(1), 41-44.

69. Castenholz RW (2001) Form-genus I *Arthrospira Stizenberger* 1852. In: Boone DR, Castenholz RW, Garrity GM (eds) Bergey's manual of systematic bacteriology, vol 1, 2nd edn. Springer, New York, pp 542–543

70. Castro-Mejía, J., Ocampo-Cervantes, J. A., Castro-Mejía,G., Cruz-Cruz, I., Monroy-Dosta ,M.C & Becerril-Cortes ,D. (2016). Laboratory production of *Daphnia magna* (Straus 1820) fed with microalgae and active dry yeast. *Journal of Entomology and Zoology Studies*, *4*, 548-553.

71. Cellamare, M., Duval, C., Drelin, Y., Djediat, C., Touibi, N., Agogué, H., Leboulanger, C., Ader, M., & Bernard, C. (2018). Characterization of phototrophic microorganisms and description of new cyanobacteria isolated from the saline-alkaline crater-lake Dziani Dzaha (Mayotte, Indian Ocean). *FEMS microbiology ecology*, 94(8): 1-25.

72. Cha, T. S., Chen, C. F., Yee, W., Aziz, A., & Loh, S. H. (2011). Cinnamic acid, coumarin and vanillin: Alternative phenolic compounds for efficient *Agrobacterium*-mediated transformation of the unicellular green alga, Nannochloropsis sp. *Journal of microbiological methods*, *84*(3), 430-434.

73. Chabet dis, C., Refes, W., & Okazaki, R. K. (2023). First biocharacterization of Artemia populations from western and northwestern Algeria. *Zoomorphology*, *143*(1), 77-88.

74. Chakdar, H., Jadhav, S.D., Dhar, D.W., Pabbi, S. (2012). Potential applications of blue green 456 algae. J. Sci. Ind. Res. 71, 13-20.

75. Champenois, J., Marfaing, H., & Pierre, R. (2015). Review of the taxonomic revision of Chlorella and consequences for its food uses in Europe. *Journal of Applied Phycology*, *27*, 1845-1851.

76. Chapman DJ, Chapman VJ (1973) The algae, 2nd edn. Macmillan, London, p 497.

77. Chen, S., & Gao, K. (2021). Microalgae continuous and semi-continuous cultures. *Research Methods of Environmental Physiology in Aquatic Sciences*, 39-46.

78. Cheng, P., Shan, S., Zhu, Z., Liu, K., Namsaraev, Z., Dubovskiy, I., & Xu, Q. (2023). The role of microalgae culture modes in aquaculture: a brief opinion. *Frontiers in Bioengineering and Biotechnology*, *11*, 1196948.

79. Cheng, P., Zhou, C., Chu, R., Chang, T., Xu, J., Ruan, R.,& Yan, X. (2020). Effect of microalgae diet and culture system on the rearing of bivalve mollusks: Nutritional properties and potential cost improvements. *Algal Research*, *51*, 102076.

80. Chiu, S. T., Shiu, Y. L., Wu, T. M., Lin, Y. S., & Liu, C. H. (2015). Improvement in non-specific immunity and disease resistance of barramundi, Lates calcarifer (Bloch), by diets containing Daphnia similis meal. *Fish & Shellfish Immunology*, *44*(1), 172-179.

81. Chojnacka, K., Chojnacki, A., & Gorecka, H. (2005). Biosorption of Cr3+, Cd2+ and Cu2+ ions by blue–green algae *Spirulina sp*.: kinetics, equilibrium and the mechanism of the process. *Chemosphere*, *59*(1), 75-84.

82. Choochote, W., Suklampoo, L., & Ochaikul, D. (2014). Evaluation of antioxidant capacities of green microalgae. *Journal of applied phycology*, *26*, 43-48.

83. Choudhary, S., Tripathi, S., & Poluri, K. M. (2022). Microalgal-based bioenergy: Strategies, prospects, and sustainability. *Energy & Fuels*, *36*(24), 14584-14612.

84. Chu, W. L. (2012). Biotechnological applications of microalgae. *IeJSME*, *6*(1), S24-S37.

85. Ciccone, M. M., Cortese, F., Gesualdo, M., Carbonara, S., Zito, A., Ricci, G. & Riccioni, G. (2013). Dietary intake of carotenoids and their antioxidant and anti-inflammatory effects in cardiovascular care. *Mediators of inflammation*, *2013*(1), 782137.

86. Cichoński, J., & Chrzanowski, G. (2022). Microalgae as a source of valuable phenolic compounds and carotenoids. *Molecules*, *27*(24), 8852.

87. Ciferri, O. (1983). Spirulina, the edible microorganism. *Microbiological reviews*, *47*(4), 551-578.

88. Coêlho, D. D. F., Tundisi, L. L., Cerqueira, K. S., Rodrigues, J. R. D. S., Mazzola, P. G., Tambourgi, E. B., & Souza, R. R. D. (2019). Microalgae: cultivation aspects and bioactive compounds. *Brazilian Archives of Biology and Technology*, *62*, e19180343

89. Conceição, L. E., Yúfera, M., Makridis, P., Morais, S., & Dinis, M. T. (2010). Live feeds for early stages of fish rearing. *Aquaculture research*, *41*(5), 613-640.

90. Cook, J. M., Tedstone, A. J., Williamson, C., McCutcheon, J., Hodson, A. J., Dayal, A., ... & Tranter, M. (2020). Glacier algae accelerate melt rates on the south-western Greenland Ice Sheet. *The Cryosphere*, *14*(1), 309-330.

91. Cordero, B. F., Obraztsova, I., Couso, I., Leon, R., Vargas, M. A., & Rodriguez, H. (2011). Enhancement of lutein production in *Chlorella sorokiniana* (Chorophyta) by improvement of culture conditions and random mutagenesis. *Marine drugs*, *9*(9), 1607-1624.

92. Costa NB, Kolman MA, Giani A. (2016). Cyanobacteria diversity in alkaline saline lakes in the Brazilian Pantanal wetland: a polyphasic approach. Journal of Plankton Research 38(6): 1389-1403.

93. Costa, J. A. V., & de Morais, M. G. (2014). An open pond system for microalgal cultivation. In *Biofuels from algae* (pp. 1-22). Elsevier.

94. D'Angelo Costa, G. M., & Maia Campos, P. M. B. G. (2024). Development of Cosmetic Formulations Containing Olive Extract and Spirulina sp.: Stability and Clinical Efficacy Studies. Cosmetics, 11(3), 68

95. Da Silva, M. R. O. B., Moura, Y. A. S., Converti, A., Porto, A. L. F., Marques, D. D. A. V., Bezerra, R. P. (2021). Assessment of the potential of Dunaliella microalgae for different biotechnological applications: A systematic review. *Algal Research*, *58*, 102396.

96. Dadheech, P.K., Ballot, A., Casper, P., Kotut, K., Novelo, E., Lemma, B., Proschold, T., & Krienitz, L. (2010). Phylogenetic relationship and divergence among planktonic strains of *Arthrospira* (Oscillatoriales, Cyanobacteria) of African, Asian and American origin deduced by 16S–23S ITS and phycocyanin operon sequences. Phycologia 49(4): 361-372

97. Daglia, M. (2012). Polyphenols as antimicrobial agents. *Current opinion in biotechnology*, *23*(2), 174-181.

98. Dan, S., Yamashita, K., Matsunari, H., Nagakura, K., & Hamasaki, K. (2022). Capability of Artemia to digest *Chlorella vulgaris* and *Nannochloropsis oculata* under stagnant culture conditions. *Aquaculture Research*, *53*(12), 4316-4326.

99. Darienko, T., Gustavs, L., Eggert, A., Wolf, W., and Pröschold, T. (2015). Evaluating the species boundaries of green microalgae (Coccomyxa, Trebouxiophyceae, Chlorophyta) using integrative taxonomy and DNA barcoding with further implications for the species identification in environmental samples. PLoS One 10:e0127838.

100. De Farias, N. F., Demarco, M., & Tribuzi, G. (2019). Drying and quality of microalgal powders for human alimentation In: Microalgae-from physiology to application. *Ed. Milada Vítová London, UK: IntechOpen*.

101. 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*(1), 835761.

102. De Mule, M. C. Z., De Caire, G. Z., & De Cano, M. S. (1996). Bioactive substances from Spirulina platensis (Cyanobacteria). *Phyton*, *58*, 93-96.

103. Dębowski, M., Zieliński, M., Kazimierowicz, J., Kujawska, N., & Talbierz, S. (2020). Microalgae cultivation technologies as an opportunity for bioenergetic system development—advantages and limitations. *Sustainability*, *12*(23), 9980.

104. Dębowski, M., Zieliński, M., Kisielewska, M., Kazimierowicz, J., Dudek, M., Świca, I., & Rudnicka, A. (2020). The cultivation of lipid-rich microalgae biomass as anaerobic digestate valorization technology—A pilot-scale study. *Processes*, *8*(5), 517.

105. Del Campo, J. A., Rodríguez, H., Moreno, J., Vargas, M. Á., Rivas, J., & Guerrero, M. G. (2001). Lutein production by Muriellopsis sp. in an outdoor tubular photobioreactor. *Journal of biotechnology*, *85*(3), 289-295.

106. Demirbas, A., & Demirbas, M. F. (2011). Importance of algae oil as a source of biodiesel. *Energy conversion and management*, *52*(1), 163-170.

107. Demirel, Z., Yilmaz-Koz, F. F., Karabay-Yavasoglu, U. N., Ozdemir, G., & Sukatar, A. (2009). Antimicrobial and antioxidant activity of brown algae from the Aegean Sea. *Journal of the Serbian Chemical Society*, *74*(6), 619-628.

108. Devi, K. U., Swapna, G., & Suman, K. (2013). Biosequestration of carbon dioxide–potential and challenges.

109. Deviram, G., Mathimani, T., Anto, S., Ahamed, T. S., Ananth, D. A., & Pugazhendhi, A. (2020). Applications of microalgal and cyanobacterial biomass on a way to safe, cleaner and a sustainable environment. *Journal of Cleaner Production*, *253*, 119770.

110. Dhiab RB, Ouada HB, Boussetta H, Franck F, Elabed A, Brouers M. (2007). Growth, fluorescence, photosynthetic O_2 production and pigment content of salt adapted cultures of *Arthrospira* (*Spirulina*) *platensis*. Journal of Applied Phycology 19: 293-301. 10.1007/s10811-006-9113-z

111. Dinev, T., Tzanova, M., Velichkova, K., Dermendzhieva, D., & Beev, G. (2021). Antifungal and antioxidant potential of methanolic extracts from Acorus calamus L., Chlorella vulgaris Beijerinck, Lemna minuta Kunth and Scenedesmus dimorphus (Turpin) Kützing. *Applied Sciences*, *11*(11), 4745

112. Dissa, A. O., Desmorieux, H., Savadogo, P. W., Segda, B. G., & Koulidiati, J. (2010). Shrinkage, porosity and density behaviour during convective drying of spirulina. *Journal of food Engineering*, *97*(3), 410-418.

113. Dolganyuk, V., Belova, D., Babich, O., Prosekov, A., Ivanova, S., Katserov, D., ... & Sukhikh, S. (2020). Microalgae: A promising source of valuable bioproducts. *Biomolecules*, *10*(8), 1153.

114. Dragone, G. (2022). Challenges and opportunities to increase economic feasibility and sustainability of mixotrophic cultivation of green microalgae of the genus Chlorella. *Renewable and Sustainable Energy Reviews*, *160*, 112284.

115. Driesen, K. (2015). *Acute aquatic toxicity test of caffeine with Daphnia magna Straus and biomonitoring of PRB and Intensive Green Filter wastewater treatment systems* (Doctoral dissertation, Masters Dissertation, Universiteit Gent).

116. Edgar RC (2004) MUSCLE: Multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res 32:1792–1797.

117. Eichler, A.W. 1883. Syllabus der Vorlesungen uber spezielle und medicinisch-pharmaceutische Botanik, 3rd Edn., 68. pp. Berlin

118. Ekonomou, G., Lolas, A., Castritsi-Catharios, J., Neofitou, C., D. Zouganelis, G., Tsiropoulos, N., & Exadactylos, A. (2019). Mortality and effect on growth of Artemia franciscana exposed to two common organic pollutants. *Water*, *11*(8), 1614.

119. El Gamal, A. A. (2010). Biological importance of marine algae. *Saudi Pharmaceutical Journal*, 18(1), 1-25

120. Eladl, S. N., Elnabawy, A. M., & Eltanahy, E. G. (2024). Recent biotechnological applications of value-added bioactive compounds from microalgae and seaweeds. *Botanical Studies*, *65*(1), 28.

121. El-Adl, M. F., Deyab, M. A., Khafaga, M. M., & Abo Ahmed, S. E. S. (2024). The freshwater alga Chlorella sorokiniana tolerates salt stress via modulating metabolites and minerals. *Scientific Journal for Damietta Faculty of Science*, *14*(2), 44-51.

122. El-Baky, H. H. A., El Baz, F. K., & El-Baroty, G. S. (2009). Production of phenolic compounds from Spirulina maxima microalgae and its protective effects. *African Journal of Biotechnology*, *8*(24).

123. Elbaz, A. M., Ahmed, A. M., Abdel-Maqsoud, A., Badran, A. M., & Abdel-Moneim, A. M. E. (2022). Potential ameliorative role of *Spirulina platensis* in powdered or extract forms against cyclic heat stress in broiler chickens. *Environmental science and pollution research*, *29*(30), 45578-45588.

124. El-Baz, F. K., & Abd El Baky, H. H. (2018). Pilot Scale of Microalgal Production Using Photobioreactor. *Photosynthesis from its Evolution to Future Improvements in Photosynthetic Efficiency Using Nanomaterials; Intech Open: London, UK*, 53.

125. El-Baz, F. K., et al. (2023). Therapeutic potential of microalgae-derived natural compounds in diabetic wound healing: A comprehensive review. *Heliyon*, 9(3), e01104

126. Elhafiz, A., Gaur, A. E. S. S., Hamdany, N., Maryam, O., & Lakshmi, T. V. R. (2015). *Chlorella vulgaris* and *Chlorella pyrenoidosa* live cells appear to be promising sustainable biofertilizer to grow rice, lettuce, cucumber and eggplant in the UAE soils. *Recent Research in Science and Technology*, 7, 14–21.

127. El-Sheekh, M. M., Daboor, S. M., Swelim, M. A., & Mohamed, S. (2014). Production and characterization of antimicrobial active substance from *Spirulina platensis*. *Iranian journal of microbiology*, *6*(2), 112.

128. Enerstvedt, K. H. (2018). Analysis of polyphenolic content in marine and aquatic angiosperms from Norwegian coastal waters.(PHD Thesis)

129. Engin, A. B., Bukan, N., Kurukahvecioglu, O., Memis, L., & Engin, A. (2011). Effect of butylated hydroxytoluene (E321) pretreatment versus l-arginine on liver injury after sub-lethal dose of endotoxin administration. *Environmental toxicology and pharmacology*, *32*(3), 457-464.

130. Erdmann N, Hagemann M (2001) Salt acclimation of algae and cyanobacteria: a comparison. In: Rai LC, Gaur JP (eds) Algal Adaptation to environmental stresses. Springer, Berlin, pp 323–361.

131. Essam, T., Amin, M. A., El Tayeb, O., Mattiasson, B., & Guieysse, B. (2007). Solar-based detoxification of phenol and p-nitrophenol by sequential TiO2 photocatalysis and photosynthetically aerated biological treatment. *Water research*, *41*(8), 1697-1704.

132. Ettl, H. and Gärtner, G. (1995) Syllabus der Boden-, Luft- und Flechtenalgen. G. Fischer, Stuttgart, Jena, New York, 722.

133. Eze, C. N., Onyejiaka, C. K., Ihim, S. A., Ayoka, T. O., Aduba, C. C., Nwaiwu, O., & Onyeaka, H. (2023). Bioactive compounds by microalgae and potentials for the management of some human disease conditions. *AIMS microbiology*, *9*(1), 55.

134. Fakhri, S., Abbaszadeh, F., Dargahi, L., & Jorjani, M. (2018). Astaxanthin: A mechanistic review on its biological activities and health benefits. *Pharmacological research*, *136*, 1-20.

135. FAO (2020), Action, S. I, World fisheries and aquaculture. *Food and Agriculture Organization*, *2020*, 1-244.

136. Farg, H., El-Makhzangy, A., & El-Shawaf, A. G. (2021). Chemical and technological studies on micro algae extracts and its utilization in some food products. 1-chemical, biochemical characteristics and nutritional value of spirulina ALGAE. *Journal of productivity and development*, *26*(1), 107-117.

137. Farkas, A., Pap, B., Zsíros, O., Patai, R., Shetty, P., Garab, G & Maróti, G. (2023). Salinity stress provokes diverse physiological responses of eukaryotic unicellular microalgae. *Algal Research*, *73*, 103155.

138. Fawley, M. W., & Fawley, K. P. (2020). Identification of eukaryotic microalgal strains. *Journal of applied phycology*, *32*, 2699-2709.

139. Fellah, Z. E., Sebaa, N., Fellah, M., Ogam, E., Mitri, F. G., Depollier, C., & Lauriks, W. (2008). Application of the Biot model to ultrasound in bone: inverse problem. *ieee transactions on ultrasonics, ferroelectrics, and frequency control*, *55*(7), 1516-1523.

140. Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783-791.

141. Ferdous, U. T., Nurdin, A., Ismail, S., & Yusof, Z. N. B. (2023). Evaluation of the antioxidant and cytotoxic activities of crude extracts from marine *Chlorella sp*. *Biocatalysis and Agricultural Biotechnology*, *47*, 102551.

142. Figler, A., B-Béres, V., Dobronoki, D., Márton, K., Nagy, S. A., & Bácsi, I. (2019). Salt tolerance and desalination abilities of nine common green microalgae isolates. *Water*, *11*(12), 2527.

143. Fihri, R. F., Ez-Zoubi, A., Mbarkiou, L., Amar, A., Farah, A., & Bouchamma, E. O. (2024). Antibacterial and antioxidant activities of *Chlorella vulgaris* and Scenedesmus incrassatulus using natural deep eutectic solvent under microwave assisted by ultrasound. *Heliyon*, *10*(15).

144. Firoozabad, M. S. M., & Nasr, M. M. (2022). Antimicrobial activities of microbial essential fatty acid against foodborne pathogenic bacteria. *Iranian Journal of Microbiology*, *14*(2), 214.

145. Foo, S.C., Mok, C.Y., Ho, S.Y., Khong, N.M.H..(2023). Microalgal culture preservation: progress, trends and future developments. Algal Res. 71, 103007.

146. Fott B, Karim AG. (1973). *Spirulina* plankton community in a lake in Jebel Marra, Sudan. Archiv fur Protistenkunde. 115: 408– 418.

147. Fott, B. (1969). A monograph of the genus Chlorella. The fresh water species. *Studies in Phycology.*, 10-70.

148. Fox RD. 1996. *Spirulina:* production & potential. Aix-en-Provence, Edisud.

149. Fradique, M., Batista, A. P., Nunes, M. C., Gouveia, L., Bandarra, N. M., & Raymundo, A. (2010). Incorporation of *Chlorella vulgaris* and *Spirulina maxima* biomass in pasta products. Part 1: Preparation and evaluation. *Journal of the Science of Food and Agriculture*, *90*(10), 1656-1664.

150. Fristiohady, A., Sadarun, B., Sutariati, G. A. K., Sundowo, A., Yodha, A. W. M., Baharum, S. N., & Idin, S. (2024). Chemical Profiles and Biological Activities of Microalga *Chlorella sp*. from Southeast Sulawesi. *Ilmu Kelautan: Indonesian Journal of Marine Sciences*, *29*(2).

151. Fritsch, F. E. (1935). *Structure and Reproduction of the Algae Volume I: Introduction, Chlorophyceae... Flagellata*. University Press.

152. Fritsch, F.E. (1922).The Structure and Reproduction of The Algae, 1st ed.; Cambridge University Press: Cambridge, UK, Volume 1.

153. Fritsch, F.E. (1922).The Structure and Reproduction of The Algae, 1st ed.; Cambridge University Press: Cambridge, UK, Volume 2.

154. Fujitani, N., Sakaki, S., Yamaguchi, Y., & Takenaka, H. (2001). Inhibitory effects of microalgae on the activation of hyaluronidase. *Journal of applied phycology*, *13*(6), 489-492.

155. Ganeson, Y., Paramasivam, P., Palanisamy, K. M., Govindan, N., & Maniam, G. P. (2024). LCMS and FTIR profiling of microalga *Chlorella sp*. for cosmetics and skin care applications. *Cleaner Water*, *2*, 100028.

156. Garcia-Gonzalez, J., & Sommerfeld, M. (2016). Biofertilizer and biostimulant properties of the microalga *Acutodesmus dimorphus*. *Journal of applied phycology*, *28*, 1051-1061.

157. Gardner NL. (1917). New Pacific Coast marine algae (Vol. 6). University of California Press.

158. Gheda, S. F., & Ismail, G. A. (2020). Natural products from some soil cyanobacterial extracts with potent antimicrobial, antioxidant and cytotoxic activities. *Anais da Academia Brasileira de Ciências*, *92*, e20190934.

159. Giebelhausen, B., & Lampert, W. (2001). Temperature reaction norms of *Daphnia magna*: the effect of food concentration. *Freshwater Biology*, *46*(3), 281-289.

160. Giménez Giménez, A., Ibáñez González, M. J., Robles Medina, A., Molina Grima, E., García Salas, S., & Esteban Cerdán, L. (1998). Downstream processing and purification of eicosapentaenoic (20: 5n-3) and arachidonic acids (20: 4n-6) from the microalga Porphyridium cruentum. *Bioseparation*, *7*, 89-99.

161. Gloria, N. F., Soares, N., Brand, C., Oliveira, F. L., Borojevic, R., & Teodoro, A. J. (2014). Lycopene and beta-carotene induce cell-cycle arrest and apoptosis in human breast cancer cell lines. *Anticancer research*, *34*(3), 1377-1386.

162. Goiris, K., Muylaert, K., Fraeye, I., Foubert, I., De Brabanter, J., & De Cooman, L. (2012). Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. *Journal of applied phycology*, *24*, 1477-1486.

163. Gomez-Villa, H., Voltolina, D., Nieves, M., & Pina, P. (2005). Biomass production and nutrient budget in outdoor cultures of *Scenedesmus obliquus* (Chlorophyceae) in artificial wastewater, under the winter and summer conditions of Mazatlan, Sinaloa, Mexico. *Vie et Milieu/Life & Environment*, 121-126.

164. Goodarzi, V., Zamani, H., Bajuli, L., & Moradshahi, A. (2014). Evaluation of antioxidant potential and reduction capacity of some plant extracts in silver nanoparticles' synthesis. *Molecular biology research communications*, *3*(3), 165.

165. Goswami, R. K., Agrawal, K., & Verma, P. (2022). Microalgae Dunaliella as biofuel feedstock and β-carotene production: An influential step towards environmental sustainability. *Energy Conversion and Management: X*, *13*, 100154.

166. Grácio, M., Ferreira, J., Steinrücken, P., Kleinegris, D. M., Sousa, I., Nunes, M. C., & Raymundo, A. (2024). The Volatile Composition and the Potential Health Benefits of Different Microalgae Strains. *Foods*, *13*(14), 2174.

167. Grignon-Dubois, M., & Rezzonico, B. (2013). The economic potential of beach-cast seagrass–Cymodocea nodosa: a promising renewable source of chicoric acid. *Botanica Marina*, *56*(4), 303-311.

168. Grosshagauer, S., Kraemer, K., & Somoza, V. (2020). The true value of Spirulina. *Journal of agricultural and food chemistry*, *68*(14), 4109-4115.

169. Gui, L., Xu, L., Liu, Z. Y., Zhou, Z. G., & Sun, Z. (2022). Carotenoid-rich microalgae promote growth and health conditions of Artemia nauplii. *Aquaculture*, *546*, 737289.

170. Guillard, R. R. (2005). Purification methods for microalgae. *Algal culturing techniques*, *117*, 32.

171. Gulati, R. A. M. E. S. H., & Demott, W. I. L. L. I. A. M. (1997). The role of food quality for zooplankton: remarks on the state-of-the-art, perspectives and priorities. *Freshwater Biology*, *38*(3), 753-768.

172. Guldas, M., Ziyanok-Demirtas, S., Sahan, Y., Yildiz, E., & Gurbuz, O. (2020). Antioxidant and anti-diabetic properties of *Spirulina platensis* produced in Turkey. *Food Science and Technology*, *41*, 615-625.

173. Guzmán, S., Gato, A., Lamela, M., Freire-Garabal, M., & Calleja, J. M. (2003). Anti-inflammatory and immunomodulatory activities of polysaccharide from *Chlorella stigmatophora* and Phaeodactylum tricornutum. *Phytotherapy Research*, *17*(6), 665-670.

174. Guzmán-Murillo, M. A., Ascencio, F., & Larrinaga-Mayoral, J. A. (2013). Germination and ROS detoxification in bell pepper (*Capsicum annuum L.*) under NaCl stress and treatment with microalgae extracts. *Protoplasma*, *250*, 33-42.

175. Hachicha, R., Elleuch, F., Ben Hlima, H., Dubessay, P., de Baynast, H., Delattre, C., ... & Fendri, I. (2022). Biomolecules from microalgae and cyanobacteria: Applications and market survey. *Applied Sciences*, *12*(4), 1924.

176. Hadjadj M. 2018. Suivi des indicateurs de pollution dans une zone aride à semi-aride: Diagnostic des environs de Gdyel suite aux dépôts des déchets ménagers et rejet d'eau usée au niveau du lac Télamine. Magister Thesis, Université d'Oran 2 : p. 109 – 126.

177. Halliwell, B., & Gutteridge, J. M. (1995). The definition and measurement of antioxidants in biological systems. *Free radical biology & medicine*, *18*(1), 125-126.

178. Hamed, I. (2016). *The evolution and versatility of microalgal biotechnology: a review. Compr Rev Food Sci Food Saf 15: 1104–1123*.

179. Harun, R., Singh, M., Forde, G. M., & Danquah, M. K. (2010). Bioprocess engineering of microalgae to produce a variety of consumer products. *Renewable and sustainable energy reviews*, *14*(3), 1037-1047.

180. Harvey WH (1836) In: Mackay JT (ed) Algae.

181. Hashmi, Z., Abbas, S. H., Osama, S. M., Muhammad, A., Usman, M. T., Jatoi, A. S., & Bozdar, M. M. (2023). Microalgae Technology in Aquaculture Applications: A Comprehensive Literature Review. *AMPLITUDO: Journal of Science and Technology Innovation*, *2*(2), 61-69.

182. Hemantkumar, J. N., & Rahimbhai, M. I. (2019). Microalgae and its use in nutraceuticals and food supplements. In *microalgae-from Physiology to Application*. IntechOpen.

183. Hermanns, A. S., Zhou, X., Xu, Q., Tadmor, Y., & Li, L. (2020). Carotenoid pigment accumulation in horticultural plants. *Horticultural Plant Journal*, *6*(6), 343-360.

184. Hernández-Urcera, J., Romero, A., Cruz, P., Vasconcelos, V., Figueras, A., Novoa, B., & Rodríguez, F. (2024). Screening of microalgae for bioactivity with antiviral, antibacterial, anti-inflammatory and anti-cancer assays. *Biology*, *13*(4), 255.

185. Hernández, H., Nunes, M. C., Prista, C., & Raymundo, A. (2022). Innovative and healthier dairy products through the addition of microalgae: a review. *Foods*, *11*(5), 755.

186. Hetta, M., Mahmoud, R., El-Senousy, W., Ibrahim, M., El-Taweel, G., & Ali, G. (2014). Antiviral and antimicrobial activities of *Spirulina platensis*. *World J Pharm Pharm Sci*, *3*, 31-39

187. Hong, W. K., Yu, A., Heo, S. Y., Oh, B. R., Kim, C. H., Sohn, J. H., ... & Seo, J. W. (2013). Production of lipids containing high levels of docosahexaenoic acid from empty palm fruit bunches by Aurantiochytrium sp. KRS101. *Bioprocess and biosystems engineering*, *36*, 959-963.

188. Hopes, A., & Mock, T. (2015). Evolution of microalgae and their adaptations in different marine ecosystems. *eLS*, *3*, 1-9.

189. Horrocks, L. A., & Yeo, Y. K. (1999). Health benefits of docosahexaenoic acid (DHA). *Pharmacological research*, *40*(3), 211-225.

190. Huheihel, M., Ishanu, V., Tal, J., & Arad, S. M. (2002). Activity of *Porphyridium sp.* polysaccharide against herpes simplex viruses in vitro and in vivo. *Journal of Biochemical and Biophysical methods*, *50*(2-3), 189-200.

191. Ibrahim, M. R., Hamouda, R. A., Tayel, A. A., & Al-Saman, M. A. (2021). Anti-cholesterol and antioxidant activities of independent and combined microalgae aqueous extracts in vitro. *Waste and Biomass Valorization*, *12*, 4845-4857.

192. Ijaz, S., & Hasnain, S. (2016). Antioxidant potential of indigenous cyanobacterial strains in relation with their phenolic and flavonoid contents. *Natural product research*, *30*(11), 1297-1300.

193. Ilieva, Y., Zaharieva, M. M., Najdenski, H., & Kroumov, A. D. (2024). Antimicrobial Activity of *Arthrospira* (Former Spirulina) and *Dunaliella* Related to Recognized Antimicrobial Bioactive Compounds. *International Journal of Molecular Sciences*, *25*(10), 5548.

194. Ismail, M. M., El-Fakharany, E. M., & Hegazy, G. E. (2023). Purification and fractionation of phycobiliproteins from *Arthrospira platensis* and Corallina officinalis with evaluating their biological activities. *Scientific Reports*, *13*(1), 14270.

195. Jacob-Lopes, E., Maroneze, M. M., Queiroz, M. I., & Zepka, L. Q. (Eds.). (2020). Handbook of microalgae-based processes and products: fundamentals and advances in energy, food, feed, fertilizer, and bioactive compounds. *Academic Press*.

196. Jacob-Lopes, E., Scoparo, C. H. G., Queiroz, M. I., & Franco, T. T. (2010). Biotransformations of carbon dioxide in photobioreactors. *Energy Conversion and Management*, *51*(5), 894-900.

197. Jedynak, P., Burczyk, J., Borowski, S., Kaszyckl, P., Halat-Las, M., Kedra, M., ... & Malec, P. (2018). Use of microalgae for treatment of post-fermentation effluent from biogas production. *PRZEMYSL CHEMICZNY*, *97*(12), 2106-2109.

198. Jeong, S. H., Kim, B. Y., Kang, H. G., Ku, H. O., & Cho, J. H. (2005). Effects of butylated hydroxyanisole on the development and functions of reproductive system in rats. *Toxicology*, *208*(1), 49-62.

199. Jerez-Martel, I., García-Poza, S., Rodríguez-Martel, G., Rico, M., Afonso-Olivares, C., & Gómez-Pinchetti, J. L. (2017). Phenolic profile and antioxidant activity of crude extracts from microalgae and cyanobacteria strains. *Journal of Food Quality*, *2017*(1), 2924508.

200. Jerney, J., & Spilling, K. (2018). Large scale cultivation of microalgae: open and closed systems. In *Biofuels from algae: methods and protocols* (pp. 1-8). New York, NY: Springer New York.

201. Jiang, Y., Lu, W., Long, S., Zhang, M., Xu, Y., & Wang, Y. (2020). Effects of dietary supplementation of *Schizochytrium sp*. on milk production, milk fatty acid profile, and antioxidant capacity of dairy cows. *Journal of Dairy Science, 103*(5), 4237–4246

202. Jones, R. H., & Flynn, K. J. (2005). Nutritional status and diet composition affect the value of diatoms as copepod prey. *Science*, *307*(5714), 1457-1459.

203. Jorge, C.M., Antonio, O.C.J., German, C.M., Irina, C.C.,Carmen M.D. and Daniel, B.C. (2016). Laboratoryproduction of *Daphnia magna* (Straus 1820) fed withmicroalgae and active dry yeast. Journal of Entomologyand Zoology Studies, 4, 548-553.

204. Jung CH, Braune S, Waldeck P, Küpper JH, Petrick I, Jung F.(2021). Morphology and Growth of *Arthrospira platensis* during Cultivation in a Flat-Type Bioreactor. Life 11(6): 536.

205. Kaggwa MN, Burian A, Oduor SO, Schagerl M. (2013). Ecomorphological variability of *Arthrospira fusiformis* (Cyanoprokaryota) in African soda lakes. Microbiology Open 2(5): 881-891.

206. Kamani, M. H., Eş, I., Lorenzo, J. M., Remize, F., Roselló-Soto, E., Barba, F. J. & Khaneghah, A. M. (2019). Advances in plant materials, food by-products, and algae conversion into biofuels: use of environmentally friendly technologies. *Green chemistry*, *21*(12), 3213-3231.

207. Kapoore R. V., Wood E. E., Llewellyn C. A. (2021). Algae biostimulants: A critical look at microalgal biostimulants for sustainable agricultural practices. Biotechnol. Adv. 49, 107754.

208. Karakaş, K., Eryalçın, K. M., Ergönül, M. B., Atıcı, T., & Atasagun, S. (2023). The evaluation of the growth performance of a cyanobacterial isolate *Phormidium lucidum* (Kützing ex Gomont 1892) grown under different environmental conditions and its use as a dietary source for Daphnia magna (Straus 1820). *Communications Faculty of Sciences University of Ankara Series C Biology*, *32*(1), 70-86.

209. Karray, A., Krayem, N., Saad, H. B., & Sayari, A. (2021). Spirulina platensis, Punica granatum peel, and moringa leaves extracts in cosmetic formulations: An integrated approach of in vitro biological activities and acceptability studies. *Environmental Science and Pollution Research*, *28*, 8802-8811.

210. Karthikeyan, D., Muthukumaran, M., & Balakumar, B. S. (2016). Mass cultivation of microalgae in open raceway pond for biomass and biochemicals production. *Int J Adv Res Biol Sci*, *3*(2), 247-260.

211. Karthikeyan, S., Balasubramanian, R., & Iyer, C. S. P. (2007). Evaluation of the marine algae Ulva fasciata and Sargassum sp. for the biosorption of Cu (II) from aqueous solutions. *Bioresource Technology*, *98*(2), 452-455.

212. Katırcıoğlu, H., Shamchı, M. P., Çeker, S., Çapık, Ö., & Ağar, G. (2020). Determination of Biological Properties of *Chlorella vulgaris* C1 Extracts. *Eastern Anatolian Journal of Science*, *6*(2), 15-21.

213. Kaur, S., Sharma, A., Bala, S., Satheesh, N., Nile, A. S., & Nile, S. H. (2025). Microalgae in the food-health nexus: Exploring species diversity, high-value bioproducts, health benefits, and sustainable market potential. *Bioresource Technology*, 132424.

214. Kaushik, P., & Chauhan, A. (2008). In vitro antibacterial activity of laboratory grown culture of *Spirulina platensis*. *Indian Journal of Microbiology*, *48*, 348-352.

215. Kebede E. (1997). Response of *Spirulina platensis* (*Arthrospira fusiformis*) from Lake Chitu, Ethiopia, to salinity stress from sodium salts. Journal of Applied Phycology 9(6): 551-558.

216. Khaligh, S. F., & Asoodeh, A. (2022). Recent advances in the bio-application of microalgae-derived biochemical metabolites and development trends of photobioreactor-based culture systems. *3 Biotech*, *12*(10), 260.

217. Khan, M. I., Shin, J. H., & Kim, J. D. (2018). The promising future of microalgae: current status, challenges, and optimization of a sustainable and renewable industry for biofuels, feed, and other products. *Microbial cell factories*, *17*, 1-21.

218. Khan, S. A., Hussain, M. Z., Prasad, S., & Banerjee, U. C. (2009). Prospects of biodiesel production from microalgae in India. *Renewable and sustainable energy reviews*, *13*(9), 2361-2372.

219. Kholif, A. E., Morsy, T. A., Gouda, G. A., Anele, U. Y., & Galyean, M. L. (2016). Effect of feeding diets containing *Chlorella vulgaris* on lactation, milk fatty acids, and blood parameters of goats. *Livestock Science, 191*, 70–76

220. Kholssi, R., Ramos, P. V., Marks, E. A., Montero, O., & Rad, C. (2021). 2Biotechnological uses of microalgae: a review on the state of the art and challenges for the circular economy. *Biocatalysis and Agricultural Biotechnology*, *36*, 102114.

221. Kim, G. M., & Kim, Y. K. (2022). Drying techniques of microalgal biomass: A review. *Applied Chemistry for Engineering*, *33*(2), 145-150.

222. Kim, S. K., et al. (2022). Microorganisms in Fish Feeds, Technological Innovations, and Key Strategies for Sustainable Aquaculture. Microorganisms, 11(2), 439.

223. Kimura M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. Journal of Molecular Evolution 16:111-120.

224. Kirst, G. O. (1989). Salinity tolerance of eukaryotic marine algae. *Annual review of plant biology*, *41*(1), 21-53.

225. Komárek J, Anagnostidis K. 2005. Cyanoprokaryota. 2. Teil/2nd Part, Oscillatoriales Elsevier GmbH, Munchen. Pp 341–353

226. Komárek, J., & Hauer, T. (2011). CyanoDB. cz-On-line database of cyanobacterial genera. *Word-wide electronic publication, Univ. of South Bohemia & Inst. of Botany AS CR*.

227. Krienitz L, Ballot A, Casper P, Kotut K, Pflugmacher S, Wiegand C. (2005). Toxic cyanobacteria and massive deaths of flamingos: What we can learn from *Arthrospira* mass-cultivation. Phycologia 44(4):59-59.

228. Krienitz, L., Hegewald, E. H., Hepperle, D., Huss, V. A., Rohr, T., & Wolf, M. (2004). Phylogenetic relationship of Chlorella and Parachlorella gen. nov.(Chlorophyta, Trebouxiophyceae). *Phycologia*, *43*(5), 529-542.

229. Krienitz, L., Huss, V. A., & Bock, C. (2015). Chlorella: 125 years of the green survivalist. *Trends in plant science*, *20*(2), 67-69.

230. Kucmanová, A., & Gerulová, K. (2019). Microalgae harvesting: a review. *Vedecké Práce Materiálovotechnologickej Fakulty Slovenskej Technickej Univerzity v Bratislave so Sídlom v Trnave*, *27*(44), 129-143.

231. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. (2018). MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547.

232. Kumar, A., Ramamoorthy, D., Verma, D. K., Kumar, A., Kumar, N., Kanak, K. R., ... & Mohan, K. (2022). Antioxidant and phytonutrient activities of *Spirulina platensis*. *Energy Nexus*, *6*, 100070.

233. Kumar, M., Kulshreshtha, J., & Singh, G. P. (2010). Growth and biopigment accumulation of *Chlorella vulgaris* under salt stress. Journal of Algal Biomass Utilization, 1(4), 20–26.

234. Łączyńska B., Palińska-Żarska K., Nowosad J., Biłas M., Krejszeff S., Müller T., Kucharczyk D., Żarski D. (2016). Effect of age, size and digestive tract development on weaning effectiveness in crucian carp, *Carassius carassius* (Linnaeus, 1758). J. Appl. Ichthyol., 32: 866–872.

235. Lam Man Kee, L. M., Lee KeatTeong, L. K., & Abdul Rahman Mohamed, A. R. M. (2012). Current status and challenges on microalgae-based carbon capture.

236. Lamaison, J. L., & Carnat, J. (1990). Teneur en principaux flavonodes does fleurs et des feuiltes de crtaegus monogyna Jacq. et. de Crataegusleavigata (Poret) DC.(Rosaceae). *Acta Pharm. Acta Pharm. Acta Helv*, *65*, 315-320.

237. Lane D. (1991). 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acids techniques in bacterial systematics. Wiley, New York, Pp 115–147.

238. Laroche, C. (2022). Exopolysaccharides from microalgae and cyanobacteria: diversity of strains, production strategies, and applications. *Marine drugs*, *20*(5), 336.

239. Lau E, Nash CZ, Vogler DR, Cullings K W. (2005). Molecular diversity of cyanobacteria inhabiting coniform structures and surrounding mat in a Yellowstone hot spring. Astrobiology 5(1): 83-92.

240. Le, T. H., Hoa, N. V., Sorgeloos, P., & Van Stappen, G. (2019). Artemia feeds: a review of brine shrimp production in the Mekong Delta, Vietnam. *Reviews in Aquaculture*, *11*(4), 1169-1175.

241. Lee RE (2008) Phycology, 4th edn. Cambridge University Press, London, p 561.

242. Lee, J. (1989). *Ireland, 1912-1985: politics and society*. Cambridge University Press.

243. Lee, K., Eisterhold, M. L., Rindi, F., Palanisami, S., & Nam, P. K. (2014). Isolation and screening of microalgae from natural habitats in the midwestern United States of America for biomass and biodiesel sources. *Journal of natural science, biology, and medicine*, *5*(2), 333.

244. Lee, Y. K., Chen, W., Shen, H., Han, D., Li, Y., Jones, H. D., ... & Hu, Q. (2013). Basic culturing and analytical measurement techniques. *Handbook of microalgal culture: applied phycology and biotechnology*, 37-68.

245. Leliaert, F., Verbruggen, H., Vanormelingen, P., Steen, F., López-Bautista, J. M., Zuccarello, G. C., & De Clerck, O. (2014). DNA-based species delimitation in algae. *European journal of phycology*, *49*(2), 179-196.

246. León-Vaz, A., León, R., Vigara, J., & Funk, C. (2023). Exploring Nordic microalgae as a potential novel source of antioxidant and bioactive compounds. *New Biotechnology*, *73*, 1-8.

247. Letunic I, Bork P (2019) Interactive Tree of Life (iToL) v4: recent updates and new developments. Nucleic Acids Res 47(W1):W256-W259.

248. Levasseur, W., Perré, P., & Pozzobon, V. (2020). A review of high value-added molecules production by microalgae in light of the classification. *Biotechnology advances*, *41*, 107545.

249. Li, A., Zhang, L., Zhao, Z. Y., Ma, S. S., Wang, M., & Liu, P. H. (2016). Prescreening, identification and harvesting of microalgae with antibacterial activity. *Biologia*, *71*(10), 1111-1118.

250. Li, H., Xu, A. N., Yang, Z., Lei, Y., Chen, J., Zhan, Z., ... & Xie, S. (2024). Assessing the changes in marine microalgae diversity in the Nanji Islands Nature Reserve over the past decades using sediment eDNA. *Frontiers in Marine Science*, *11*, 1466434.

251. Li, S., Li, F., Zhu, X., Liao, Q., Chang, J. S., & Ho, S. H. (2022). Biohydrogen production from microalgae for environmental sustainability. *Chemosphere*, *291*, 132717.

252. Li, X., Yuan, Y., Cheng, D., Gao, J., Kong, L., Zhao, Q& Sun, Y. (2018). Exploring stress tolerance mechanism of evolved freshwater strain Chlorella sp. S30 under 30 g/L salt. *Bioresource Technology*, *250*, 495-504.

253. Lima, S. A., Raposo, M. F. J., Castro, P. M., & Morais, R. M. (2004). Biodegradation of p-chlorophenol by a microalgae consortium. *Water Research*, *38*(1), 97-102.

254. Lin, L., Chan, G. Y. S., Jiang, B. L., & Lan, C. Y. (2007). Use of ammoniacal nitrogen tolerant microalgae in landfill leachate treatment. *Waste management*, *27*(10), 1376-1382.

255. Liu, Q., Li, W., & Qin, S. (2020). Therapeutic effect of phycocyanin on acute liver oxidative damage caused by X-ray. *Biomedicine & Pharmacotherapy*, *130*, 110553.

256. Ljubic, A. (2020). Novel microalgal based ingredients.PHD Thesis, Technical University of Denmark, page 21

257. Long, X., Zhang, C., Yang, Q., Zhang, X., Chen, W., Zhu, X., ... & Tan, Q. (2024). Photoheterotroph improved the growth and nutrient levels of *Chlorella vulgaris* and the related molecular mechanism. *Applied Microbiology and Biotechnology*, *108*(1), 269.

258. Luo, W., Pröschold, T., Bock, C., and Krienitz, L. (2010). Generic concept in C*hlorella*-related coccoid green algae (Chlorophyta, Trebouxiophyceae). Plant Biol. 12, 545–553.

259. Ma, M., & Hu, Q. (2023). Microalgae as feed sources and feed additives for sustainable aquaculture: prospects and challenges. *Reviews in Aquaculture*, *16*(2), 818-835.

260. Maan, M. E., & Sefc, K. M. (2013, June). Colour variation in cichlid fish: developmental mechanisms, selective pressures and evolutionary consequences. In *Seminars in Cell & Developmental Biology* (Vol. 24, No. 6-7, pp. 516-528). Academic Press.

261. Madkour, K., Dawood, M. A., & Sewilam, H. (2022). The use of Artemia for aquaculture industry: An updated overview. *Annals of Animal Science*, *23*(1), 3-10.

262. Mahajan S, Tuteja N. (2005). Cold, salinity and drought stresses: an overview. Arch Biochem Biophys 444:139–158.

263. Mahmudi, M., Arsad, S., Fitrianesia, F., Ramadhan, S. F., Arif, A. R., Savitri, F. R., ... & Lusiana, E. D. (2023). Microalgae diversity in varying habitat characteristics in Pasuruan and Sidoarjo coastal areas, East Java, Indonesia. *Biodiversitas Journal of Biological Diversity*, *24*(8).

264. Maity, S., & Mallick, N. (2022). Bioprospecting marine microalgae and cyanobacteria as alternative feedstocks for bioethanol production. *Sustainable Chemistry and Pharmacy*, *29*, 100798.

265. Majee, S.B., Avlani, D., Biswas, G.R., 2017. Pharmacological, pharmaceutical, cosmetic 596 and diagnostic applications of sulfated polysaccharides from marine algae and bacteria. 597 Afr. J. Pharm. Pharmacol. 11, 68-77.

266. Majee, S.B., Avlani, D., Biswas, G.R., 2017. Pharmacological, pharmaceutical, cosmetic 596 and diagnostic applications of sulfated polysaccharides from marine algae and bacteria. 597 Afr. J. Pharm. Pharmacol. 11, 68-77.

267. Makarewicz, M., Drożdż, I., Tarko, T., & Duda-Chodak, A. (2021). The interactions between polyphenols and microorganisms, especially gut microbiota. *Antioxidants*, *10*(2), 188.

268. Makridis, P., & Vadstein, O. (1999). Food size selectivity of *Artemia franciscana* at three developmental stages. *Journal of plankton research*, *21*(11), 2191-2201.

269. Mansour, F. B., Guermazi, W., Chamkha, M., Bellassoued, K., Salah, H. B., Harrath, A. H., ... & Ayadi, H. (2024). Bioactive Potential of the Sulfated Exopolysaccharides From the Brown Microalga Halamphora sp.: Antioxidant, Antimicrobial, and Antiapoptotic Profiles. *Analytical Science Advances*, e202400030.

270. Marrez, D. A., Naguib, M. M., Sultan, Y. Y., & Higazy, A. M. (2019). Antimicrobial and anticancer activities of Scenedesmus obliquus metabolites. *Heliyon*, *5*(3).

271. Martelli, F., Cirlini, M., Lazzi, C., Neviani, E., & Bernini, V. (2020). Edible seaweeds and spirulina extracts for food application: In vitro and in situ evaluation of antimicrobial activity towards foodborne pathogenic bacteria. *Foods*, *9*(10), 1442.

272. Mata T.M., Martins A.A and Caetano N.S (2010). Microalgae for biodiesel production and other applications, Renew. Sust. Energ. Rev., 14(1), 217- 32.

273. Mc Gee, D., Archer, L., Smyth, T. J., Fleming, G. T., & Touzet, N. (2020). Bioprospecting and LED-based spectral enhancement of antimicrobial activity of microalgae isolated from the west of Ireland. *Algal Research*, *45*, 101704.

274. Mehwish, A. U., Manzoor, M., Aleem, K., Shafi, F., Shamoon, F., & Rani, A. (2023, April). *Rohail (2023) A review exploring the incorporation of microalgae to enhance the nutritional profile and health benefits of dairy products. Agrobiol Rec 13: 92–100*.

275. Mendiola, J. A., Jaime, L., Santoyo, S., Reglero, G., Cifuentes, A., Ibañez, E., & Señoráns, F. J. (2007). Screening of functional compounds in supercritical fluid extracts from Spirulina platensis. *Food chemistry*, *102*(4), 1357-1367.

276. Mezgebu, A., Liu, X., Mingist, M., & Ban, S. (2025). Dietary lipid constraints of Arthrospira platensis for survival and growth of Daphnia magna. *Inland Waters*, 1-12.

277. Mócsai, R., Helm, J., Polacsek, K., Stadlmann, J., & Altmann, F. (2024). The Diversity of N-Glycans of Chlorella Food Supplements Challenges Current Species Classification. *Foods*, *13*(19), 3182.

278. Moisander PH, McClinton E, Paerl HW. (2002). Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. Microbial Ecology 43(4): 432-442.

279. Molina Grima E, Belarbi EH, Acien-Fernandez FG, Robles-Medina A, Yusuf C (2003) Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol Adv 20: 491-515.

280. Molina Grima, E., Garcia Camacho, F., & Acien Fernandez, F. G. (1999). Production of EPA from *Phaeodactylum tricornutum*. *Chemicals from microalgae*, 57-92.

281. Mudimu, O., Rybalka, N., Bauersachs, T., Born, J., Friedl, T., & Schulz, R. (2014). Biotechnological screening of microalgal and cyanobacterial strains for biogas production and antibacterial and antifungal effects. *Metabolites*, *4*(2), 373-393.

282. Muñoz, R., & Guieysse, B. (2006). Algal–bacterial processes for the treatment of hazardous contaminants: a review. *Water research*, *40*(15), 2799-2815.

283. Muñoz, R., Köllner, C., & Guieysse, B. (2009). Biofilm photobioreactors for the treatment of industrial wastewaters. *Journal of hazardous materials*, *161*(1), 29-34.

284. Mustapa, M., Sallehudin, N. J., Mohamed, M. S., Noor, N. M., & Raus, R. A. (2016). Decontamination of *Chlorella sp*. culture using antibiotics and antifungal cocktail treatment. *ARPN J. Eng. Appl. Sci*, *11*, 104-109.

285. Naseema Rasheed, R., Pourbakhtiar, A., Mehdizadeh Allaf, M., Baharlooeian, M., Rafiei, N., Alishah Aratboni, H., Morones-Ramire,J.R & Winck, F. V. (2023). Microalgal co-cultivation-recent methods, trends in omic-studies, applications, and future challenges. *Frontiers in Bioengineering and Biotechnology*, *11*, 1193424.

286. Neelam Arun, N. A., Shalini Gupta, S. G., & Singh, D. P. (2012). Antimicrobial and antioxidant property of commonly found microalgae *Spirulina platensis*, *Nostoc muscorum* and *Chlorella pyrenoidosa* against some pathogenic bacteria and fungi, : 4866-4875.

287. Nelis, H. J., Lavens, P., Moens, L., Sorgeloos, P., Jonckheere, J. A., Criel, G. R., & De Leenheer, A. P. (1984). cis-Canthaxanthins. Unusual carotenoids in the eggs and the reproductive system of female brine shrimp artemia. *Journal of Biological Chemistry*, *259*(10), 6063-6066.

288. Neustupa, J., Němcová, Y., Eliáš, M., & Škaloud, P. (2009). Kalinella bambusicola gen. et sp. nov.(Trebouxiophyceae, Chlorophyta), a novel coccoid Chlorella-like subaerial alga from Southeast Asia. *Phycological research*, *57*(3), 159-169.

289. Nezafatian, E., Farhadian, O., Daneshvar, E., & Bhatnagar, A. (2024). Investigating the effects of salinity and light stresses on primary and secondary metabolites of *Tetraselmis tetrathele*: Total phenolic compounds, fatty acid profile, and biodiesel properties. *Biomass and Bioenergy*, *181*, 107050.

290. Norsker, N. H., Barbosa, M. J., Vermuë, M. H., & Wijffels, R. H. (2011). Microalgal production—a close look at the economics. *Biotechnology advances*, *29*(1), 24-27.

291. Novoveská, L., Nielsen, S. L., Eroldoğan, O. T., Haznedaroglu, B. Z., Rinkevich, B., Fazi, S., ... & Einarsson, H. (2023). Overview and challenges of large-scale cultivation of photosynthetic microalgae and cyanobacteria. *Marine drugs*, *21*(8), 445.

292. Nowicka-Krawczyk P, Mühlsteinová R, Hauer T. (2019). Detailed characterization of the *Arthrospira* type species separating commercially grown taxa into the new genus *Limnospira* (Cyanobacteria). Scientific Reports 9(1): 1-11.

293. Nozaki, H., Katagiri, M., Megumi, Nakagawa, M &, Aizawa, K. (1995). Taxonomic Re-examination of the Two Strains labeled" Chlorella" in the Microbial Culture Collection at the National Institute for Environmental Studies (NIES-Collection). *Microbiology and Culture Collections: official publication of the Japan Society for Culture Collections: 11*(1), 11-18.

294. Nübel U, Garcia-Pichel F, Muyzer G. 1997. PCR primers to amplify 16S rRNA genes from cyanobacteria. Applied and Environmental Microbiology 63(8): 3327-3332.

295. Nur Fadillah, S., Natsir, H., Ahmad, A., Karim, A., & Taba, P. (2023). Extraction and fractionation of active protein from microalgae *Nitzschia sp*. as antimicrobial agent. *Egyptian Journal of Chemistry*, *66*(6), 95-100.

296. O'Sullivan, J.J., Norwood, E.-A., O'Mahony, J.A., Kelly, A.L., 2019. Atomisation technologies used in spray drying in the dairy industry: a review. J. Food Eng. 243, 57–69.

297. Oliveira, E. G. D., Rosa, G. S. D., Moraes, M. A. D., & Pinto, L. A. D. A. (2009). Characterization of thin layer drying of *Spirulina platensis* utilizing perpendicular air flow. *Bioresource technology*, *100*(3), 1297-1303.

298. O'Neill, J. (2016). Tackling drug-resistant infections globally: final report and recommendations.

299. Ördög, V., Stirk, W. A., Lenobel, R., Bancířová, M., Strnad, M., Van Staden, J& Németh, L. (2004). Screening microalgae for some potentially useful agricultural and pharmaceutical secondary metabolites. *Journal of applied phycology*, *16*, 309-314.

300. Ötleş, S., & Pire, R. (2001). Fatty acid composition of Chlorella and Spirulina microalgae species. *Journal of AOAC international*, *84*(6), 1708-1714.

301. Pacheco Vega, J. M., Cadena Roa, M. A., Ascencio, F., Rangel Davalos, C., & Contreras, M. R. (2015). Assessment of endemic microalgae as potential food for *Artemia franciscana* culture.

302. Papalia, T., Sidari, R., & Panuccio, M. R. (2019). Impact of different storage methods on bioactive compounds in *Arthrospira platensis* biomass. *Molecules*, *24*(15), 2810.

303. Parker SP (1982) Synapsis and classification of living organisms, vol 1 & 2. McGraw Hill, New York, p 2424

304. Pascher, A. (1931). Systematische Übersicht über die mit Flagellaten in Zusammenhang stehenden Algenreihen und Versuch einer Einreihung dieser Algenstämme in die Stämme des Pflanzenreiches. *Beihefte zum Botanischen Centralblatt*, *48*, 317.

305. Paulo, M. C., Cardoso, C., Coutinho, J., Castanho, S., & Bandarra, N. M. (2020). Microalgal solutions in the cultivation of rotifers and artemia: scope for the modulation of the fatty acid profile. *Heliyon*, *6*(11).

306. Pereira, A. G., Jimenez-Lopez, C., Fraga, M., Lourenço-Lopes, C., García-Oliveira, P., Lorenzo, J. M., ... & Simal-Gandara, J. (2020). Extraction, properties, and applications of bioactive compounds obtained from microalgae. *Current Pharmaceutical Design*, *26*(16), 1929-1950.

307. Pereira, H., Páramo, J., Silva, J., Marques, A., Barros, A., Maurício, D., ... & Varela, J. (2018). Scale-up and large-scale production of *Tetraselmis sp*. CTP4 (Chlorophyta) for CO2 mitigation: From an agar plate to 100-m3 industrial photobioreactors. *Scientific reports*, *8*(1), 1-11.

308. Pereira, L., Cotas, J., & Valado, A. (2024). Antioxidants from microalgae and their potential impact on human well-being. *Exploration of Drug Science*, *2*(3), 292-321.

309. Person, J., Lando, D., & Mathieu, D. (2011). Livre turquoise: algues, filières du futur. In *Actes du Colloque Adebiotech, Romainville*.

310. Peter, A. P., Khoo, K. S., Chew, K. W., Ling, T. C., Ho, S. H., Chang, J. S., & Show, P. L. (2021). Microalgae for biofuels, wastewater treatment and environmental monitoring. *Environmental Chemistry Letters*, *19*, 2891-2904.

311. Phang SM. (2002). *Arthrospira* Stizenb. ex Gomont. Plant Resources of South-East Asia 15(1): 88-94.

312. Płaczek, M., Patyna, A., & Witczak, S. (2017). Technical evaluation of photobioreactors for microalgae cultivation. In *E3S web of conferences* (Vol. 19, p. 02032). EDP Sciences.

313. Pozzobon, V., Levasseur, W., & Camarena-Bernard, C. (2024). *Chlorella vulgaris* cold preservation (4∘ C) as a means to stabilize biomass for bioreactor inoculation: A six-month study. *Aquacultural Engineering*, *107*, 102449.

314. Prakash, J., Pushparaj, B., Carlozzi, P., Torzillo, G., Montaini, E., & Materassi, R. (1997). Microalgal biomass drying by a simple solar device∗. *International journal of solar energy*, *18*(4), 303-311.

315. Prasad, R. N., Sanghamitra, K., Antonia, G. M., Juan, G. V., Benjamin, R. G., Luis, I. M. J., & Guillermo, V. V. (2013). Isolation, identification and germplasm preservation of different native Spirulina species from Western Mexico. *American Journal of Plant Sciences*, *2013*.

316. Pratita, A. T. K., Fathurohman, M., Ruswanto, R., & Suhartati, R. (2019, July). Potential of autotroph microalgae (spirulina plantentis) as antimicrobial agent. In *Journal of Physics: Conference Series* (Vol. 1179, No. 1, p. 012173). IOP Publishing.

317. Pratiwy, F. M., Grandiosa, R., & Arifah, F. N. (2021). The enrichment of live feeds: An inquiry for feeding at early stages of fish. *International Journal of Fisheries and Aquatic Studies*, *9*(1), 131-134.

318. Pratt, R., Daniels, T. C., Eiler, J. J., Gunnison, J. B., Kumler, W. D., Oneto, J. F., & Strain, H. H. (1944). Chlorellin, an antibacterial substance from Chlorella. *Science*, *99*(2574), 351-352.

319. Price, K., & Farag, I. H. (2013). Resources conservation in microalgae biodiesel production. International Journal of Engineering and Technical Research, 1(8), 49-56.

320. Prusińska M., Nowosad J., Jarmołowicz S., Mikiewicz M., Duda A., Wiszniewski G., Sikora M., Biegaj M., Samselska A., ArciuchRutkowska M., Targońska K., Otrocka-Domagała I., Kucharczyk D. (2020). Effect of feeding barbel larvae (Barbus barbus (L, 1758)) Artemia sp. nauplii enriched with PUFAs on their growth and survival rate, blood composition, alimentary tract histological structure and body chemical composition. Aquac. Rep., 18: 100492.

321. Qaranjıkı, A., & Kırkağaç, M. (2022). The Evaluation of Different Feeds on Brine Shrimp, Artemia parthenogenetica Culture. *Çanakkale Onsekiz Mart University Journal of Marine Sciences and Fisheries*, *5*(2), 109-118.

322. Qin, L., Qin, Y., Cui, N., Han, X., Lu, H., Yang, T., & Liang, W. (2024). Photosynthetic microalgae microbial fuel cells for bioelectricity generation and microalgae lipid recovery using Gd-Co@ N-CSs/NF as cathode. *Chemical Engineering Journal*, *490*, 151647.

323. Randhawa, S., & Bahna, S. L. (2009). Hypersensitivity reactions to food additives. *Current opinion in allergy and clinical immunology*, *9*(3), 278-283.

324. Rani, A., Saini, K. C., Bast, F., Mehariya, S., Bhatia, S. K., Lavecchia, R., & Zuorro, A. (2021). Microorganisms: a potential source of bioactive molecules for antioxidant applications. *Molecules*, *26*(4), 1142.

325. Rashad, S., A Elchaghaby, M., & A El-Chaghaby, G. (2020). Aquatic microalgae "Anabaena oryzae": phenolic compounds, antioxidant activity and antibacterial activity against Streptococcus mutans oral bacteria. *Egyptian Journal of Aquatic Biology and Fisheries*, *24*(7-Special issue), 203-210.

326. Rawat, I., Kumar, R.R., Mutanda, T., Bux, F., 2011. Dual role of microalgae: phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. Appl. Energy 88 (10), 3411e3424.

327. Razzak, S. A., Bahar, K., Islam, K. O., Haniffa, A. K., Faruque, M. O., Hossain, S. Z., & Hossain, M. M. (2024). Microalgae cultivation in photobioreactors: Sustainable solutions for a greener future. *Green Chemical Engineering*, *5*(4), 418-439.

328. Reed RH, & Stewart WDP. (1985). Osmotic adjustment and organic solute accumulation in unicellular cyanobacteria from freshwater and marine habitats. Marine Biology 88(1): 1-9.

329. Ribeiro, M. C. M., Salles, T. S., Moreira, M. F., Barbarino, E., do Valle, A. F., & Couto, M. A. P. G. (2022). Antiviral activity of microalgae extracts against Mayaro virus. *Algal Research*, *61*, 102577.

330. Romay, C. H., Gonzalez, R., Ledon, N., Remirez, D., & Rimbau, V. (2003). C-phycocyanin: a biliprotein with antioxidant, anti-inflammatory and neuroprotective effects. *Current protein and peptide science*, *4*(3), 207-216.

331. Ronga, D., Biazzi, E., Parati, K., Carminati, D., Carminati, E., & Tava, A. (2019). Microalgal biostimulants and biofertilisers in crop productions. *Agronomy*, *9*(4), 192.

332. Roque, J., Brito, Â., Rocha, M., Pissarra, J., Nunes, T., Bessa, M., ... & Tamagnini, P. (2023). Isolation and characterization of soil cyanobacteria and microalgae and evaluation of their potential as plant biostimulants. *Plant and Soil*, *493*(1), 115-136.

333. Ross, E., & Dominy, W. (1990). The nutritional value of dehydrated, blue-green algae (Spirulina platensis) for poultry. *Poultry Science, 69*(5), 794–800.

334. Roussel, T., Halary, S., Duval, C., Piquet, B., Cadoret, J. P., Vernès, L., Bernard, C., & Marie, B. (2023). Monospecific renaming within the cyanobacterial genus *Limnospira* (*Spirulina*) and consequences for food authorization. *Journal of Applied Microbiology*, *134*(8) :1-15

335. Rout NP, Khandual S, Gutierrez-Mora A, Ibarra-Montoya JL, Vega-Valero G. (2015). Divergence in three newly identified *Arthrospira* species from Mexico. World Journal of Microbiology and Biotechnology 31(7): 1157-1165.

336. Ru, I. T. K., Sung, Y. Y., Jusoh, M., Wahid, M. E. A., & Nagappan, T. (2020). Chlorella vulgaris: A perspective on its potential for combining high biomass with high value bioproducts. *Applied Phycology*, *1*(1), 2-11.

337. Ruiz-Domínguez, M. C., Espinosa, C., Paredes, A., Palma, J., Jaime, C., Vílchez, C., & Cerezal, P. (2019). Determining the potential of *Haematococcus pluvialis* oleoresin as a rich source of antioxidants. *Molecules*, *24*(22), 4073.

338. Rybner, T. V. (2016). Improving the biomass productivity and phycocyanin concentration by mixotrophic cultivation of *Arthrospira platensis* (Doctoral dissertation, Section for Sustainable Biotechnology, Aalborg University Copenhagen).

339. Ryu, B. G., Kim, K., Kim, J., Han, J. I., & Yang, J. W. (2012). Use of organic waste from the brewery industry for high-density cultivation of the docosahexaenoic acid-rich microalga, Aurantiochytrium sp. KRS101. *Bioresource technology*, *129*, 351-359.

340. Ryu, B., Himaya, S.W.A., Kim, S-K.(2015). Applications of Microalgae-Derived Active Ingredients as Cosmeceuticals. In: Kim, S-K (ed.) Handbook of Marine Microalgae, Academic Press, pp. 309-316.

341. Saadaoui, I., Rasheed, R., Aguilar, A., Cherif, M., Al Jabri, H., Sayadi, S., & Manning, S. R. (2021). Microalgal-based feed: promising alternative feedstocks for livestock and poultry production. *Journal of Animal Science and Biotechnology*, *12*(1), 76.

342. Safafar, H., Van Wagenen, J., Møller, P., & Jacobsen, C. (2015). Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. *Marine drugs*, *13*(12), 7339-7356.

343. Sahin, S. C. (2018). The potential of Arthrospira platensis extract as a tyrosinase inhibitor for pharmaceutical or cosmetic applications. *South African Journal of Botany*, *119*, 236-243.

344. Sahle-Demessie, E., Hassan, A. A., & El Badawy, A. (2019). Bio-desalination of brackish and seawater using halophytic algae. *Desalination*, *465*, 104-113.

345. Sahoo, N. K., Gupta, S. K., Rawat, I., Ansari, F. A., Singh, P., Naik, S. N., & Bux, F. (2017). Sustainable dewatering and drying of self-flocculating microalgae and study of cake properties. *Journal of Cleaner Production*, *159*, 248-256.

346. Saide, A., Martínez, K. A., Ianora, A., & Lauritano, C. (2021). Unlocking the health potential of microalgae as sustainable sources of bioactive compounds. *International Journal of Molecular Sciences*, *22*(9), 4383.

347. Saikia, D. K., Ahmed, R., Chikkaputtaiah, C., & Velmurugan, N. (2023). Commercialization of *Haematococcus*-based products: current status and future forecast. In *Haematococcus: Biochemistry, Biotechnology and Biomedical Applications* (pp. 321-337). Singapore: Springer Nature Singapore.

348. Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., & Alqumber, M. A. (2023, January). Antimicrobial resistance: a growing serious threat for global public health. In *Healthcare* (Vol. 11, No. 13, p. 1946). Multidisciplinary Digital Publishing Institute.

349. Salamatullah, A. (2014). Characterization of extraction methods to recover phenolic-rich antioxidants from blue green algae (spirulina) using response surface approaches.

350. Samarakoon, K. W., O-Nam, K., Ko, J. Y., Lee, J. H., Kang, M. C., Kim, D., ... & Jeon, Y. J. (2013). Purification and identification of novel angiotensin-I converting enzyme (ACE) inhibitory peptides from cultured marine microalgae (*Nannochloropsis oculata*) protein hydrolysate. *Journal of Applied Phycology*, *25*, 1595-1606.

351. Sanchez M, Bernal-Castillo J, Rozo C, Rodríguez I. 2003. *Spirulina* (*Arthrospira*): an edible microorganism: a review. Universitas Scientiarum 8(1): 7-24.

352. *Sánchez, J. F., Fernández-Sevilla, J. M., Acién, F. G., Cerón, M. C., Pérez-Parra, J., & Molina-Grima, E. (2008). Biomass and lutein productivity of Scenedesmus almeriensis: influence of irradiance, dilution rate and temperature. *Applied microbiology and biotechnology*, *79*, 719-729.

353. Sangeetha, R., Raj, V. M., & George, S. (2024). An Overview of the Freshwater Cladoceran Daphnia magna (Straus, 1820) and Its Application in Fish Larviculture. *Asian Journal of Fisheries and Aquatic Research*, *26*(12), 180-185.

354. Sankarapandian, V., Nitharsan, K., Parangusadoss, K., Gangadaran, P., Ramani, P., Venmathi Maran, B. A., & Jogalekar, M. P. (2022). Prebiotic potential and value-added products derived from Spirulina laxissima SV001—a step towards healthy living. *BioTech*, *11*(2), 13.

355. Sansone, C., & Brunet, C. (2019). Promises and challenges of microalgal antioxidant production. *Antioxidants*, *8*(7), 199.

356. Santos,, A.C., Morais, F., Simões, A., Pereira, I., Sequeira, J.A.D., Pereira-Silva, M., Veiga, F., Ribeiro, A.(2019). Nanotechnology for the development of new cosmetic formulations. Expert. Opin. Drug. Deliv. 1-36.

357. Santoyo, S., Herrero, M., Senorans, F. J., Cifuentes, A., Ibáñez, E., & Jaime, L. (2006). Functional characterization of pressurized liquid extracts of *Spirulina platensis*. *European Food Research and Technology*, *224*, 75-81.

358. Sarada, D. V., Sreenath Kumar, C., & Rengasamy, R. (2011). Purified C-phycocyanin from *Spirulina platensis* (Nordstedt) Geitler: a novel and potent agent against drug resistant bacteria. *World Journal of Microbiology and Biotechnology*, *27*, 779-783.

359. Sativa, H. A., Faridah, D. N., & Andarwulan, N. (2024). Methods validation and analysis of *Spirulina* (*Arthrospira platensis*) microcystin contaminants by Enzyme-Linked Immunosorbent Assay (ELISA). *Brazilian Journal of Food Technology*, *27*, e2024005.

360. Satriaji, D. E., Zainuri, M., & Widowati, I. (2016). Study of Growth and N, P Content of Microalgae *Chlorella vulgaris* Cultivated in Different Culture Media and Light Intensity. *Jurnal Teknologi (Sciences & Engineering)*, 78(5).

361. Schagerl, M., Kaptejna, A., Polz, F., Ali, S. S., Huo, S., Seneca, J & Hechtl, V. (2025). Testing the Purity *of Limnospira fusiformis* Cultures After Axenicity Treatments. *Cells*, *14*(2), 136.

362. Schmid, B., Navalho, S., Schulze, P. S., Van De Walle, S., Van Royen, G., Schüler, L. M., Maia, I.N., Bastos, C.R.V., Baune, M.C., Januschewski, E., Coelho, A., Pereira, H., Varela, J., Navalho J & Cavaco Rodrigues, A. M. (2022). Drying microalgae using an industrial solar dryer: a biomass quality assessment. *Foods*, *11*(13), 1873

363. Seghiri, R., Kharbach, M., & Essamri, A. (2019). Functional composition, nutritional properties, and biological activities of Moroccan Spirulina microalga. *Journal of Food Quality*, *2019*(1), 3707219.

364. Seixas, P., Coutinho, P., Ferreira, M., & Otero, A. (2009). Nutritional value of the cryptophyte Rhodomonas lens for Artemia sp. *Journal of Experimental Marine Biology and Ecology*, *381*(1), 1-9.

365. Sena, L., Rojas, D., Montiel, E., González, H., Moret, J., & Naranjo, L. (2011). A strategy to obtain axenic cultures of *Arthrospira spp*. cyanobacteria. *World Journal of Microbiology and Biotechnology*, *27*, 1045-1053.

366. Senousy, H. H., Abd Ellatif, S., & Ali, S. (2020). Assessment of the antioxidant and anticancer potential of different isolated strains of cyanobacteria and microalgae from soil and agriculture drain water. *Environmental Science and Pollution Research*, *27*, 18463-18474.

367. Shaaban, M. M. (2001). Nutritional status and growth of maize plants as affected by green microalgae as soil additives.

368. Shaaban, M. M., El-Saady, A. K. M., & El-Sayed, A. B. (2010). Green microalgae water extract and micronutrients foliar application as promoters to nutrient balance and growth of wheat plants. *J. Am. Sci*, *6*(9), 631-636.

369. Shahid, A., Malik, S., Zhu, H., Xu, J., Nawaz, M. Z., Nawaz, S. & Mehmood, M. A. (2020). Cultivating microalgae in wastewater for biomass production, pollutant removal, and atmospheric carbon mitigation; a review. *Science of the Total Environment*, *704*, 135303.

370. Shaima, A. F., Yasin, N. H. M., Ibrahim, N., Takriff, M. S., Gunasekaran, D., & Ismaeel, M. Y. (2022). Unveiling antimicrobial activity of microalgae Chlorella sorokiniana (UKM2), Chlorella sp.(UKM8) and Scenedesmus sp.(UKM9). *Saudi journal of biological sciences*, *29*(2), 1043-1052.

371. Shannon, E., & Abu-Ghannam, N. (2016). Antibacterial derivatives of marine algae: An overview of pharmacological mechanisms and applications. *Marine drugs*, *14*(4), 81.

372. Sharma G, Kumar M, Ali MI, Jasuja ND. (2014). Effect of carbon content, salinity and pH on *Spirulina platensis* for phycocyanin, allophycocyanin and phycoerythrin accumulation. Microbial and Biochemical Technology 6(4): 202-206.

373. Sharma, I., Khare, N., & Rai, A. (2024). Carotenoids: Sources, Bioavailability and Their Role in Human Nutrition. In *Dietary Carotenoids-Sources, Properties, and Role in Human Health*. IntechOpen.

374. Sharma, R., & Rana, A. (2024). Algalelixirs: unraveling the multifaceted impact of spirulina in human health. *Food Bioscience*, 105365.

375. Shen, Y., Yuan, W., Pei, Z. J., Wu, Q., & Mao, E. (2009). Microalgae mass production methods. *Transactions of the ASABE*, *52*(4), 1275-1287.

376. Show KY, Lee DJ, Tay J, Lee T, Chang JS.(2015). Microalgal drying and cell disruption—recent advances. Bioresour Technol 184:258–266

377. Siddhnath, Surasani, V. K. R., Singh, A., Singh, S. M., Hauzoukim, Murthy, L. N., & Baraiya, K. G. (2024). Bioactive compounds from micro-algae and its application in foods: a review. *Discover Food*, *4*(1), 27.

378. Sili C, Torzillo G, Vonshak A. 2012. *Arthrospira* (*Spirulina*). In: Ecology of Cyanobacteria II Springer, Dordrecht. Pp. 677-705.

379. Silva, A. R., Oliveira, R. L., Nascimento, T. V. C., Bezerra, L. R., Ribeiro, C. V. D. M., & Bagaldo, A. R. (2023). *Chlorella vulgaris* supplementation in goat nutrition: Effects on performance, reproduction, and milk quality. *Animals, 13*(5), 745.

380. Silva, M., Geada, P., Pereira, R. N., & Teixeira, J. A. (2025). Microalgae biomass–a source of sustainable dietary bioactive compounds towards improved health and well-being. *Food Chemistry Advances*, 100926.

381. Silva, M., Kamberovic, F., Uota, S. T., Kovan, I. M., Viegas, C. S., Simes, D. C., ... & Barreira, L. (2022). Microalgae as potential sources of bioactive compounds for functional foods and pharmaceuticals. *Applied Sciences*, *12*(12), 5877.

382. Sinetova, M. A., Kupriyanova, E. V., & Los, D. A. (2024). *Spirulina/Arthrospira/Limnospira*—Three names of the single organism. *Foods*, *13*(17), 2762.

383. Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, *16*(3), 144-158.

384. Slama, I., Abdelly, C., Bouchereau, A., Flowers, T., & Savouré, A. (2015). Diversity, distribution and roles of osmoprotective compounds accumulated in halophytes under abiotic stress. *Annals of botany*, *115*(3), 433-447.

385. Solovchenko, A. E., Khozin-Goldberg, I., Didi-Cohen, S., Cohen, Z., & Merzlyak, M. N. (2008). Effects of light intensity and nitrogen starvation on growth, total fatty acids and arachidonic acid in the green microalga Parietochloris incisa. *Journal of applied Phycology*, *20*, 245-251.

386. Soltani, N., Khavari-Nejad, R. A., Tabatabaei Yazdi, M., Shokravi, S., & Fernández-Valiente, E. (2005). Screening of soil cyanobacteria for antifungal and antibacterial activity. *Pharmaceutical biology*, *43*(5), 455-459.

387. Sorgeloos, P., Lavens, P., Leger, P., Tackaert, W., & Versichele, D. (1986). Manual for the culture and use of brine shrimp Artemia in aquaculture.

388. Sousa, V., Hijo, A. A. T., Lüdtke, F. L., Vicente, A. A., Dias, O., & Geada, P. (2024). Recovery and encapsulation of Dunaliella salina β-carotene through a novel sustainable approach: Sequential application of an ionic liquid as naturally-derived solvent and emulsifier. *Food Chemistry*, *458*, 140232.

389. Souza, C. M. M., de Lima, D. C., Bastos, T. S., de Oliveira, S. G., Beirão, B. C. B. & Félix, A. P. (2019). Microalgae *Schizochytrium sp*. as a source of docosahexaenoic acid (DHA): Effects on diet digestibility, oxidation and palatability and on immunity and inflammatory indices in dogs. Animal Science Journal, 90(12), 1567–1574.

390. Spínola, M. P., Mendes, A. R., & Prates, J. A. (2024). Chemical Composition, Bioactivities, and Applications of Spirulina (Limnospira platensis) in Food, Feed, and Medicine. *Foods*, *13*(22), 3656.

391. Spolaore, P., Joannis-Cassan, C., Duran, E. & Isambert, A. (2006). Commercial applications of microalgae. Journal of Bioscience and Bioengineering, 101(2), 87-96.

392. Sruthy, E. S., & Baiju, E. C. K. (2024). Exploration of secondary metabolites from green algae as antimicrobial agents: A comprehensive review. *Botanica Serbica*, *48*(2), 127-140.

393. Stirk, W. A., Bálint, P., Tarkowská, D., Novák, O., Strnad, M., Ördög, V., & Van Staden, J. (2013b). Hormone profiles in microalgae: gibberellins and brassinosteroids. *Plant Physiology and Biochemistry*, *70*, 348-353.

394. Stirk, W. A., Ördög, V., Novák, O., Rolčík, J., Strnad, M., Bálint, P., & van Staden, J. (2013 a). Auxin and cytokinin relationships in 24 microalgal strains1. *Journal of phycology*, *49*(3), 459-467.

395. Stoyneva-Gärtner, M., Uzunov, B., & Gärtner, G. (2024). A Spotlight on the Potential of Microscopic Motile Algae as Novel Sources for Modern Cosmetic Products. *Cosmetics (2079-9284)*, *11*(4).

396. Su, G., Jiao, K., Chang, J., Li, Z., Guo, X., Sun, Y., ... & Lin, L. (2016). Enhancing total fatty acids and arachidonic acid production by the red microalgae Porphyridium purpureum. *Bioresources and Bioprocessing*, *3*, 1-9.

397. Su, M., Bastiaens, L., Verspreet, J., & Hayes, M. (2023). Applications of microalgae in foods, pharma and feeds and their use as fertilizers and biostimulants: Legislation and regulatory aspects for consideration. *Foods*, *12*(20), 3878.

398. Suchithra, M. R., Muniswami, D. M., Sri, M. S., Usha, R., Rasheeq, A. A., Preethi, B. A., & Dineshkumar, R. (2022). Effectiveness of green microalgae as biostimulants and biofertilizer through foliar spray and soil drench method for tomato cultivation. *South African Journal of Botany*, *146*, 740-750.

399. Sudhir P, Murthy SDS. (2004). Effects of salt stress on basic processes of photosynthesis. Photosynthetica 42(4): 481-486.

400. Sudhir PR, Pogoryelov D, Kovács L, Garab G, Murthy SD. (2005). The effects of salt stress on photosynthetic electron transport and thylakoid membrane proteins in the cyanobacterium *Spirulina platensis*. BMB Reports 38(4):481-485.

401. Sukačová, K., Lošák, P., Brummer, V., Máša, V., Vícha, D., & Zavřel, T. (2021). Perspective design of algae photobioreactor for greenhouses—A comparative study. *Energies*, *14*(5), 1338.

402. Sukenik, A. (1999). Production of eicosapentaenoic acid by the marine eustigmatophyte Nannochloropsis. *Chemicals from microalgae*, 41-56.

403. Sukhikh, S., Prosekov, A., Ivanova, S., Maslennikov, P., Andreeva, A., Budenkova, E., & Babich, O. (2022). Identification of metabolites with antibacterial activities by analyzing the FTIR spectra of microalgae. *Life*, *12*(9), 1395.

404. Sulistiyarto, B., & Bakrie, R. (2024). Survival, growth, and biomass of brine shrimp (Artemia franciscana) fed with spirulina powder and soybean flour. International Journal of Fisheries and Aquatic Studies 2024; 12(1): 13-18.

405. Takyar, M. B. T., Khajavi, S. H., & Safari, R. (2019). Evaluation of antioxidant properties of Chlorella vulgaris and Spirulina platensis and their application in order to extend the shelf life of rainbow trout (Oncorhynchus mykiss) fillets during refrigerated storage. *Lwt*, *100*, 244-249.

406. Talebi, A. F., Tabatabaei, M., Mohtashami, S. K., Tohidfar, M., & Moradi, F. (2013). Comparative salt stress study on intracellular ion concentration in marine and salt-adapted freshwater strains of microalgae. *Notulae Scientia Biologicae*, *5*(3), 309-315.

407. Tamura K, Nei M, Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences 101(30): 11030-11035.

408. Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular biology and evolution*, *38*(7), 3022-3027.

409. Tan, J. S., Lee, S. Y., Chew, K. W., Lam, M. K., Lim, J. W., Ho, S. H., & Show, P. L. (2020). A review on microalgae cultivation and harvesting, and their biomass extraction processing using ionic liquids. *Bioengineered*, *11*(1), 116-129.

410. Taniyama, Y., & Griendling, K. K. (2003). Reactive oxygen species in the vasculature: molecular and cellular mechanisms. *Hypertension*, *42*(6), 1075-1081.

411. Tannin-Spitz, T., Bergman, M., Van-Moppes, D., Grossman, S., & Arad, S. (2005). Antioxidant activity of the polysaccharide of the red microalga Porphyridium sp. *Journal of Applied Phycology*, *17*, 215-222.

412. Tarlan, E., Dilek, F. B., & Yetis, U. (2002). Effectiveness of algae in the treatment of a wood-based pulp and paper industry wastewater. *Bioresource technology*, *84*(1), 1-5.

413. Tarraf, S. A., Talaat, I. M., El-Sayed, A. E. K. B., & Balbaa, L. K. (2015). Influence of foliar application of algae extract and amino acids mixture on fenugreek plants in sandy and clay soils. *Nusantara Bioscience*, *7*(1).

414. Tep, B., Sokmen, M., Akpulat, H. A., & Sokmen, A. (2006). Screening of the antioxidant potentials of six Salvia species from Turkey. *Food chemistry*, *95*(2), 200-204.

415. Thajuddin N, Subramanian G. (2005). Cyanobacterial biodiversity and potential applications in biotechnology. Current Science 89(1): 47-57.

416. Thangaraj, M., Saravana, B. P., Thanasekaran, J., Joen-Rong, S., Manubolu, M., & Pathakoti, K. (2022). Phytochemicals of algae, *Arthospira platensis (spirulina) Chlorella vulgaris* (chlorella) and Azolla pinnata (azolla). *GSC Biological and Pharmaceutical Sciences*, *19*(2), 023-043.

417. Thyagarajan, T., Puri, M., Vongsvivut, J., & Barrow, C. J. (2014). Evaluation of bread crumbs as a potential carbon source for the growth of Thraustochytrid species for oil and omega-3 production. *Nutrients*, *6*(5), 2104-2114.

418. Tien, C. J. (2002). Biosorption of metal ions by freshwater algae with different surface characteristics. *Process Biochemistry*, *38*(4), 605-613.

419. Tiong, I. K. R., Nagappan, T., Wahid, M. E. A., Muhammad, T. S. T., Tatsuki, T., Satyantini, W. H., & Sung, Y. Y. (2020). Antioxidant capacity of five microalgae species and their effect on heat shock protein 70 expression in the brine shrimp Artemia. *Aquaculture Reports*, *18*, 100433.

420. Torky, A., Saad, S., & Eltanahy, E. (2023). Microalgae as dietary supplements in tablets, capsules, and powder. In *Handbook of food and feed from microalgae* (pp. 357-369). Academic Press.

421. Toyomizu, M., Sato, K., Taroda, H., Kato, T., & Akiba, Y. (2001). Effects of dietary spirulina on meat color in muscle of broiler chickens. *British Poultry Science, 42*(2), 197–202.

422. Tripathi, S., Srivastava, P., Devi, R. S., & Bhadouria, R. (2020). Influence of synthetic fertilizers and pesticides on soil health and soil microbiology. In *Agrochemicals detection, treatment and remediation* (pp. 25-54). Butterworth-Heinemann.

423. Turcihan, G., Isinibilir, M., Zeybek, Y. G., & Eryalçın, K. M. (2022). Effect of different feeds on reproduction performance, nutritional components and fatty acid composition of cladocer water flea (Daphnia magna). *Aquaculture Research*, *53*(6), 2420-2430.

424. Turcihan, G., Turgay, E., Yardımcı, R. E., & Eryalçın, K. M. (2021). The effect of feeding with different microalgae on survival, growth, and fatty acid composition of Artemia franciscana metanauplii and on predominant bacterial species of the rearing water. *Aquaculture International*, *29*(5), 2223-2241.

425. Turpin, P. J. F. (1827). Spirulina oscillarioide. *Dict. Sci.* **1827**, *50*, 309–310.

426. Uduman, N., Qi, Y., Danquah, M. K., Forde, G. M., & Hoadley, A. (2010). Dewatering of microalgal cultures: a major bottleneck to algae-based fuels. *Journal of renewable and sustainable energy*, *2*(1).

427. Unagul, P., Assantachai, C., Phadungruengluij, S., Suphantharika, M., Tanticharoen, M., & Verduyn, C. (2007). Coconut water as a medium additive for the production of docosahexaenoic acid (C22: 6 n3) by Schizochytrium mangrovei Sk-02. *Bioresource Technology*, *98*(2), 281-287.

428. Usharani, G., Srinivasan, G., Sivasakthi, S., & Saranraj, P. (2015). Antimicrobial activity of Spirulina platensis solvent extracts against pathogenic bacteria and fungi. *Advances in Biological Research*, *9*(5), 292-298.

429. Valderrama, L. T., Del Campo, C. M., Rodriguez, C. M., de-Bashan, L. E., & Bashan, Y. (2002). Treatment of recalcitrant wastewater from ethanol and citric acid production using the microalga Chlorella vulgaris and the macrophyte Lemna minuscula. *Water research*, *36*(17), 4185-4192.

430. Varshney P, Mikulic P, Vonshak A, Beardall J, Wangikar PP. (2015). Extremophilic micro-algae and their potential contribution in biotechnology. Bioresource Technology 184: 363-372.

431. Velazquez-Lucio, J., Rodríguez-Jasso, R. M., Colla, L. M., Sáenz-Galindo, A., Cervantes-Cisneros, D. E., Aguilar, C. N., & Ruiz, H. A. (2018). Microalgal biomass pretreatment for bioethanol production: a review.

432. Velioglu, Y., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of agricultural and food chemistry*, *46*(10), 4113-4117.

433. Vieira, M. V., Pastrana, L. M., & Fuciños, P. (2020). Microalgae encapsulation systems for food, pharmaceutical and cosmetics applications. *Marine drugs*, *18*(12), 644.

434. Vijayaram, S., Ringø, E., Ghafarifarsani, H., Hoseinifar, S. H., Ahani, S., & Chou, C. C. (2024). Use of algae in aquaculture: a review. *Fishes*, *9*(2), 63.

435. Vikneshan, M., Saravanakumar, R., Mangaiyarkarasi, R., Rajeshkumar, S., Samuel, S. R., Suganya, M., & Baskar, G. (2020). Algal biomass as a source for novel oral nano-antimicrobial agent. *Saudi Journal of Biological Sciences*, *27*(12), 3753-3758.

436. Visuddho, V., Halim, P., Helen, H., Muhar, A. M., Iqhrammullah, M., Mayulu, N., & Nurkolis, F. (2024). Modulation of apoptotic, cell cycle, DNA repair, and senescence pathways by marine algae peptides in cancer therapy. *Marine Drugs*, *22*(8).

437. Von Alvensleben, N., Stookey, K., Magnusson, M., & Heimann, K. (2013). Salinity tolerance of Picochlorum atomus and the use of salinity for contamination control by the freshwater cyanobacterium Pseudanabaena limnetica. *PloS one*, *8*(5), e63569.

438. Von Lintig, J., Hessel, S., Isken, A., Kiefer, C., Lampert, J. M., Voolstra, O., & Vogt, K. (2005). Towards a better understanding of carotenoid metabolism in animals. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1740*(2), 122-131.

439. Vornoli, A., Grande, T., Lubrano, V., Vizzarri, F., Gorelli, C., Raffaelli, A., ... & Echeverria, C. (2023). In vitro characterization of antioxidant, antibacterial and antimutagenic activities of the green microalga Ettlia pseudoalveolaris. *Antioxidants*, *12*(6), 1308.

440. Vuorela, S. (2005). *Analysis, isolation, and bioactivities of rapeseed phenolics* (Doctoral dissertation, Helsingin yliopisto).

441. Wan Afifudeen, C. L., Teh, K. Y., & Cha, T. S. (2022). Bioprospecting of microalgae metabolites against cytokine storm syndrome during COVID-19. *Molecular Biology Reports*, *49*(2), 1475-1490.

442. Wan, X., Li, X., Liu, D., Gao, X., Chen, Y., Chen, Z., & Zhao, C. (2021). Physicochemical characterization and antioxidant effects of green microalga *Chlorella pyrenoidosa* polysaccharide by regulation of microRNAs and gut microbiota in Caenorhabditis elegans. *International Journal of Biological Macromolecules*, *168*, 152-162.

443. Wang, B., Lan, C. Q., & Horsman, M. (2012). Closed photobioreactors for production of microalgal biomasses. *Biotechnology advances*, *30*(4), 904-912.

444. Wang, F., Cao, Y., Guo, Y., Zhu, Z., & Zhang, C. (2021). Evaluation of antioxidant and antibacterial activities of lipid extracts from Eustigmatos cf. polyphem (Eustigmatophyceae) and preliminary identification of bioactive compound. *Algal Research*, *59*, 102446.

445. Wang, T., Ge, H., Liu, T., Tian, X., Wang, Z., Guo, M., & Zhuang, Y. (2016). Salt stress induced lipid accumulation in heterotrophic culture cells of *Chlorella protothecoides*: mechanisms based on the multi-level analysis of oxidative response, key enzyme activity and biochemical alteration. *Journal of biotechnology*, *228*, 18-27.

446. Wang, X., & Zhang, X. (2013). Separation, antitumor activities, and encapsulation of polypeptide from Chlorella pyrenoidosa. *Biotechnology progress*, *29*(3), 681-687.

447. Wellburn AR. (1994). The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Journal of Plant Physiology 144(3): 307-313.

448. Williams-Nguyen, J., Sallach, J. B., Bartelt-Hunt, S., Boxall, A. B., Durso, L. M., McLain, J. E., & Zilles, J. L. (2016). Antibiotics and antibiotic resistance in agroecosystems: state of the science. *Journal of environmental quality*, *45*(2), 394-406.!

449. Williamson, C. J., Cook, J., Tedstone, A., Yallop, M., McCutcheon, J., Poniecka, E., & Anesio, A. (2020). Algal photophysiology drives darkening and melt of the Greenland Ice Sheet. *Proceedings of the National Academy of Sciences*, *117*(11), 5694-5705.

450. Winkel, M., Trivedi, C. B., Mourot, R., Bradley, J. A., Vieth-Hillebrand, A., & Benning, L. G. (2022). Seasonality of glacial snow and ice microbial communities. *Frontiers in microbiology*, *13*, 876848.

451. Wittrock VB, Nordstedt CFO (1884) Algae aquae dulcia exiccata . Fasc 14, 679:59

452. Wong, C. Z. E., Teoh, M. L., & Chan, S. W. (2023). Chlorella across latitudes: investigating biochemical composition and antioxidant activities for biotechnological applications. *Adv Polar Sci*, *34*(4), 340-351.

453. Wu, F., Zhang, Y., Gu, J. D., He, D., Zhang, G., Liu, X., & Feng, H. (2022). Community assembly, potential functions and interactions between fungi and microalgae associated with biodeterioration of sandstone at the Beishiku Temple in Northwest China. *Science of The Total Environment*, *835*, 155372.

454. Yadavalli, R., Peasari, J. R., Mamindla, P., Mounika, S., & Ganugapati, J. (2018). Phytochemical screening and in silico studies of flavonoids from *Chlorella pyrenoidosa*. *Informatics in Medicine Unlocked*, *10*, 89-99.

455. Yan, C., & Zheng, Z. (2013). Performance of photoperiod and light intensity on biogas upgrade and biogas effluent nutrient reduction by the microalgae *Chlorella sp*. *Bioresource Technology*, *139*, 292-299.

456. Yan, N., Zhao, J., Xiong, K., Yang, C., Li, J., & Chen, Q. (2024). Terrestrial algae: pioneer organisms of carbonate rock solutional weathering in South China karst. *Frontiers in Microbiology*, *15*, 1329695.

457. Yang, N., Zhang, Q., Chen, J., Wu, S., Chen, R., Yao, L., ... & Zhang, Z. (2023). Study on bioactive compounds of microalgae as antioxidants in a bibliometric analysis and visualization perspective. *Frontiers in plant science*, *14*, 1144326.

458. Yarkent, Ç., Gürlek, C., Oncel, S.S. (2020). Potential of microalgal compounds in trending natural cosmetics: A review. Sustain. Chem. Pharm. 17, 100304.

459. Yewalkar, S. N., Dhumal, K. N., & Sainis, J. K. (2007). Chromium (VI)-reducing Chlorella spp. isolated from disposal sites of paper-pulp and electroplating industry. *Journal of Applied Phycology*, *19*, 459-465.

460. Yoshie-Stark, Y., Hsieh, Y. P., & Suzuki, T. (2003). Distribution of flavonoids and related compounds from seaweeds in Japan. *Journal-Tokyo University of Fisheries*, *89*, 1-6.

461. Zainoddin, H. A. H., Hamzah, A., Jamari, Z., & Omar, W. A. W. (2018). Chemical profiles of methanolic extracts from two species of microalgae, *Nannochloropsis sp*. and *Spirulina sp*. *Pertanika Journal of Tropical Agricultural Science*, *41*(3).

462. Zeng, M.T. & Vonshak, A. (1998). Adaptation of *Spirulina platensis* to salinity-stress. Comp. Biochem. Physiol. A, 120, 113–118.

463. Zheng, J., Inoguchi, T., Sasaki, S., Maeda, Y., McCarty, M. F., Fujii, M., & Takayanagi, R. (2013). Phycocyanin and phycocyanobilin from Spirulina platensis protect against diabetic nephropathy by inhibiting oxidative stress. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *304*(2), R110-R120.

464. Zeybek, Y. G., Sayar, M., & Eryalçın, K. M. (2023). Effect of microalgal based diets on growth, proximate, fatty acid and amino acid composition of water flea (*Daphnia magna*).

465. Zhang, B. Y., Geng, Y. H., Li, Z. K., Hu, H. J., & Li, Y. G. (2009). Production of astaxanthin from Haematococcus in open pond by two-stage growth one-step process. *Aquaculture*, *295*(3-4), 275-281.

466. Zielinski, D., Fraczyk, J., Debowski, M., Zielinski, M., Kaminski, Z. J., Kregiel, D., & Kolesinska, B. (2020). Biological activity of hydrophilic extract of *Chlorella vulgaris* grown on post-fermentation leachate from a biogas plant supplied with stillage and maize silage. *Molecules*, *25*(8), 1790.

467. Zitelli GC, Rodolfi L, Biondi N, Tredici MR (2006) Productivity and photosynthetic efficiency of outdoor cultures of *Tetraselmis suecica* in annular columns. Aquaculture 261: 932-943.

468. Zmora, O., Grosse, D. J., Zou, N., & Samocha, T. M. (2013). Microalga for aquaculture: practical implications. *Handbook of microalgal culture: Applied phycology and biotechnology*, 628-652.

469. Zohir, W. F., Makhlof, M. E., Abdallah, A. M., & El-Sheekh, M. M. (2023). Algae Cultivation Systems. In *Value-added Products from Algae: Phycochemical Production and Applications* (pp. 11-41). Cham: Springer International Publishing.

470. Zou, S., Fei, C., Song, J., Bao, Y., He, M., & Wang, C. (2016). Combining and comparing coalescent, distance and character-based approaches for barcoding microalgaes: a test with Chlorella-like species (Chlorophyta). *PLoS One*, *11*(4), e0153833.

471. Zuccaro, G., Yousuf, A., Pollio, A., & Steyer, J. P. (2020). Microalgae cultivation systems. *Microalgae cultivation for biofuels production*, 11-29.

Appendix

Appendix 1: Culture Medium (BG11)

BG11 medium (containing the following nutrients in g/l: $NaNO_3$, 1.5; K_2HPO_4, 0.04; $MgSO_4.7H_2O$, 0.075; $CaCl_2.2H_2O$, 0.036; citric acid, 0.006; ferric ammonium citrate, 0.006; Na_2EDTA, 0.001; Na_2CO_3, 0.02; and 1 ml of trace element mix was added (containing in g/l: H_3BO_3, 2.86; $MnCl_2.4H_2O$, 1.81; $ZnSO_4.7H_2O$, 0.222; $NaMoO_4.2H_2O$, 0.39; $CuSO_4.5H_2O$, 0.079; $Co(NO_3)_2.6H_2O$, 0.0494), NaCl, NaOH, Agar Agar.

Appendix 2: Table polyphenol standards used for HPLC analysis

	Standards numbers	Classe	Retention time
1	Ascorbic acid	Phenolic acid	3.660
2	Tannic acid	Phenolic acid	6.62
3	Protocatechuic acid	Phenolic acid	8.547
4	Galic acid	Phenolic acid	10.343
5	Robinin	Flavanol	17.3
6	Catechin	Flavanol	17.887
7	Rutin	Flavonol	17.970
8	Lutéolin3'-7 diglucoside	Flavone	24.253
9	Vanillic acid	Phenolic acid	24.710
10	Cafeic acid	Phenolic acid	24.832
11	Naringenin-7-glucoside	Flavanone	24.972
12	Apegin	Flavones	25.243
13	*Luteolin* 7,3'-*diglucoside*	Flavone	26.116
14	Isorhamnetin-3-rutinoside	Hydroxy-flavone	28.052
15	*isorhamnetin-3-glucoside*	Hydroxy-flavone	28.120
16	Hesperidin	Flavanone	28.284
17	*Apigenin 7-glucoside*	Flavone	29.207
18	Xanthotoxin	Furocoumarine	31.72
19	Hespertine	Flavanone	30.047
20	Naringenin	Flavanone	32.461
21	Luteolin	Flavone	33.53
22	Myricetin	Flavonol	33.853
23	Quercetin	Flavonol	35.703
24	penta-hydroxyflavone	Flavone	36.203
25	Morin	Flavonol	36.5
26	Rhamnetin	Flavonol	36.818
27	Galangin	Flavonol	47.293
28	Acacetin	Flavone	49.153
29	Chrysin	Flavone	49.253
30	Kaempferol	Flavonol	49.303
31	Kaempferide	Flavone	49.3
32	Diosmin	Flavone	49.770
33	Tangeretin	Flavone	51.703
34	3-Hydro-flavon	Flavone	52.220
35	5-Hydroxy-flavon	Flavone	54.243

1. Publication internationale

- **Guenachi, B.,** Korteby Mefti, H., Benfares, R., Abderrahmani, K., Boudjema, K., Achour, H. Y., Toumatia ,O., Azmane,B.,& Lamari, L. (2025). Morphological and molecular identification of a new halotolerant cyanobacterial strain, Limnospira fusiformis TL03, isolated from Telamine Lake in the northwest of Algeria. *Acta Botanica Brasilica*, *39*, e20230137.
- **Guenachi, B.,** Korteby, Mefti , H., & Lamari, L. (2024). Phenolic content, antioxidant, and antibacterial activities of hydromethanolic extract of *Limnospira fusiformis* TL03 isolated from Telamine Lake, northwest Algeria. *Bulletin of Pharmaceutical Sciences Assiut University*, *47*(2), 899-916.

2. International communication

- **B. Guenachi**, O. IAZZOUGUENE , H. Korteby Mefti,L. Lamari. Poster. Testing of an extract of Arthrospira platensis (Spirulina isolated from Tamanrasset region) against some microbial activities. International Agricultural, Biological & Life Science Conference 17-20 September, 2023, Edirne, Turkey.
- **B. Guenachi**[1,2], H. Korteby Mefti[2], A. Zitouni[3], L. Lamari3. Poster. effect of different concentration of sodium chlorid on spirulina growth. (1) Laboratory of Research of Aromatic and Medicinal Plants, Blida 1 University, Algeria. (2) National Centre for Research and Development of Fisheries and Aquaculture (CNRDPA), Bou Ismail, Algeria. (3) Laboratoire de Biologie des Systèmes Microbiens, Ecole Normale Supérieure de Kouba, Algeria. Biotechnology and green economy. *1st International Symposium 'Environment & Sustainable Development*, Relizane, Algeria, 10-11 February 2020.
- **B. Guenachi**[1, 2].*, H. Korteby Mefti[1],A. Zitouni[3], L. Lamari[3].EFFECT OF DIFFERENT CONCENTRATION OF SODIUM CHLORID ON CHLOROPHYLL AND CAROTENOIDS OF SPIRULINA. poster (1) Blida 1 University, Laboratory of Research of Aromatic and Medicinal Plants, Faculty of natural sciences and life, B.P 270 Soumaa's Road,Blida,Algeria.(2) National Centre for Research and Development of Fisheries and Aquaculture (CNRDPA), Bou Ismail, Algeria.(3) Laboratoire de Biologie des Systèmes Microbiens, Ecole Normale Supérieure de Kouba, Alegria.Extremophilic Prokaryotes: From diversity to biotechnology.Tlemcen, Algeria,23rd -24th November 2019.
- **B. Guenachi**[1,2], H. Korteby Mefti[2], N. Sabaou[3], A. Zitouni[3], L. Lamari[3]. Poster (Isolation of some microalgae from certain water bodies of Algeria).poster. (1) Laboratory of Research of Aromatic and Medicinal Plants, Blida 1 University, Algeria. (2) National Centre for Research and Development of Fisheries and Aquaculture (CNRDPA), Bou Ismail, Algeria. (3) Laboratoire de Biologie des Systèmes Microbiens, Ecole Normale Supérieure de Kouba, Algeria. IAC- 2018-3rd Africa-International Allelopathy Congress November, 24th-26th, 2018 Blida-Algeria.

3. National and international communication

- **B. Guenachi**, O. IAZZOUGUENE , H. Korteby Mefti,L. Lamari. poster. Antimicrobial activities of extracts of a microalgae Arthrospira Arthrospira (Spirulina) isolated from the Tamanrasset region. 4th Conference on the Biology of Microbial Systems, to be held on December 3, 2022, at the "Ecole Normale Supérieure El Cheikh Mohamed El Bachir El Ibrahimi (ENS-Kouba)", Algiers, Algeria.

- **B. Guenachi**[1,2], H. Korteby Mefti[2], N. Sabaou[3], A. Zitouni[3], L. Lamari[3]. Poster EFFECT OF DIFFERENT CONCENTRATION OF SODIUM NITRATE AND SODIUM BICARBONATE ON SPIRULINA GROWTH. (1) Laboratory of Research of Aromatic and Medicinal Plants, Blida 1 University, Algeria. (2) National Centre for Research and Development of Fisheries and Aquaculture (CNRDPA), Bou Ismail, Algeria. (3) Laboratoire de Biologie des Systèmes Microbiens, Ecole Normale Supérieure de Kouba, Algeria. 1 er Symposium National Biomolécules & Biotechnologies& 1 ères Doctoriales LRPMA 18th-19th, 2019 Blida-Algeria.

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PHENOLIC CONTENT, ANTIOXIDANT, AND ANTIBACTERIAL ACTIVITIES OF HYDROMETHANOLIC EXTRACT OF *LIMNOSPIRA FUSIFORMIS* TL03 ISOLATED FROM TELAMINE LAKE, NORTHWEST ALGERIA

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This study was conducted to evaluate the total phenolic, flavonoid content, antioxidant, and antibacterial activity of hydomethanolic Limnospira fusiformis TL03 .

The total phenolics and flavonoids were determined by using the Folin-Ciocalteu and aluminum chloride methods, respectively. The YL 9100 HPLC was used to identify the phenolic compounds.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging activity and beta-carotene-linoleic acid tests were used to evaluate antioxidant activity in vitro. The antimicrobial activities were tested against six bacteria using the disc diffusion method and the agar dilution method.

The total phenolic and flavonoids contained in the hydromethanolic extract were 18.41 ±0.18 mg GAE/g DW and 2.11 ±0.12 mg quercetin/g DW, respectively. The result of polyphenol analysis showed the presence of: Ascorbic acid, Gallic acid, Luteolin , Quercetine, Kaempferol.

This extract shows strong DPPH radical scavenging and β-carotene bleaching activities with a percentage of 86.19±1.61% and 85.87±1.65%, respectively, in addition to activity antibacterial, in which the zone inhibition diameters (ZID) range from 33.33±1.53 to 11±1 mm, with the higher value antibacterial activity (33.33±1.53 mm) registered against S. aureus and the lower minimum inhibitory concentrations (MIC) observed against K pneumonia (0.78 mg/ml). In conclusion, it was revealed that the hydromethanolic extract of L. fusiformis TL03. has significant antioxidant and antibacterial properties

Keyswords: *Limnospira fusiformis TL03, Total phenolic and flavonoid, HPLC, DPPH, Beta carotene, Antibacterial activity*

INTRODUCTION

There is growing interest in the global health community about oxidative stress and the spread of infectious diseases. The oxidative stress, caused by a variety of stressors and free radical inducers, may be responsible, to varying degrees, for the onset and/or development of many diseases, including cancer, diabetes, metabolic disorders, atherosclerosis, and cardiovascular disease [1]. The treatment of oxidative stress through the long-term use of synthetic antioxidants may cause some health problems, such as skin allergies, digestive problems, and, in some cases, an increased risk of cancer[2-6]. The global outbreak of infectious bacterial diseases with the widespread prevalence of antibiotic-resistant bacteria poses another threat to human and animal health.

Plants are a sustainable source of bioactive substances that have been used as

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folk medicines by humans since ancient times [7,8]to treat various diseases, and also their diversity of chemical compounds has been used in the production of various drugs. Microalgae and Cyanobacteria are also valuable sources of bioactive compounds, with a tenfold greater diversity of bioactive substances compared to land plants[9].

In the recent years, there has been an increased interest in microalgae and cyanobacteria research due to their wide biotechnological applications including, wastewater treatment, carbon dioxide fixation, production of biodiesel and methane[10-16]. In addition in agriculture as biofertilizers[17, 18] and biostimulants[19].

Cyanobacterium (Limnospira and / or Arthrospira), commercially known as Spirulina[20-22], is one of the most produced microalgae and cyanobacteria worldwide[23;] about 30% of the world global algal biomass production comes from the genus *Arthrospira*[24]. The global production was 89,000 tons in 2016 (FAO, 2018)[25]. Spirulina has been consumed by humans since ancient civilizations such as the Aztecs, who were the first to realize the nutritional value of these organisms[26 -27] and is still traditionally consumed in Africa, near Lake Chad [28].

Spirulina have high nutritional value[29]; it contains a high amount of proteins with all essential amino acids[30-35] and essential fatty acids, minerals, pigments, vitamins, and phenolic compounds. It was proven to be used as a food supplement, animal feed, cosmetics, and natural coloring ; food colors [36-42] .Furthermore, the biological and therapeutic study of *spirulina* has also received a lot of attention due to the fact that it contains many bioactive compounds with therapeutic properties[27,41, 43, 45- 51] including weight control, intestinal flora, anticancer and immune modulating activities, Anti-inflammatory, antioxidant, antiviral, and antibacterial[41,52, 53].

In this paper, the first study on the biological activity of *Limnospira fusiformis* TL03, recently isolated from Telamine Lake in the northwest of Algeria. Therefore, the current study was carried out to assess the antioxidant activities of *Limnospira fusiformis* TL03 hydromethanolic extract and the characterization of its polyphenolic compounds in addition to evaluate its antimicrobial activity using agar well-diffusion assay and minimum inhibition concentration (MIC) against selected Gram positive and Gram negative bacteria.

MATERIAL AND METHODS

Chemicals Carrageenan, linoleic acid, ascorbic acid, butylated hydroxytoluene, Tween 40, potassium ferricyanide, DPPH, Ferric chloride, β-carotene, Methanol, Galic acid, Rutin, Vanillic acid, Flavone, Galangin, Caffeine, Stearic acid, Tannic acid, Myricetin, Alpha-tocopherol, Morin, Apigenin, Diosmin, Catechin, Tangeretin, 5-Hydroxy-flavon, Chrysin, Kaemferide, Robinin, 3-Hydroxy-flavon, Acacetin, Hesperetin, Quercetin, 6.methoxyflavone, Aspirin, Acetic acid used in the experiment were procured from Sigma (Sigma-Aldrich, Germany).

Biological material

Cyanobacterium strain: The cyanobacterium *Limnospira. fusiformis* TL03 (accession number MZ215991.1) was isolated from Telamine Lake in northwest Algeria.

L. fusiformis TL03 Culture conditions

L. fusiformis TL03 was cultured in Bleu Green medium BG11[54], where the pH was adjusted to 9.5 and the medium was autoclaved at 121 °C for 20 min.

The incubation was performed under controlled conditions: a temperature of 30 ± 2 °C and continuous light of 2.7 Klux provided by white fluorescent tubes. The mixing of the cells was carried out by aeration pump in order to keep the cells in suspension and to avoid their agglomeration. After incubating for 20 days, the cells were harvested, washed with distilled water, and dried using a lyophilizer. (ALPHA 1- 2 LD plus).

Bacterial strains

The tested bacteria were provided by the Microbial Systems Biology Laboratory, Kouba Higher Normal School of Kouba, Algeria. The antibacterial activity of hydromethanolic extracts of *L. fusiformis* TL03 was tested against six strains of pathogenic bacteria **(Table 1)**.

Table 1 : Tested bacterial strains.

Bacteria	Strains	Origin
Bacteria gram positive	*Bacillus subtilis* (*B. subtilis*)	ATCC 6633
	Staphylococcus aureus (*S. aureus*)	CIP 7625
	Listeria monocytogenes (*L. monocytogenes*)	CIP 82110
Bacteria gram negative	*Escherichia coli* (*E. coli*)	ATCC 10536
	Pseudomonas aeruginosa(*P. aeruginosa*)	CIP A22
	Klepseilla pneumonia (*K. pneumonia*)	CIP 82.91

ATCC : American Type Culture Collection, CIP : Collection de l'Institut Pasteur (Institut Pasteur Collection).

Extract preparation

A crude hydromethanolic extract of *L. fusiformis* TL03 was prepared by macerating 2 g of freeze-dried algae in 100 mL of methanol/water (7 v: 3 v). The mixture was kept at room temperature in the dark for 24 hours and then filtered through Wathman No. 1 paper. These procedures were repeated three times to extract the maximum amount of compounds. The three filtrates were collected in an opaque glass vial and then dried at 40 °C using a rotary vacuum evaporator (Buchi R-210). The dried extracts were stored at 4°C in the dark until use.

Total phenolic assay

The total phenolic content of the extract was assessed using spectrophotometric methods[55] based on the Folin-Ciocalteu reagent. 3.9 ml of distilled water was combined with 0.1 ml of diluted hydromethanolic crude extract (10 mg/mL), and the mixture was thoroughly mixed with 0.25 mL of Folin-Ciocalteu reagent for 3 min. Then, 0.75 mL of 20% (w/v) sodium carbonate (Na_2CO_3) was added to the mixture. After thorough mixing, samples were incubated in a water bath at 40 °C for 40 min. The absorbance was measured at 765 nm against a blank sample consisting of distilled water instead of the diluted sample. The total phenolic content of the extracts was determined and expressed as mg of Gallic acid equivalents (GAE) per gram of sample in dry weight (mg GEA/g) using the regression equation of the calibration curve. The analysis was conducted three times.

Equation: y = 0.0795X - 0.1033

Total flavonoid content

The total content of flavonoids in the crude extract was determined by the aluminum chloride colorimetric method [56] Briefly, 0.75 mL of hydromethanolic crude extract (10 mg/mL) was made up to 1.5 mL with 2% $AlCl_3$ solution methanol. The mixture was well mixed before incubation at room temperature in the dark for 10 minutes. The mixture absorbance was measured at 440 nm against the standard containing a diluted solution of $AlCl_3$.

A calibration curve for quercetin was used to determine the total flavonoid concentration and the result was expressed as milligram equivalents of quercetin per gram of dry microalgae (mg QE/g). Equation: Y = 0.5658X – 0.1155

Polyphenol analysis

The HPLC analysis of crude hydromethanolic extract of *L. fusiformis* TL03 was performed using a Young Lin YL 9100 HPLC chromatograph with a UV-Vis detector (YL9120). The separation was achieved by a reversed-phase Agilent XDB Eclipse C8 column (250 mm/4.6 mm/5 m). The mobile phase was bi-distilled water enriched with 0.1 % acetic acid (Eluent A) and gradient methanol (Eluent B). The flow rate was adjusted at 1 mL/min, the temperature was regulated at 30 °C, and the injection volume was 20 µL. The HPLC gradient elutiens were as follows: 0–50 min, 95% elution A and 5% elution B; 50–60 min, 5% A and 95% elution B. The chromatograms were recorded at 254 and 280 nm.

Each compound of phenolic acids and flavonoids contained in hydromethanolic extract was identified by comparing its

retention time (Rt) and the UV spectra to those of the following standards (Galic acid, Rutin, Vanillic acid, Ascorbic acid, Flavone, Galangin Caffeine, Stearic acid, Tannic acid, Myricetin, Alpha-tocopherol, Morin, Apigenin, Diosmin, Catechin, Tangeretin**,** 5-Hydroxy-flavon, Chrysin, Kaemferide, Robinin, 3-Hydro-flavon, Acacetin, Hesperetin, Quercetin, and 6.methoxy-flavon, Xanthotoxine, Luteolin, Kaempferol)

Antioxidant Activity

DPPH radical scavenging activity

The DPPH assay was performed according to Tep [57], the Crude hydromethanolic extracts (10 mg/ml) were prepared in a series of dilutions (0.125, 0.250, 0.5, 1, 2, 3, 4, and 5 mg/ml) by adding methanol.

An equal volume of DPPH solution dissolved in methanol (0.004%) was added to 1.5 ml of each diluted test extract (0.125 to 5 mg/ml). After vigorous mixing for 10 seconds, the solution was incubated at room temperature in the dark for 30 minutes. The absorbance was read at a wavelength of 517 nm. Reference standards of BHT, Ascorbic acid, Trolox, Vitamin E were used as benchmarks.

All samples were performed in triplicate.

The following formula was used to determine the percentage of free radical DPPH inhibition

$$(I\ \%) = [(Ac - As)/ (Ac)] \times 100\ [Tep]^{57.}$$

Where As is the sample absorbance and Ac represents the absorbance of the control reaction, which uses all the reagents except the test substance.

The IC50 value was used to determine the antiradical activity of the samples; this value is the concentration of the sample required for 50% inhibition of DPPH radicals

Beta-carotene determination

Two mg of beta-carotene was dissolved in 10 ml of chloroform, and 2 ml of this solution was added to a vial previously containing 40 mg of linoleic acid and 400 mg of Tween 80. The mixture was agitated, and then the chloroform was completely evaporated using a rotary vacuum evaporator. Afterward, 100 ml of distilled water was added to the residue. After thoroughly mixing, a volume of 4.8 mL of the obtained emulsion was transferred to test tubes, each one containing 0.2 mL of the extract (10 mg/mL) or the same volume of control antioxidants (BHT).

The tubes were placed in a water bath at 50 °C and the absorbance was measured at 470 nm at intervals of 30, 60, 90 and 120 minutes. Samples were performed in triplicate

The bleaching rate of beta-carotene (R) is calculated according to Al-Shaikhan[58] as follows:

$$Rt = In\ (Abst0/Abst)/t$$

Rt: the bleaching rate of beta-carotene at times (30, 60, 90, and 120 minutes); In: natural logarithm; Abst0 corresponds to the initial absorbance of the emulsion immediately after sample preparation (t = 0 minutes); Abst: the absorbance of the emulsion at times (30, 60, 90, and 120 minutes). The percentage of antioxidant activity is calculated using the following equation:

$$\text{Antioxidant activity \%} = [(\text{R control} - \text{R sample})/\text{R control}] \times 100.$$

R control and R sample are the average rates of control and sample (extract) bleaching, respectively.

Antibacterial activity test

Two methods were used to evaluate the antimicrobial activity of crude hydromethanolic extract of L. fusiformis TL03: disc diffusion and *agar dilution method*

Disc diffusion method

Disc diffusion preparation

The disc diffusion technique was used to assess the antibacterial activity of the hydromethanolic extract of *L fusiformus* TL03. Sterile Whatman No. 1 discs (5.5 mm in diameter) were impregnated with 10 µl of hydromethanolic extracts (50 mg/ml).

Ampicillin (1 mg/mL) was used as a control

The tested bacterial strains were plated individually on nutrient agar. After 18 hours of incubation at 35°C, the colonies were transferred to saline (0.9% NaCl) to obtain a suspension turbidity equivalent to 1.5 x 108 CFU/mL [Alagawany] [59]

One hundred microliters of bacterial aliquots were spread on the surface of Mueller-

Hinton agar (MHA) Petri dishes and then, three sterilized dried paper discs containing crude hydromethanolic extract or control (ampicillin) were placed equidistantly on the surface of agar Petri dishes (90 mm diameter) for each bacterial strain tested. Prior to incubation, the Petri dishes were placed in the dark at 4°C for 1 hour to allow the hydromethanolic extract and the ampicillin to spread. Then the plates were incubated at 37 °C for 24 h, and the inhibition zones diameters (mm) around the discs were measured by the transparent ruler.

***Agar dilution method:*Minimum inhibition concentration (MIC)**

From the initial concentration of crude hydromethanolic extract (50 mg/ml), a series of double dilutions (25, 12.5, 6.25, 3.125, 1.56, 0.78 mg/ml) were prepared by addition to Mueller-Hinton broth. The control was prepared using the same method as described above with double dilution of ampicillin (0.5, 0.25, 0.125, 0.0625, 0.032, and 0.016 mg/mL).

One hundred µL of each tested bacterial cell suspension (1.5 108 CFU/ml) was placed separately on the Petri agar surface. All plates were incubated at 37 °C for 24 hours.

The MIC values were defined as the lowest concentration of antibacterial agents that inhibited the growth of bacteria [Ashour] [60]

Statistical analysis

The statistical analysis was conducted using IBM SPSS 25.0 software. The data were presented as means ± standard error (S.D.). The differences between groups were analyzed using a one-way analysis of variance (ANOVA) test, followed by the Tukey test.

RESULTS AND DISCUSSION

Results

Quantification of TPC and TFC

This study showed that the hydromethanol extract of *L fusiformis* TL03 contained a high total phenolic and flavonoid. The total phenolic and flavonoid compounds were 18.41±0.18 GAE mg/g and 2.11±0.12 QE mg/g DW, respectively.

Polyphenol analysis

The phenolic compounds of the hydromethanolic extract of *L. fusiformis* TL03 were investigated by HPLC chromatography.

The phenolic compounds contained in the extract were identified by comparing the retention time and UV spectra of the peaks obtained from the extract with those of the standards.

The results of HPLC analysis of the crude extract showed the presence of seven compounds including: Ascorbic acid, Gallic acid, Luteolin, Quercetin, Kaempferol. **(Fig. 1, Table 2)**.

Antioxidants activities

In this study, two assays (DPPH and beta-carotene/linoleic acid test) were used for the evaluation of the antioxidant activity of the hydromethanolic extract of L. fusiformis TL03 and the results are shown in **Table (3)**.

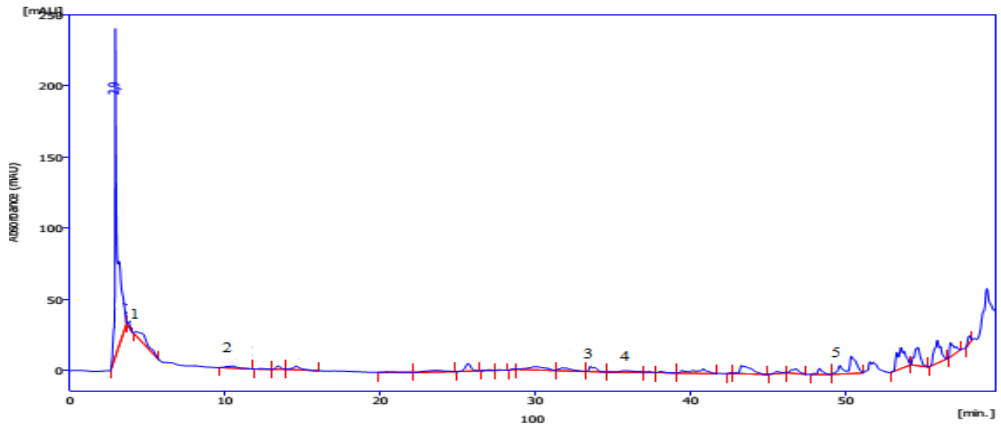

Fig.1: HPLC chromatogram of *L. fusiformis* TL03 hydromethanolic extracts. Phenolic compounds identified: (1): Ascorbic acid, (2): Gallic acid, (3): Luteolin, (4): Quercetine, (5): Kaempferol.

Table 2: The phenolic compounds identified by HPLC-DAD in hydromethanolic extract of *L. fusiformis* TL03.

N0	Compound	Area	Retention time	Nature of compound
1	Ascorbic acid	0.5	3.66	Vitamine C
2	Gallic acid	1	10.34	phenolic acid.
3	Luteolin	1.6	33.53	Flavon
4	Quercetine	1	35.703	Flavonol
5	Kaempferol	0.1	49.303	Flavonol

Table 3: Antioxidant activity (DPPH assay) and IC_{50} value in hydromethanolic extract of L.fusiformis TL03.

Extracts /standars	IC50 (µg ml $^{-1}$)DPPH	% inhibition of DPPH
Hydromethanolic extract	470	86.19 ± 1.61 (a)
BHT	72,16 ± 0,1	82,36 ± 0,94 (b)
Ascorbic acid	4 ± 0,1	98,54 ± 0,25©
Trolox	6,86 ± 0,05	98,03 ± 0,09©
vitamine E	9,55 ± 0,07	94,08 ± 0,1(d)

DPPH

The crude hydromethanolic extract of *L fusiformis* TL03 show strong antioxidant activity with percentage inhibition of DPPH radical reached to 86.19±1.61 %.

Comparing this result with the standards (antioxidant references), the percentage of free radical inhibition of the extract was higher than that of BHT (82.36 ± 0.94%), while it was lower than that of other standards such as Ascorbic acid (98.54 ± 0.25%), Trolox (98.03 ± 0.09%), and Vitamin E (94.08 ± 0.1%).

The IC50 value for TL03 hydromethanolic extracts was 470 µg/ml. While their values were 72.16 ± 0.1, 4 ± 0.1, 6.86 ± 0.05 and 9.55 ± 0.07 µg.ml-1 for -BHT, ascorbic acid, Trolox and vitamin E, respectively.

Beta carotene

The results of the percentages of antibleaching activities showed that the hydromethanolic extract of Limnospira fusiformis was significantly more effective in inhibiting the oxidation of linoleic acid (60. 35 ± 1.33 %) ($p< 0.05$) than that of BHT (52.5 ±0.82 %):

Antibacterial activities

The antibacterial activities of crude hydromethanolic extract of *L. fusiformus* TL03 were evaluated against six strains of bacteria by measuring the diameter of the inhibition zones, and the results were registered in **Table 4**.and **Fig. 2**.

The tested bacterial strains revealed different results, in which the diameters of the inhibition zone (ZID) ranged from 16.33±0.58 to 33.33±1.53 mm at a concentration of 50 mg/ml. The highest mean of zone inhibition diameter was registered against the Gram-positive bacterium *S. aureus (33.33±1.53 mm)*, followed by *B. subtilis* (16.67±0.58 mm) and *L. monocytogenes* (16.33±0.58 mm).

For Gram-negative bacteria, the highest value of zone inhibition diameter (16.33±1.15mm) against *K. pneumonia was* followed by *P. aeruginosa* (14.67±0.58 mm) and the lowest *E. coli* (11±1mm)

Table 4: Antibacterial activity of crude hydromethanolic extract of *L. fusiformus* TL03 tested by disc diffusion and Agar dilution method.

Bacterial strains		*L. fusiformis TL03* extract		Ampicilline	
		ZID(mm)	MIC(mg/ml)	ZID(mm)	MIC(mg/ml)
Bacteria gram positive	*B.* subtilis	16.67±0.58(a)	6.25	19.67± 0.58(d)	< 0.016
	S. aureus	33.33±1.53(b)	6.25	40±00(e)	0.5
	L. monocytogenes	16.33±0.58(a)	6.25	35±00(b)	0.5
Bacteria gram negative	*E. coli*	11±1(c)	12.5	24.33±1.15(f)	<0.016
	P. aeruginosa	14.67±0.58(a)	1.56	9.67±0.58(c)	< 0.016
	K. pneumonia	16.33±1.15(a)	0.78	40.33±0.58(e)	0.25

MIC: Minimum inhibitory concentration, ZID: zone inhibition diameter.
Different letters indicate significant difference (P< 0.5) . Error bar represents standard error (n = 4).

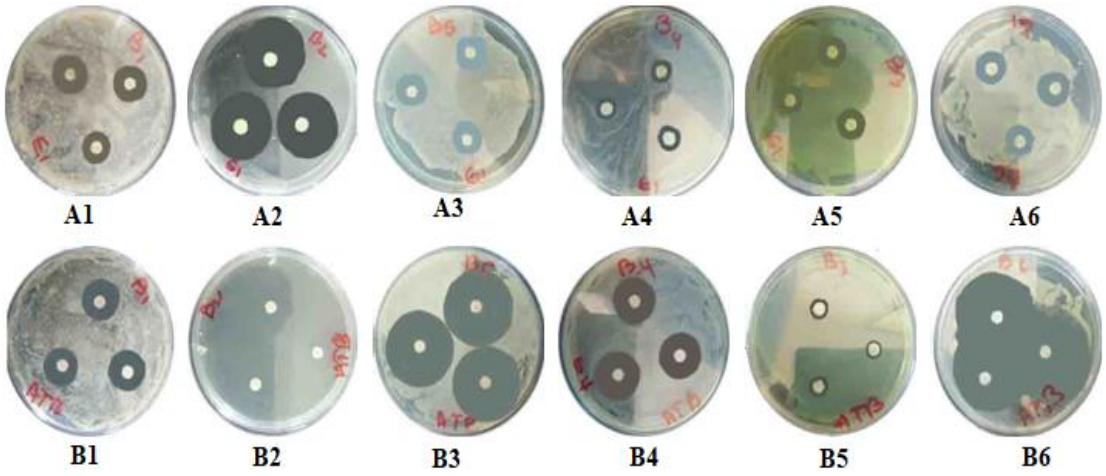

Fig. 2: Zone inhibition diameters (ZID) of the hydromethanolic extract of *L. fusiformis TL03* (A) and the antibiotic Ampicilline (B) against six bacterial strains. 1: *B. subtilis*; 2: *S. aureus*; 3: *L. monocytogenes*; 4: *E. coli*; 5: *P. aeruginosa*; 6: *K. pneumonia*.

Diffusion method

The minimum inhibitory concentrations (MIC) of L. fusiformus TL03 **extracts** are shown in **Table 4** and **Fig. 3**

The results showed that the MICs (minimum inhibitory concentrations) of the methanolic extract**(Table 5)** ranged from 0.78 mg/ml to 12.5 mg/ml. The lowest MIC value (0.78 mg/ml) was registered against *K. pneumonia,* followed by *P. aeruginosa* (1.56 mg/ml. whereas the highest value (12.5 mg/ml) was against *E. coli.* However, the MIC of this extract was 6.25 mg/ml against three bacteria gram+, *B. subtilis* , *S. aureus* and *L. monocytogenes*. The MIC of ampicillin ranged from 0.5 mg/ml to less than 0.016 mg/ml, where the highest value was registered against *S. aureus* and *L. monocytogenes,* followed by 0.25 mg/ml against *K. pneumonia,* while the lowest value (less than >0.016 mg/ml) was obtained against *B. subtilis*, *P. aeruginosa*, and *E. coli.*

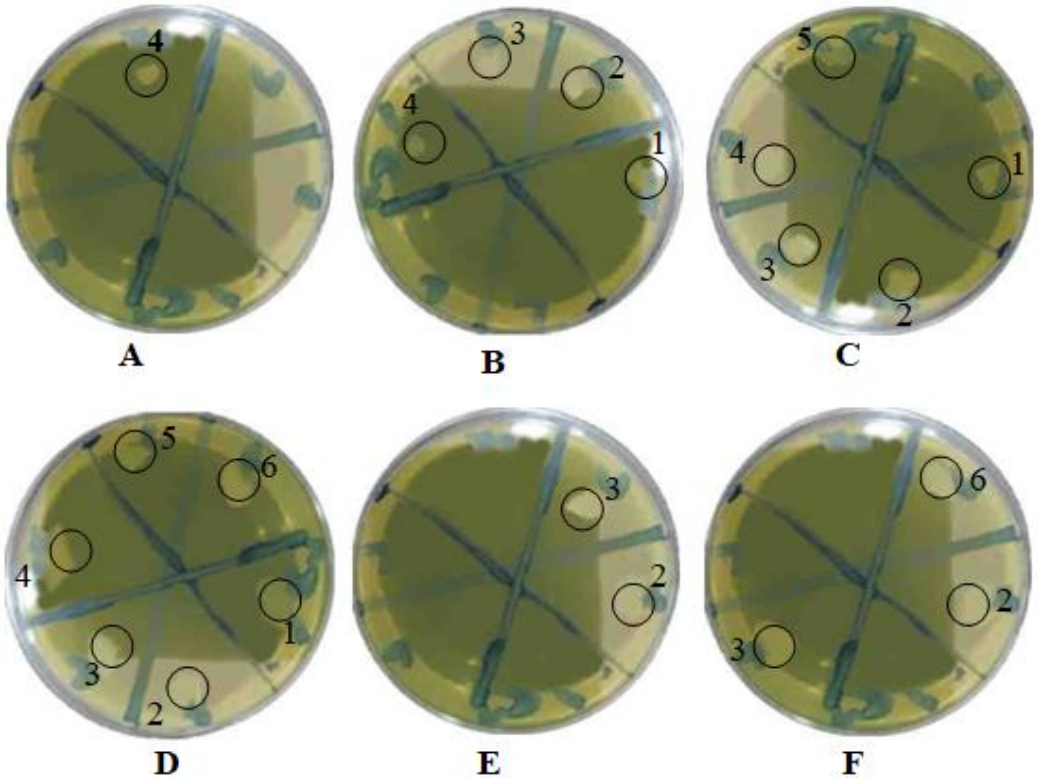

Fig. 3: Minimum inhibition concentration of the hydromethanolic extract of *L. fusiformis TL03* (A-D) and the antibiotic Ampicilline (E-F) against six bacterial strains. 1: *B. subtilis*; 2: *S. aureus*; 3: *L. monocytogenes*; 4: *E. coli*; 5: *P. aeruginosa*; 6: *K. pneumonia*.
A : 12.5 mg /ml , B : 6.25 mg /ml, C: 1.56 mg /ml, D: 12.5 mg /ml, E: 0.5 mg /ml, F: 0.016 mg /ml.

Table 5: Antibacterial activity of crude hydromethanolic extract of *L. fusiformus* TL03 **tested by** disc diffusion and Agar dilution method.

Bacterial strains		*L. fusiformis TL03* extract		Ampicilline	
		ZID(mm)	MIC(mg/ml)	ZID(mm)	MIC(mg/ml)
Bacteria gram positive	*B.* subtilis	16.67±0.58(a)	6.25	19.67± 0.58(d)	< 0.016
	S. aureus	33.33±1.53(b)	6.25	40±00(e)	0.5
	L. monocytogenes	16.33±0.58(a)	6.25	35±00(b)	0.5
Bacteria gram negative	*E. coli*	11±1(c)	12.5	24.33±1.15(f)	<0.016
	P. aeruginosa	14.67±0.58(a)	1.56	9.67±0.58(c)	< 0.016
	K. pneumonia	16.33±1.15(a)	0.78	40.33±0.58(e)	0.25

MIC: Minimum inhibitory concentration, ZID: zone inhibition diameter.
Different letters indicate significant difference (P< 0.5) .Error bar represents standard error (n = 4).

Discussion

Phenolic compounds are secondary metabolites essential for plant physiological processes, stress responses, and survival under harsh conditions [61]. The phenolic content of *S. platensis* is also influenced by a number of parameters, such as algal species, origin, culture conditions, stress factors, time of biomass collection, and age, as well as the methods of their extraction and solvents utilized[62]. They are classified as simple phenols, phenolic acids, flavonoids, xanthones, stilbenes, and lignans[63,64]. Flavonoids and phenolic acids are the main classes of phenolic compounds reported in *Spirulina*[43].

The total phenol content in the hydromethanolic extract of *L. fusiformis* TL03 agrees well with the results of other previously reported spirulina species[63, 65 ,66], while being higher than those documented in several research[67-73].

Total contents of flavonoids

By comparing our findings of total flavonoids in this study (2.11 ± 0.12 QE mg/g DW) with those reported by several authors, we noticed that the level of flavonoids in the hydromethanolic extract of the examined algae was higher than that found in other studies.

[Bellahcen][67] found that the total contents of flavonoids in ethanolic and aqueous extracts (0.21 ± 0.01 and 0.15 ± 0.01 mg quercetin/g dw, respectively) of Moroccan *Spirulina.* [Thangaraj][70] reported that the total flavonoids in the methanolic extract of *Arthospira platensis* (*spirulina*) were 1.42 ± 0.05 mg/g dry weight.

Other studies reported that the amount of flavonoids present in a *spirulina* extract was determined to be 142.23 mg quercetin/kg of extract[71] and 176.3 ± 7.65 mg QE/100 g in the aqueous extract of *spirulina* powder[65].

On the other hand, some studies indicated a high amount of total flavonoids compared to our findings. [Zainoddin][64] declared that the total flavonoid content in *spirulina* was 11.19 ± 0.07 mg QE/g DW. [Seghiri][69] found the content in the methanolic extract of Moroccan *Spirulina* to be 15.60 ± 2.74 mg RE/g dw.

Hplc Analysis

The polyphenol chromatographic analysis of the crude hydromethanolic extract of *L. fusiformis* TL03 revealed the presence of various compounds, of which five were identified **(Fig 1 and Table**

2). These results are similar to those of previous studies in which the authors mentioned the presence of ascorbic acid, gallic acid, luteotin, quercetin, kaempferol, and other phenolic compounds in different species of *spirulina*[67-69, 74-76, 78, 80].

Antioxydant Activities

DPPH

The DPPH assay results of this study **(Table 3)** showed that the hydromethanolic extracts of the TL03 strain possessed strong radical scavenging activity. The result of this study is similar in part to other study on the effective scavenging activity of DPPH radicals in *Spirulina platensis* extract[81], where the inhibition values of extracts prepared from 50%, 70%, and 96% methanol/water were 91.81%, 78.94%, and 58.61%, respectively[80]. In agreement, other study recorded the high percentage inhibition of DPPH radicals (97.37, 89.47, and 77.66%) in methanolic extract of *S. obliquus*, *B. eriensis*, followed by *P. pyrenoidosa*, respectively [74].

While the DPPH assay finding is greater than that of a methanolic extract of *Arthrospira platensis* reported by GHEDA [82] and those reported for green microalgae including *Chlorella sp.*E53 , *Chlorella sp.*ED53 and *Chlorococcum sp.*C53[83].

The potent antiradical activity may be due to the presence of phycobilin pigments (C-phycocyanin, allophycocyanin, and C-phycoerytherin) as well as phenolic and flavonoid compounds in the methanolic extract of *Arthrospira*[84].

The obtained results of IC 50 are higher compared to those mentioned by Abd El-Baky[77], who reported that the IC 50 of extracts derived from *S. maxima* cultured in Zarrouk media containing 2.5, 1.875, 1.25, 0.625, and 0 NaNO3 g/L revealed an IC50 value of 30.0, 28.0, 26.0, 23.0, and 22.0 µg /ml, respectively. Furthermore, the inhibition percentage of this study was higher than those (ranging from 60.33 ± 1.12 to 81.14 ± 1.25%) reported in different media of the same study.

Beta carotene

Carotenoids are important groups of pigments (red, orange, or yellow colors) that are present in plants, fungi, bacteria, and algae and cyanobacteria[85,86]. They are also key metabolites for human nutrition and health[87].

Natural carotenoids (lutein, β-carotene, and lycopene) have beneficial effects on both human and animal health[88,89]. B-carotene remains their essential source of vitamin A, which is necessary for the functions of the retina. In addition, it possesses anticancer, and antiviral and antibacterial properties[90].

The effectinesse of hydromethanolic extract to inhibit β-carotene bleaching was 85.87 ± 1.65% at 10mg/mL **(Table 4)**. The result of

this study of beta-carotene bleaching **(Table 4)** was consistent with that reported in *Spirulina* extract produced from various solvents (ethyl acetate, hexane, and chloroform extracts), in which *β*-carotene bleaching rates were 93.64±0.25%, 73.36±0.35%, and76.77±0.12%, respectively[91].

Antibacterial Activities

Several studies have investigated the antimicrobial activity of *Spirulina* extracts against the bacterial strains tested in this study, in addition to other pathogenic bacterial strains. *B. cereus*[68,92] and *S. aureus*[68,93-99], *L. monocytogenes*, *E. coli*[68, 93-95, 97, 99, 100], *P. aeruginosa*[93, 94, 97] and *K. pneumonia*[94, 95, 97, 99]. Those authors reported that there are different levels of effectiveness depending on the algae species, culture condition, process, and solvents used for the extraction.

Our findings **(Table 5)** showed that *L fusiformis* TL03 extracts had strong antibacterial activity against *S. aureus, B. cereus, K. pneumonia, P. aeruginosa,* and *L. monocytogenes,* whereas they had less activity against *E. coli*.

These results are also in agreement with previous studies[99,101, 102], which confirmed that the methanol extract of *Spirulina platensis* showed high activity against several positive and negative gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonaeruginosa*, *Klebsiella pneumoniae, Streptococcus pyogenes, Streptococcus epidermidis, Proteus mirabilis, Salmonella typhi,* and *Shigella flexneri*).

According to Abdel-Moneim[103], The methanolic extract of *Spirulina platensis* exhibited greater total phenolic content, antioxidant activity, and antibacterial activity in comparison to other extracts (hexane and acetone extracts). Furthermore, it has been noted that the methanolic extract of *Spirulina platensis* has potent antimicrobial and antifungal activities against some pathogenic microbial strains[104].

Martelli[68] demonstrated similar results as our findings, where the hydroethanolic extracts of *A. platensis* showed the highest antimicrobial activity (ZID = 15 mm) against gram-positive bacteria (*L. monocytogenes, S. aureus, and B. cereus*) and interesting inhibition results for gram-negative bacteria (*E. coli, Salmonella* spp.).

Kaushik[99] reported that the ZID of methanolic extract of *Spirulina platensis against* three bacteria (*S. typhi*, *P. aeruginosa*, *E. coli*, *S. aureus*) ranged from 11.52±.18 to 15.21±.1mm but no activity was observed against *K. pneumonia.*

Abdel-Moneim[103] stated Reported that the strong antimicrobial activity of the methanolic extract may be due to its high phenolic content.

According to some published papers[105,106], the antimicrobial activities of algae against a variety of bacteria and fungus are linked to polyphenols, terpenes, flavonoids, alkaloids, pigments,amino acids, polysaccharides (such as depolymerized fucoidans), lipids, and other lipid-soluble substances.

Spirulina bioactive compounds can damage bacterial cell integrity by increasing membrane permeability, which leads to loss of cytoplasmic content and then cell death[103]. On the other hand, Demule[107] reported that the antibacterial activity of the methanolic extract of *S. platensis* is caused by the presence of a significant amount of linolenic acid.

The minimum inhibitory concentration (MIC) of hydromethanolic extract of *L* fusiformis TL03 **(Table 5)** *ranging* from **0.78 to 12.56 mg/ml.** These results agree in part with previous studies by Abdel-Moneim[103], which recorded that the MIC values of *Spirulina* extracts (methanol, hexane, and acetone) against tested bacteria ranged from 1.2 to 10 mg/ml, with the lowest level MIC in methanol extracts (1.2 to 2 mg/ml).

These data are also in agreement with the previous study by Usharani[102], who reported that the MIC value of methanolic extract of *Spirulina platensis against eleven bacterial strains (Staphylococcus aureus, Streptococcus epidermidis, Streptococcus pyogenes, Bacillus cereus, Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholerae, Salmonella typhi, Klebsiella pneumoniae, and Shigella flexneri*) ranged from 1.25 mg/ml to 5 mg/ml.

Kaushik reported that .The MIC of methanol extract of *Spirulina platensis* against *S. aureus* and *E. coli* were 128 μg /ml and 256 μg /ml, respectively.

Conclusion

In conclusion, the results of this work showed that the methanolic extract of *L. fusiformis* TL03 contains a variety of bioactive compounds and has potent antioxidant and antibacterial properties against several pathogenic bacteria. Consequently, further research should be conducted to identify, isolate and characterize the molecules responsible for these activities, which may be used as alternatives to antibiotics and other conventional chemical drugs to treat various diseases. It is useful to conduct further studies to investigate its benefits in various pharmaceutical and food industry applications.

REFERENCES

1. Y. Taniyama and K. K. Griendling, "Reactive oxygen species in the vasculature:molecular and cellular mechanisms", ***Hypertension***, 42(6), 1075–1081 (2003).
2. S. H. Jeong, B. Y. Kim, H. G. Kang, H. O. Ku and J. H. Cho, "Effects of butylated hydroxyanisole on the development and functions of reproductive system in rats", ***Toxicology***, 208(1), 49–62(2005).
3. A. B. Engin, N. Bukan, O. Kurukahvecioglu, L. Memis and A. Engin, "Effect of butylated hydroxytoluene (E321) pretreatment versus l-arginine on liver injury after sub-lethal dose of endotoxin administration", ***Environ Toxicol Pharmacol***, 32, 457–464 (2011)
4. A. Botterweck, H. Verhagen, R. Goldbohm, J. Kleinjans and P. V. D. Brandt, "Intake of butylated hydroxyanisole and butylated hydroxytoluene and stomach cancer risk: results from analyses in the Netherlands Cohort Study", ***Food Chem Toxicol***, 38(7), 599–605(2000).
5. S. Randhawa and S. L. Bahna, "Hypersensitivity reactions to food additives", ***Curr Opin Allergy Clin Immunol***, 9(3), 278–283(2009).
6. M. Bleve, L. Ciurlia, E. Erroi, G. Lionetto, *et al.*, "Lionetto An innovative method for the purification of anthocyanins from grape skin extracts by using liquid and sub-critical carbon dioxide", ***Sep Purif Technol,*** 64(2), 192–197(2008).
7. L. Angiolella, G. Sacchetti and T. Efferth, "Antimicrobial and antioxidant activities of natural compounds", ***Evid Based Complement Alternat Med***, 2018(1), 1–3 (2018).
8. N. Chaachouay and L. Zidane, "Plant-Derived Natural Products: A Source for Drug Discovery and Development", ***Drugs Drug Candidates***, 3(1), 184–207 (2024).
9. W. Fu, D. R. Nelson, Z. Yi, M. Xu, B. Khraiwesh, K. Jijakli and K. Salehi-Ashtiani, "Bioactive compounds from microalgae: Current development and prospects", ***Studies In Natural Products Chemistry***, 54, 199–225 (2017).
10. M. A. Zaki, M. Ashour, A. M. Heneash, M. M. Mabrouk, A. E. Alprol, H. M. Khairy and M. E. Elshobary, "Potential Applications of native cyanobacterium isolate (Arthrospira platensis NIOF17/003) for biodiesel production and utilization of its byproduct in marine rotifer (Brachionus plicatilis) production", ***Sustainability***, 13(4), 1769(2021).
11. M. A. Rahman, M. A. Aziz, R. A. Al-Khulaidi, N. Sakib and M. Islam, "Biodiesel production from microalgae Spirulina maxima by two step process: Optimization of process variable", ***JRRAS***, 10(2), 140–147(2017).
12. S. S. Mostafa and N. S El-Gendy, "Evaluation of fuel properties for microalgae Spirulina platensis bio-diesel and its blends with Egyptian petro-diesel", ***Arab J Chem***, 10(2), 2040–2050 (2017).
13. U. Rajak, P. Nashine and T. N. Verma, "Characteristics of microalgae spirulina biodiesel with the impact of n-butanol addition on a CI engine", ***Energy***, 189, 116311(2019).
14. M. E. Murad and M. F. Al-Dawody, "Biodiesel production from spirulina microalgae and its impact on diesel engine characteristics" ***Rev Al-Qadisiyah J Eng Sci***, 13, 158–166(2020).
15. E. G. D. Morais, A. P. A Cassuriaga, N. Callejas, N. Martinez, I. Vieitez, I. Jachmanián and J. A. V. Costa, "Evaluation of CO 2 biofixation and biodiesel production by Spirulina

(Arthospira) cultivated in air-lift photobioreactor", ***Braz Arch Biol Technol***, 61, 18161339 (2018).

16. A. Rempel, F. de Souza Sossella, A. C. Margarites, A. L.Astolfi, R. L. R. Steinmetz, A. Kunz, and L. M. Colla, "Bioethanol from Spirulina platensis biomass and the use of residuals to produce biomethane: An energy efficient approach", ***Bioresour Technol***, 288, 121588(2019).
17. R. Dinesh kumar, M. Duraimurugan, N. Sharmiladevi, L. P. Lakshmi, A. A. Rasheeq, A. Arumugam and P. Sampathkumar, " Microalgal liquid biofertilizer and biostimulant effect on green gram (Vigna radiata L) an experimental cultivation", ***Biomass Conv Bioref***, 1–21, (2020).
18. F. Arahou, I. Lijassi, A. Wahby, L. Rhazi, M. Arahou and I. Wahby, "Spirulina-Based Biostimulants for Sustainable Agriculture: Yield Improvement and Market Trends", ***BioEnergy Research***, 16(3), 1401–1416 (2022).
19. C. Wuang, M. C. Khin, P. Q. D. Chua and Y. D. Luo, "Use of Spirulina biomass produced from treatment of aquaculture wastewater as agricultural fertilizers", ***Algal Research***, 15, 59–64(2016).
20. M. Castillo, G. Guevara, S. Baldanta, P. S. Rodríguez, L. Agudo, J. Nogales and J. M. N. Llorens, "Characterization of Limnospira platensis PCC 9108 RM and CRISPR-Cas systems", ***Microbiol Res***, 279, 127572 (2024).
21. M. Pelagatti, G. Mori, S. Falsini, R. Ballini, L. Lazzara and A. Papini, "Blue and Yellow Light Induce Changes in Biochemical Composition and Ultrastructure of Limnospira fusiformis (Cyanoprokaryota)", ***Microorganisms***, 11(5), 1236(2023).
22. T. Roussel, S. Halary, C. Duval, B. Piquet, J. P. Cadoret, L.Vernès and B. Marie, "Monospecific renaming within the cyanobacterial genus Limnospira (Spirulina) and consequences for food authorization", ***J Appl Microbiol***, 134(8), lxad 159(2023).
23. S. C. Silva, I. C. Ferreira, M. M. Dias and M. F. Barreiro, "Microalgae-derived pigments: A 10-year bibliometric review and industry and market trend analysis", ***Molecules***, 25(15), 3406(2020).
24. W. A. Metekia, A. G. Usman, B. H. Ulusoy, S. I. Abba and K. C. Bali, "Artificial intelligence-based approaches for modeling the effects of spirulina growth mediums on total phenolic compounds", ***Saudi J Biol Sci***, 29(2), 1111–1117(2022).
25. FAO. 2018,"The State of World Fisheries and Aquaculture: Meeting the sustainable development goals", ***Rome Food and Agriculture Organization of the United Nations,*** 2018(2018).
26. S. A. Pareek, B. R. Kasvan and N. Singh, "Effect of a novel dietary supplement Khejri, and Spirulina supplementation on lipid profile in cricket players", ***Front Sports Act Living***, 4, 1075388(2023).
27. R. K. Singh, S. P. Tiwari, A. K. Rai and T. M. "Mohapatra, Cyanobacteria: an emerging source for drug discovery", ***J Antibiot***, 64(6), 401–412(2011).
28. M. Carcea, M. Sorto, C. Batello, V. Narducci, A. Aguzzi, E. Azzini and V. Turfani, "Nutritional characterization of traditional and improved dihé, alimentary blue-green algae from the lake Chad region in Africa", ***LWT-Food Science and Technology***, 62(1), 753–763 (2015).
29. M. M. Ramírez-Rodrigues, C. Estrada-Beristain, J. Metri-Ojeda, A. Pérez-Alva, and D. K. Baigts-Allende," Spirulina platensis protein as sustainable ingredient for nutritional food products development", ***Sustainability***, 13(12), 6849(2021).
30. M. Raczyk, K. Polanowska, B. Kruszewski, A. Grygier and D. Michałowska, "Effect of Spirulina (Arthrospira platensis) Supplementation on Physical and Chemical Properties of Semolina (Triticum durum) Based Fresh Pasta", ***Molecules***, 27(2), 355(2022).
31. F. Jung, A. Krüger-Genge, P. Waldeck and J. H. Küpper, "Spirulina platensis, a super food", ***J Cell Biotechnol***, 5(1), 43–54(2019).

32. V. Saharan and S. Jood, "Nutritional composition of Spirulina platensis powder and its acceptability in food products", ***Int J Adv Res*** , 5(6), 2295–2300(2017).
33. S. Priyanka, R. Varsha, R. Verma and S. B. Ayenampudi, "Spirulina: a spotlight on its nutraceutical properties and food processing applications", ***J Microbiol Biotech Food Sci*** , 12(6), e4785 (2023).
34. A. M. Sharoba, "Nutritional value of spirulina and its use in the preparation of some complementary baby food formulas", ***J Dairy Sci Biotechnol***, 5(8), 517–538(2014).
35. N.Seyidoglu, S. Inan and C. A Aydin, "prominent superfood: Spirulina platensis. In Superfood and Functional Food the Development of Superfoods and Their Roles as Medicine", ***Intech Open London UK***, 22(1), 1–27(2017).
36. K. D. Athiyappan, W. Routray and B. Paramasivan, "Phycocyanin from Spirulina: A comprehensive review on cultivation, extraction, purification, and its application in food and allied industries", ***Food and Humanity***, 100235 (2024).
37. J. J. Oliveira and H. Ribeiro, "Food market trends: the cases of spirulina and bee pollen 56th", ***Int Sci Conf Econ Soc Dev,*** 246–258(2020).
38. F. Shahidi and C. Alasalvar, (Eds.), "Handbook of functional beverages and human healt", ***CRC Press,*** 11, (2016).
39. S. Bleakley and M. Hayes, "Algal proteins: extraction, application, and challenges concerning production", ***Foods***, 6(5), 33 (2017).
40. M. Tavakoli, M. B. H. Najafi and M. Mohebbi, " Effect of the milk fat content and starter culture selection on proteolysis and antioxidant activity of probiotic yogurt", ***Heliyon***, 5(2), e01204(2019).
41. N. K. AlFadhly, N. Alhelfi, A. B. Altemimi, D. K. Verma, F. Cacciola and A. Narayanankutty, "Trends and technological advancements in the possible food applications of Spirulina and their health benefits", ***Molecules***, 27(17), 5584(2022).
42. D. K. Koli, S. G. Rudra, A.Bhowmik and S. Pabbi,"Nutritional, functional, textural and sensory evaluation of Spirulina enriched green pasta: A potential dietary and health supplement", ***Foods***, 11(7), 979 (2022).
43. D. G. Bortolini, G. M. Maciel, I. D. A. A. Fernandes, A. C. Pedro, F. T. V. Rubio, I. G. Brancod and C. W. I. Haminiuk, "Functional properties of bioactive compounds from Spirulina spp.: Current status and future trends", ***Food Chem Mol Sci***, 100134 (2022).
44. R. Fernandes, J.Campos, M.Serra, J. Fidalgo, H. Almeida, A. Casas and A. I. Barros, Exploring the benefits of phycocyanin: From Spirulinacultivation to its widespread applications, ***Pharmaceuticals***, 16(4), 592 (2023).
45. G. Gentscheva, K. Nikolova, V. Panayotova, K Peycheva, L. Makedonski, P Slavov and I Yotkovska,"Application of Arthrospira platensis for Medicinal Purposes and the Food Industry A Review of the Literature", ***Life***, 13(3), 845 (2023).
46. S. Kumar, V. K. R.Surasani, A. Singh, S. M. Singh, Hauzoukim, L. N. Murthy and K. G. Baraiya , "Bioactive compounds from micro-algae and its application in foods : a review", ***Discov Food***, 4(1), 4-27 (2024).
47. M. H. Saad, E. M. El-Fakharany, M. S. Salem and N. M. Sidkey,"The use of cyanobacterial metabolites as natural medical and biotechnological tools", ***J Biomol Struct Dyn***, 40(6), 2828-2850(2022).
48. S. Hassan, R. Meenatchi, K. Pachillu, S. Bansal, P. Brindangnanam, J. Arockiaraj and J. Selvin, "Identification and characterization of the novel bioactive compounds from microalgae and cyanobacteria for pharmaceutical and nutraceutical applications", ***J Basic Microbiol***, 62(9), 999-1029 (2022).
49. S. A. Khalifa, E. S. Shedid, E. M. Saied, A. R. Jassbi, F. H. Jamebozorgi, M. E. Rateb and H. R. El-Seedi, "Cyanobacteria—From the oceans to the potential biotechnological and biomedical applications", ***Mar Drugs***, 19(5), 241(2021).
50. S. Kini, M. Divyashree, M. K. Mani and B. S. Mamatha, "Algae and cyanobacteria

as a source of novel bioactive compounds for biomedical applications", ***Adv cyanobact Biol***, 173-194 (2020).
51. S. Vijayakumar and M. Menakha, "Pharmaceutical applications of cyanobacteria — A review ", ***J Acute Med***, 5(1), 15-23(2015).
52. B. Thevarajah, G. K. S. H.Nishshanka, M. Premaratne, P. H. V. Nimarshana, D. Nagarajan, J. S Chang and T. U. Ariyadasa, "Large-scale production of Spirulina-based proteins and c-phycocyanin: A biorefinery approach", ***Biochem Eng J***, 185, 108541(2022).
53. G. M. Hamad, A. El-Baky, M. M. Sharaf and A. A. Amara,"Volatile compounds, fatty acids constituents, and antimicrobial activity of cultured Spirulina (Arthrospira fusiformis) isolated from Lake Mariout in Egypt", ***Sci World J vol***, 2023(2), 9919814 (2023).
54. M. M. Allen and R. Y. Stanier, "Growth and division of some unicellular blue-green algae", ***J Gen Microbiol***, 51(2), 199-202(1968).
55. S. V. L.ingleton and J. A. Rossi, "Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents", ***Am J Enol Vitic***, 16(3), 144-158 (1965).
56. J. L. C. Lamaison and A. Carnet, "Teneurs en Principaux Flavonoides des fleurs de Crataegus Monogyna Jacq et de Crataegus Laevigata (Poiret D. C) en Fonction de la Vegetation. Pharmaceutica", ***Pharm Acta Helv***, 65, 315-320 (1990).
57. B. Tepe, M. Sokmen, H. A. Akpulat and A. Sokmen, "Screening of the antioxidant potentials of six Salvia species from Turkey", ***Food Chem***, 95(2), 200-204(2006).
58. M. Al-Saikhan, L. Howard and J. C. Miller," Antioxidant activity and total phenolics in different genotypes of potato (Solanum tuberosum, L.)", ***J Food Sci***, 60(2), 341-343(1995).
59. M Alagawany, M. Madkour, E. M. T. l-Saadony,and, F. M. Reda," Paenibacillus polymyxa (LM31) as a new feed additive: Antioxidant and antimicrobial activity and its effects on growth, blood biochemistry, and intestinal bacterial populations of growing", ***Anim Feed Sci Technol***, 276(2), 114920 (2021).
60. E. A. Ashour, M. E. A. El-Hack, M. E. Shafi, W. Y. Alghamdi, A. E. Taha, A. A. Swelum and M. T. El-Saadony,"Impacts of green coffee powder supplementation on growth performance, carcass characteristics, blood indices, meat quality and gut microbial load in broilers", ***Agriculture***, 10(10), 457, (2020).
61. J. Cichoński and G. Chrzanowski, "Microalgae as a source of valuable phenolic compounds and carotenoids", ***Molecules***, 27(24), 8852(2022).
62. K. Korzeniowska, B. Łęska and P. P. Wieczorek, "Isolation and determination of phenolic compounds from freshwater Cladophora glomerata", ***Algal Res***, 48, 101912 (2020).
63. M. M. Vuolo, V. S. Lima and M. R. M. Junior, "Phenolic compounds: Structure, classification, and antioxidant power. Bioactive compounds: health benefits and potential applications, 33-50 (2019).
64. T. Papalia, R. Sidari and M. R. Panuccio, "Impact of different storage methods on bioactive compounds in Arthrospira platensis biomass", ***Molecules***, 24(15), 2810(2019).
65. A. M. Elbaz, A. M. Ahmed, A. Abdel-Maqsoud, A. M. Badran and A. M. E. Abdel-Moneim, "Potential ameliorative role of Spirulina platensis in powdered or extract forms against cyclic heat stress in broiler chickens", ***Environ Sci Pollut Res Int***, 29(30), 45578-45588 (2022).
66. H. A. H Zainoddin, A. Hamzah, Z. Jamari and W. A. W. Omar, "Chemical profiles of methanolic extracts from two species of microalgae, Nannochloropsis sp. and Spirulina sp.", ***Pertanika J Trop Agric Sci***, 41(3), 1085-1096 (2018).
67. T. O. Bellahcen, A. AAmiri, I. Touam, F. Hmimid, A. E. Amrani, A. Cherif and M. Cherki, "Evaluation of Moroccan microalgae: Spirulina platensis as a potential source of natural antioxidants", ***J Complement Integr Med***, 17(3), 20190036 (2020).

68. F. Martelli, M. Cirlini, C. Lazzi, E. Neviani and V. Bernini, "Edible seaweeds and spirulina extracts for food application: In vitro and in situ evaluation of antimicrobial activity towards foodborne pathogenic bacteria", ***Foods***, 9(10), 1442 (2020).
69. R. Seghiri, M. Kharbach and A. Essamri, "Functional composition, nutritional properties, and biological activities of Moroccan Spirulina microalga", ***J Food Qual***, 2019(3), 11 (2019).
70. M. Thangaraj, B. P. Saravana, J. Thanasekaran, S. Joen-Rong, M. Manubolu and K. Pathakoti, "Phytochemicals of algae, Arthospira platensis (spirulina) Chlorella vulgaris (chlorella) and Azolla pinnata (azolla)", ***GSC Biol Pharm Sci***, 19(2), 023-043 (2022)..
71. G. A. El-Chaghaby, S Rashad, S. F. Abdel-Kader, , E. S. A. Rawash and M. Abdul Moneem, "Assessment of phytochemical components, proximate composition and antioxidant properties of Scenedesmus obliquus, Chlorella vulgaris and Spirulina platensis algae extracts", ***Egypt J Aquat Biol Fish***, 23(4), 521-526 (2019).
72. A. Salamatullah," Characterization of extraction methods to recover phenolic-rich antioxidants from blue green algae (spirulina) using response surface approaches", (2014).
73. A. Kumar, D Ramamoorthy, D. K. Verma, A. Kumar, N. Kumar, K. R. Kanakn and K. Mohan, "Antioxidant and phytonutrient activities of Spirulina platensis", ***Energy Nexus***, 6, 100070 (2022).
74. M. Guldas, S. Ziyanok, Y. Sahan, E. Yildiz and O. Gurbuz, "Antioxidant and anti-diabetic properties of Spirulina platensis produced in Turkey", ***Food Sci Technol***, 41(3), 615-625 (2020).
75. M. Akbarizareh, H. Ofoghi, and M. Hadizadeh, "Assessment of Phenolic Components of the microalgae Spirulina platensis using two methods of Chromatography, TLC and HPLC", ***J Mar Biol***, 2019, (2014).
76. A. Pagnussatt, E. M. Del Ponte, J. Garda-Buffon and E. Badiale-Furlong, "Inhibition of Fusarium graminearum growth and mycotoxin production by phenolic extract from Spirulina sp", ***Pestic Biochem Physiol***, 108(1), 21-26 (2014).
77. H. H. Abd El-Baky, F. K. El Baz and G. S. El-Baroty, "Production of phenolic compounds from Spirulina maxima microalgae and its protective effects in vitro toward hepatotoxicity model", ***Afr J PharmacyPharmacol***, 3(4), 133-139 (2009).
78. S. C. Sahin, "The potential of Arthrospira platensis extract as a tyrosinase inhibitor for pharmaceutical or cosmetic applications", ***S Afr J Bot***, 119, 236-243 (2018).
79. H. Farg, A. El-Makhzangy and A. G. El-Shawaf," Chemical and technological studies on micro algae extracts and its utilization in some food products. 1- Chemical, biochemical characteristics and nutritional value of spirulina algae.", ***J Product Dev***, 26(1), 107-117 (2021).
80. N. H. El-Tablawy, H. A .Mansour and A. E. S. M. Shaaban, "Antioxidant activities of some edaphic algae in Egypt", ***Beni-Suef univ j basic appl Sci***, 9(1), 1-11 (2020).
81. P. H. Riyadi, E. Susanto, A. D. Anggo, M. H. Arifin and L. Rizki,"Effect of methanol solvent concentration on the extraction of bioactive compounds using ultrasonic-assisted extraction (UAE) from Spirulina platensis", ***Food Res***, 7(3), 59-66 (2023).
82. S. Gheda, E. H. Abd El-Zaher, A. M. Abou-Zeid, N. A. Bedair and L. Pereira, "Potential Activity of Arthrospira platensis as Antioxidant, Cytotoxic and Antifungal against Some Skin Diseases: Topical Cream Application", ***Mar Drugs***, 2023(21), 160 (2023).
83. W. Choochote, L. Suklampoo and D. Ochaikul, "Evaluation of antioxidant capacities of green microalgae", ***J Appl Phycol***, 26(1), 43-48 (2014).
84. E. A. Shalaby and S. M. Shanab, "Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of Spirulina platensis", ***Indian J Geo Mar Sci***, 42(5), 556-564 (2013).

85. A. Hermanns, X. Zhou, Q. Xu, Y. Tadmor and L. Li, "Carotenoid pigment accumulation in horticultural plants", ***Hortic* Plant *J***, 6(6), 343-360 (2020).
86. T. Bhatt and K. Patel," Carotenoids: potent to prevent diseases review", ***Nat Prod Bioprospect***, 10(3), 109-117 (2020).
87. I. Sharma, N. Khare and A. Rai, "Carotenoids: Sources, Bioavailability and Their Role in Human Nutrition",In Dietary Carotenoids-Sources, Properties, and Role in Human Health. IntechOpen, (2024).
88. J. Amengual, E. Gouranton, Y. G J van Helden, S. Hessel, J. Ribot, E. Kramer and J. von Lintig, "Beta-carotene reduces body adiposity of mice via BCMO1", ***PloS One***, 6(6), e20644 (2011).
89. J. von Lintig, S. Hessel, A. Isken, C. Kiefer, J. M. Lampert, O. Voolstra and K. Vogt, "Towards a better understanding of carotenoid metabolism in animals", ***Biochim Biophys Acta***, 1740(2), 122-131 (2005)..
90. N. F. Gloria, N. Soares, C. Brand, F. L. Oliveira, R. Borojevic and A. J. Teodoro," Lycopene and beta-carotene induce cell-cycle arrest and apoptosis in human breast cancer cell lines", ***Anticancer Res***, 34(3), 1377-1386(2014).
91. H. E. A. Ali, S. M. M. Shanab, M. A. M. Abo-State, E. A. A. Shalaby, U. M. N. El Demerdash and M. A. Abdullah, "Evaluation of antioxidants, pigments and secondary metabolites contents in Spirulina platensis", ***Appl Mech Mater***, 625, 160-163 (2014).
92. K. Velichkova, I. Sirakov, N. Rusenova, G. Beev, S. Denev, N. Valcheva and T. Dinev, "In vitro antimicrobial activity on Lemna minuta, Chlorella vulgaris and Spirulina sp. extracts", ***Fresenius Environ Bull***, 27(8), 5736-5741(2018).
93. A. Karray, N. Krayem, H. B. Saad and A. Sayari,"Spirulina platensis, Punica granatum peel, and moringa leaves extracts in cosmetic formulations: an integrated approach of in vitro biological activities and acceptability studies", ***Environ Sci Pollut Res Int***, 28(7), 8802-8811(2021).
94. V. Sankarapandian, K. Nitharsan, K. Parangusadoss, P. Gangadaran, P. Ramani, B. A. Venmathi Maran and M. P. Jogalekar, "Prebiotic Potential and Value-Added Products Derived from Spirulina laxissima SV001—A Step towards Healthy Living", ***BioTech***, 11(2), 13 (2022).
95. M. A. Alshuniaber, R. Krishnamoorthy and W. H. Alqhtani,"Antimicrobial activity of polyphenolic compounds from Spirulina against food-borne bacterial pathogens", ***Saudi J Biol Sci***, 28(1), 459-464 (2021).
96. A. A. Al-Ghanayem,"Antimicrobial activity of Spirulina platensis extracts against certain pathogenic bacteria and fungi", ***Adv Biores***, 8(6), 96-101 (2017).
97. S. Dvl, C. Sreenath Kumar and R. Rengasamy, "Purified C-phycocyanin from Spirulina platensis (Nordstedt) Geitler: a novel and potent agent against drug resistant bacteria", ***World J Microbiol Biotechnol***, 27(4), 779-783 (2011).
98. S. Santoyo, M. Herrero, F. J. Senorans, A. Cifuentes, E. Ibáñez and L. Jaime," Functional characterization of pressurized liquid extracts of Spirulina platensis", ***Eur Food Res Technol***, 224(1), 75-81 (2006).
99. P. Kaushik and A. Chauhan, "antibacterial activity of laboratory grown culture of In vitro Spirulina platensis***", Indian J Microbiol***, 48(3), 348-352 (2008).
100. J. A. Mendiola, L. Jaime, S. Santoyo, G. Reglero, A. Cifuentes, E. Ibañez and F. J. Señoráns, "Screening of functional compounds in supercritical fluid extracts from Spirulina platensis", ***Food chem***, 102(4), 1357-1367 (2007).
101. S. F. Gheda and G. A. Ismail, "Natural products from some soil cyanobacterial extracts with potent antimicrobial, antioxidant and cytotoxic activities", ***An Acad Bras Cienc***, 92(2), e20190934 (2020).
102. G. Usharani, G. Srinivasan, S. Sivasakthi and P. Saranraj, "Antimicrobial activity of Spirulina platensis solvent extracts against pathogenic bacteria and fungi", ***Biol Res***, 9(5), 292-298(2015).
103. A. M. E. Abdel-Moneim, M. T. El-Saadony, A. M. Shehata, A. M. Saad, S. A. Aldhumri, S. M. Ouda and N. M.

Mesalam,"Antioxidant and antimicrobial activities of Spirulina platensis extracts and biogenic selenium nanoparticles against selected pathogenic bacteria and fungi", ***Saudi J Biol Sci***, 29(2), 1197-1209 (2022).

104. M. Hetta, R. Mahmoud, W. El-Senousy, M. Ibrahim, G. El-Taweel and G. Ali, "Antiviral and antimicrobial activities of Spirulina platensis", ***World J Pharm Pharm Sci***, 3(6), 31-39 (2014).
105. S. Afzal, A. K. Yadav, A. K. Poonia, K. Choure, A. N. Yadav and A. Pandey,"Antimicrobial therapeutics isolated from algal source: retrospect and prospect", ***Biologia,*** 78(2),291-305 (2023).
106. M. Bartolomeu, C. Vieira, M. Dias, T. Conde, D. Couto, D. Lopes and M. R. Domingues, "Bioprospecting antibiotic properties in photodynamic therapy of lipids from Codium tomemtosum and Chlorella vulgaris", ***Biochimie***, 203, 32-39 (2022).
107. M. C. Z. Demule, G. Z. Decaire and M. S. Decano, "Bioactive substances from Spirulina platensis (Cyanobacteria)", ***Phyton-Int J Exp Bot***, 58, 93-96(1996).

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نشرة العلوم الصيدلية
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المحتوى الفينولي والأنشطة المضادة للأكسدة والمضادة للبكتيريا للمستخلص الهيدروميثانولي لنبات ليمنوسبيرا فيوزيفورميس لت ٠٣ المعزول من بحيرة التلامين، شمال غرب الجزائر

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أجريت هذه الدراسة لتقييم المحتوى الفينولي الكلى والفلافونويد والنشاط المضاد للأكسدة والمضاد للبكتيريا للمستخلص الهيدروإيثانولي للطحلب الأخضر المزرق ليمنوسبيرا فيوزيفوريمس لت ٠٣ . وقد تم تحديد إجمالي الفينولات والفلافونويدات فى المستخلص وذلك باستخدام طريقتي فولين–سيكالتو وكلوريد الألومنيوم، على التوالي. و تم استخدام تقنية YL 9100 HPLC للتعرف على المركبات الفينولية. وتم استخدام ٢، ٢–ثنائي فينيل –١ بيكريل هيدرازيل (DPPH) لتحديد نشاط الشوارد الجذرية واختبارات حمض اللينوليك بيتا كاروتين لتقييم نشاط مضادات الأكسدة في المختبر. وتم اختبار النشاط المضاد للميكروبات ضد ستة أنواع من البكتيريا باستخدام طريقة الانتشار القرصي وطريقة تخفيف الأجار. و كان إجمالي الفينول والفلافونويدات الموجودة في المستخلص الهيدروميثانولي ١٨,٤١ ± ٠,١٨ مجم حمض الجاليك / جم DW و٢,١١ ± ٠,١٢ مجم كيرسيتين / جمDW ، على التوالي. و أظهرت نتيجة تحليل البوليفينول وجود: حمض الأسكوربيك، حمض الجاليك، للوتيولين، كيرسيتين، كيمبفيرول .

وأظهر هذا المستخلص نشاطًا قويًا لتطهير جذري DPPH وتبييض البيتا كاروتين بنسبة ٨٦,١٩ ± ١,٦١٪ و ٨٥,٨٧ ± ١,٦٥٪ على التوالي، بالإضافة إلى النشاط المضاد للبكتيريا، حيث تتراوح أقطار تثبيط المنطقة (ZID) من ٣٣,٣٣ ± ١,٥٣ إلى ١١±١ ملم، وك١لك أظهر نشاط مضاد للجراثيم ذو قيمة أعلى (٣٣,٣٣±١,٥٣ ملم) ضد المكورات العنقودية الذهبية والحد الأدنى من التركيزات المثبطة (MIC) التي لوحظت ضد بكتريا الالتهاب الرئوي ٠,٧٨) ملجم / مل). وأخيرا تم التوصل إلى أن المستخلص الهيدروميثانولي لنبات ليمنوسبيرا فيوزيفورميس لت ٠٣ له نشاط مضاد للأكسدة ومضاد للبكتيريا.

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Original Article

Morphological and molecular identification of a new halotolerant cyanobacterial strain, *Limnospira fusiformis* TL03, isolated from Telamine Lake in the northwest of Algeria

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ABSTRACT

This study examines a new strain, *Limnospira fusiformis* TL03, isolated from Lake Telamine in northwest Algeria. The strain was morphologically identified using a light microscope and molecularly characterized using its 16S rRNA gene sequence. The effect of salinity on growth and photosynthetic pigments was studied using a spectrophotometric method; different sodium chloride concentrations (0, 5, 15, 30, 45, and 60 g/l) in blue-green medium (BG11) cultured uniformly as homogenized cell suspension were tested. A morphological examination confirmed that the isolated strain belonged to the *Limnospira* genus. It has trichome lengths ranging from 210–2027 µm, as well as pitch and coil diameters ranging from 30–137 µm and 20–60 µm, respectively. The data from the 16S rRNA gene sequence analysis confirmed that the isolated strain was *Limnospira fusiformis* TL03 with 100% sequence similarity to *Limnospira fusiformis* SAG 85.79. The results also indicate that this strain can grow at various salt concentrations, with the highest optical density values (1.58 ± 0.014 and 1.56 ± 0.003) obtained in cultures containing 15 g/L and 5 g/L NaCl, respectively.

Keywords: Identification; Isolation; *Limnospira fusiformis* TL03; Salt stress; Tolerance.

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Introduction

The demand for cyanobacteria and microalgae has increased rapidly in recent years due to their numerous potential applications in fields such as nutrition, biopharmacy, and renewable energy. Extremophile cyanobacteria and microalgae are gaining popularity due to their ability to grow in harsh environments, allowing for outdoor cultivation with minimal contamination risks (Hirooka *et al*., 2014; Varshney *et al*., 2015; D'Alessandro & Antoniosi Filho, 2016; Sydney *et al*., 2019).

Among the phytoplankton microorganisms, the phylum Cyanobacteria is the most ancient, being one of the most versatile and ecologically successful groups, living in a variety of environments (Abed *et al*., 2009), including oceans, freshwater, soil, bare rocks, ice shelves, hot springs, and hypersaline to alkaline lakes. They can also grow in environments with high metal concentrations and low water availability, such as desert regions, by forming endolithic communities (Sanchez-Baracaldo *et al*., 2005; Thajuddin & Subramanian, 2005; Rastogi & Sinha, 2009). Among the numerous cyanobacteria species, we highlight *Limnospira* (= *Arthrospira*), also known commercially as *Spirulina*.

Limnospira is a newly established genus with three validated species that were reclassified from *Arthrospira* based on morphological, molecular, and ecological differences (Nowicka-Krawczyk *et al*., 2019). *Arthrospira fusiformis, Arthrospira maxima,* and *Arthrospira indica* were reclassified to *Limnospira fusiformis, Limnospira maxima,* and *Limnospira indica* respectively (Nowicka-Krawczyk *et al*., 2019). *Limnospira* and *Arthrospira* are cyanobacteria (blue-green algae), multicellular, photosynthetic prokaryotes, and filamentous. They have a high protein content that ranges from 55 to 70% of dry weight and contains all essential amino acids (Babadzhanov *et al*., 2004; Volkmann *et al*., 2008; Sotiroudis & Sotiroudis, 2013; Da Silva *et al*., 2019; Jung *et al*., 2019). They also contain all the B vitamins, as well as vitamins C, E, D, and provitamin A (Mukhopadhyay, 2015; Jung *et al*., 2019). Among the numerous cyanobacteria species, we highlight *Limnospira* (= *Arthrospira*), also known commercially as *Spirulina*.

Limnospira and *Arthrospira* have numerous advantages and can be used in a variety of applications, including human food, animal feed, aquaculture, bioenergy, wastewater treatment, cosmetics, bioplastics, and agriculture. *Limnospira* and *Arthrospira* have anti-inflammatory properties (Wu *et al*., 2016; Aladaileh *et al*., 2020), as well as antibacterial and antifungal properties (Borowitzka, 1995; Furmaniak *et al*., 2017; Falaise, 2019; Jung *et al*., 2019). The extract of *A. maxima* cultured in deep sea water effectively suppressed the expression of the Bcl-2 gene in A549 human lung adenocarcinoma cells, as well as inhibited various human cancer cells (Choi *et al*., 2013), stimulating the immune system and lowering hyperlipidemia and obesity (Jiménez *et al*., 2003; Costa *et al*., 2004; Ghaeni & Roomiani, 2016).

Arthrospira extracts and compounds may be useful in preventing cardiovascular disease (Memije-Lazaro *et al*., 2018). They reduce both blood pressure and plasma lipid concentration (Furmaniak *et al*., 2017). Several international organizations, including the World Health Organization (WHO), Food and Agriculture Organization (FAO), and United Nations Children's Fund (UNICEF), have recognized *Limnospira* and *Arthrospira* as dietary supplements (FAO Fisheries and Aquaculture Circular. No. 1034, Joint FAO/WHO Expert Committee on Food Additives - JECFA, 86th meeting 2018) (Cho *et al*., 2020).

Arthrospira spp. was recommended by the WHO for inclusion in the diet of National Aeronautics and Space Administration (NASA) astronauts in space because it is an ideal and compact food for space travel (Koyandea *et al*., 2019). *Spirulina* was declared the best food for the future by the United Nations World Food Conference, and it is gaining popularity today (Pulz & Gross, 2004). In comparison to other cyanobacteria and microalgae, *Arthrospira* production is the most

widespread in the world (Villaró *et al*., 2022). The efficiency of dry mass production reached 90,000 tons in 2010 and remained there until the last FAO report in 2016 (Furmaniak *et al*., 2017). The main populations of *Limnospira* and *Arthrospira* have been reported in 30 countries worldwide: 11 in Africa, nine in Asia, five in America, and five in Europe (Sili *et al*., 2012).

Numerous studies of *Limnospira fusiformis* habitat have revealed that it is more commonly found in alkaline environments and can survive in a wide range of salinities (Kebede, 1997; Sudhir & Murthy, 2004; Thajuddin & Subramanian, 2005; Dadheech *et al*., 2010; Asulabh *et al*., 2012; Benavente-Valdés *et al*., 2016; Costa *et al*., 2016; Cellamare *et al*., 2018). *Arthrospira* was observed for the first time in Algeria in 1996 at Tamanrasset Pond in the Southern region of this country (Fox, 1996), and it was also found in Telamine Lake in Northwest Algeria, where the latter genus will be studied for the first time in this publication.

Telamine Lake is characterized by high alkalinity and, depending on the season, middle to high salinity. This lake has a high biodiversity of plants and phytoplankton (Hadjadj, 2018). The purpose of this study was to isolate a strain of *Limnospira fusiformis* TL03 from Telamine Lake and investigate its morphological and molecular characteristics, as well as the effect of sodium chloride concentration on its growth, chlorophyll, and carotenoid content.

Materials and Methods

Sampling site description and *Limnospira fusiformis* TL03 isolation

Telamine brackish water Lake is located in northwest Algeria, approximately 20 km East of Oran city and exactly northwest of the Arzew salt flats. It is located at 35º 42' 32'' North and 00º 22' 30'' West, and it is approximately 8.5 km long and 0.5 to 1.5 km wide (Fig. 1A). The Telamine Lake has a surface area of 2.399 ha and a perimeter of 20 km. The majority of its water is alkaline, with a pH range of 7.43 to 8.95 (Hadjadj, 2018). Telamine Lake is a protected wetland (Ramsar list on 12/12/2004) due to its high animal and plant biodiversity.

A phytoplankton bloom (Fig. 1B) of cyanobacteria (*Arthrospira* sp., *Phormidium* sp., and *Microcystis* sp.), diatoms (*Navicula* sp.), and chlorophyte (*Chlorella* sp.) were also observed. Water samples were collected from Telamine Lake about 30 cm below the surface using a phytoplankton net with a mesh size of 50 µm. Samples were collected at two locations: p1 (35°43'53.9 "N 0°23'17.7" W) and p2 (35°42'12.9 "N 0°23'38.2" W). The samplings were completed at the end of April 2018. The samples were immediately transported to the laboratory for analysis. The microbiology laboratory at the National Center for Research and Development of Fisheries and Aquaculture (CNRDPA), Bou Ismail, Algeria, handled the samples and isolated the cyanobacteria. We performed centrifugation at 1000 rpm for 2 min as a first treatment step to remove unwanted microorganisms and solid residues from a sample, then one hundred microliters of the sample were spread aseptically on the surface of Petri dishes containing BG11 (Allen & Stanier, 1968) sterile solid media with different NaCl concentrations (0, 5, 15, and 25 g/l), and the pH was adjusted to 9.5. The plates were incubated at room temperature and illuminated 24 hours a day with 1 Klux light-emitting diodes (LEDs, ABALIGHT 18 W, 220V, 50 Hz). Individual cyanobacteria colonies formed on the surface of the agar medium were picked up and streaked in new, similar solid medium under aseptic conditions. This procedure was repeated several times until pure isolates were obtained.

Description and morphological measurements of the isolated strain

Using a Zeiss microscope connected to a camera (CMEX1, DC 1300C), the isolates were observed and examined directly to try to detect

the *Limnospira* strains morphologically similar to the TL03-like isolate, as described in the literature (Komárek & Anagnostidis, 2005; Sili *et al*., 2012; Nowicka-Krawczyk *et al*., 2019). The morphological properties of 610 filaments (trichomes) were measured using the method depicted in Fig. (2). The diameter and the length of the cell (Kaggwa *et al*., 2013) were measured at 1000 x magnification, the coil diameter and pitch at 400 x magnification, and the filament length at 100 x.

Molecular identification

DNA extraction

Two mL of cyanobacterium culture were mixed with 2 µL of Poloxamer (https://www.sigmaaldrich.com/catalog/product/sial/p2164009?lang=fr&region=FR). After 10 min of centrifugation at 11,000 g, the supernatant was removed, and the DNA was extracted according to the kit's instructions: http://www.mn-net.com/media/pdf/5b/d0/d9/Instruction-NucleoSpin-Tissue.pdf.

PCR amplification and sequencing

The PCR amplification (polymerase chain reaction) for 16S rRNA gene fragments was carried out using the universal bacterial primers 27F (5'- AGA-GTT-TGA-TCC-TGG-CTC-AG -3') and 1492R (5'- GGT-TAC-CTT-GTT-ACG-ACT-T -3') (Lane, 1991). The PCR reaction mixture contained 15 µl of Phusion, 0.9 µl DMSO, 0.75 µl of each primer, 11.6 µL of deionized water, and 1 µl of DNA genome for a total volume of 30 µl of the reaction mixture. The PCR conditions were as

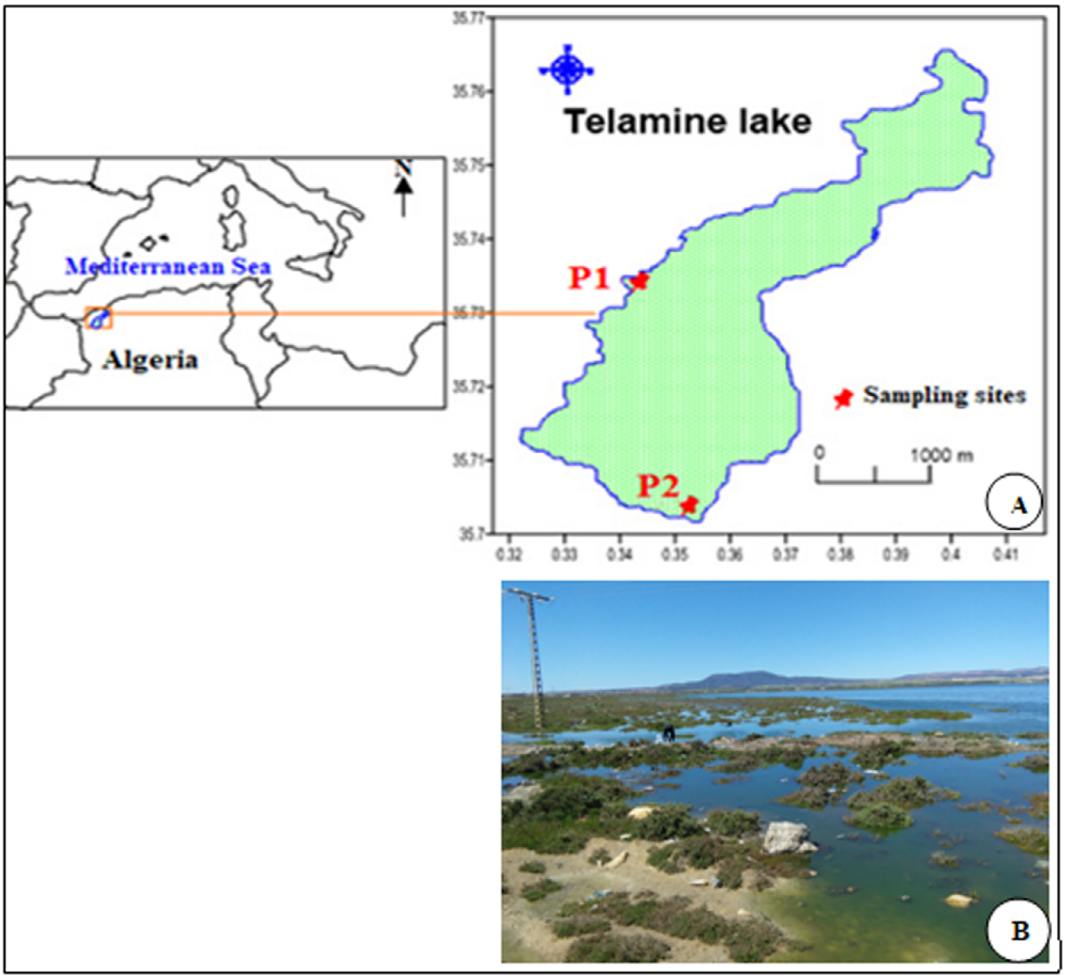

Figure 1. (A) Site description and geographic location of Telamine Lake. (B) Phytoplankton bloom in Telamine Lake (April 2018).

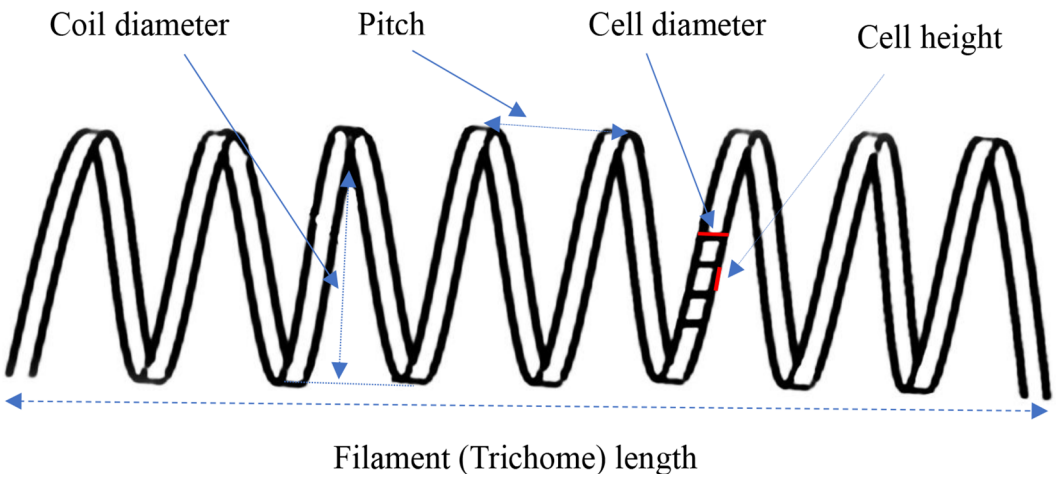

Figure 2. Schematic diagram for cell and filament morphological measurements of Arthrospira (Kaggwa et al., 2013).

follows: 1 cycle at 98 °C for 30 s, followed by 35 cycles of 98 °C for 30 s, 58 °C for 30 s, 72 °C for 1 min, and a final extension at 72 °C for 10 min. PCR was used to amplify the 16SrRNA gene sequence from isolated genomic DNA using the Applied Biosystems GeneAmp PCR 9700 Thermal Cycler. PCR products were examined on 0.8% agarose gel stained with ethidium bromide, and the migration was performed in an electrophoresis chamber. The lecture was done on an ImageQuant Las 4000 (GE HealthCare).

The PCR products were purified using Exosap-it PCR product clean-up (https://www.thermofisher.com/fr/fr/home/life-science/sequencing/sanger-sequencing/sanger-sequencing-kits-reagents/exosap-it-pcr-product-cleanup.html) and reamplified using primers specific to cyanobacteria: forward primer 359F (GGG GAA TYT TCC GCA ATG GG) and reverse primer 781R(GAC-TAC-WGG-GGT-ATC-TAA-TCC-CWT-T) (Nübel *et al.*, 1997; Lau *et al.*, 2005).

Exosap mixture for 1 primer contains 2 µl of the PCR gene product, 1 µl ExoSap-IT, and 2 µl Eau millliQ, incubated at 37 °C for 4 min and 80 °C for 1 min and directly sequenced in both directions with primers 27F, 359F, 1492R, 781R. The sequencing was performed by Macrogen (https://dna.macrogen-europe.com/eng/). The sequences were assembled in the software Geneious.

Phylogenetic analysis

Using the Basic Local Alignment Search Tool (BLAST), the 16S rRNA gene sequences were aligned with reference sequences obtained from the National Center for Biotechnology Information database (NCBI database, http://www.ncbi.nlm.nih.gov/). The alignments were manually checked and edited in the BioEdit sequence alignment editor (version 7.2.6.1). The neighbor-joining (NJ) algorithm and bootstrap resampling (1000 replicates) were used for phylogenetic analysis and tree construction. Next to the branches is the percentage of replicate trees (numbers at nodes) in which the associated taxa clustered together in the bootstrap test (Felsenstein, 1985). The MEGA package database was used to build the phylogenetic tree, and the ClustalW algorithm with the MEGA version was used (Kumar *et al.*, 2018). The evolutionary distances were calculated using the Maximum Composite Likelihood method (Tamura *et al.*, 2004) and are in base substitutions per site (Bar, 0.01 nucleotide substitution per nucleotide position). The tree was established using *Planktothrix agardhii* NIES-204^T as an outgroup. The 16S rRNA gene sequences were entered into the GenBank database.

GenBank accession numbers and taxonomic data for all species included in the 16S rRNA gene sequence alignments in the present study are listed in Table 1.

Table 1. strain, taxonomy ID, and NCBI GenBank accession number of taxa in 16S rRNA gene sequence alignments in this study.

Species	strain	Taxonomy ID	Accession number
Limnospira fusiformis	*TL03*	NCBI:txid2873793	MZ970329
Limnospira fusiformis	*SAG 85.79*	NCBI:txid1521533	KM0119968
Limnospira indica	PCC 8005	NCBI:txid376219	X70769
Limnospira maxima	UTEX2720	NCBI:txid2596819	AF260509
Arthrospira platensis	**PCC 7345**	NCBI:txid129975	*JN831265*
Phormidium terebriforme	**AB2002/07**	NCBI:txid284926	AB2002/07 (AY575933)
Lyngbya hieronymusii	CN-3	NCBI:txid443941	AB045906
Lyngbya aestuarii	**PCC 7419**	NCBI:txid65095	AJ000714
Planktothrix agardhii	**NIES-204**	NCBI:txid282423	AB045954

Culturing conditions

The isolated strain was maintained and cultured in BG11 medium (Allen & Stanier, 1968), containing the following nutrients in g/l: $NaNO_3$, 1.5; K_2HPO_4, 0.04; $MgSO_4.7H_2O$, 0.075; $CaCl_2.2H_2O$, 0.036; citric acid, 0.006; ferric ammonium citrate, 0.006; Na_2EDTA, 0.001; Na_2CO_3, 0.02; and 1 ml of trace element mix was added (containing in g/l: H_3BO_3, 2.86; $MnCl_2.4H_2O$, 1.81; $ZnSO_4.7H_2O$, 0.222; $NaMoO_4.2H_2O$, 0.39; $CuSO_4.5H_2O$, 0.079; $Co(NO_3)_2.6H_2O$, 0.0494). The pH was adjusted to 9.5. The media were autoclaved at 121º C for 20 min. The isolated strain was maintained in a liquid BG11 medium containing 5 g/l of NaCl and subcultured in the same fresh medium every month. The experiment on the effect of salinity on *L. fusiformis* TL03 was done in 500-ml Erlenmeyer flasks with 250 ml of fresh BG11 medium and different NaCl concentrations (0, 5, 15, 25, 35, 45, and 60 g/l). Before sterilizing at 121 °C for 20 min, the pH of all media was adjusted to 9.5. All culture media were uniformly inoculated by adding 25 mL of homogenized cell culture (stock culture) with an initial optical density of 0.220 ± 0.002 at 750 nm.

This experiment uses BG11 with 15 g/l NaCl, the same salinity as its natural habitat, as a control. The cultures were incubated in a culture chamber at a temperature of 27 ± 2 °C, with continuous illumination provided by light-emitting diodes (LEDs) with an intensity of (2.7 ± 0.2 Klux). A Mini Light Meter UT383 was used to measure the intensity of the light. The culture was continuously agitated with an air pump to keep cells suspended and prevent biomass cell agglomeration. Four replicates were used in the experiments. The cultures were kept under these conditions for 20 days.

Growth and pigment measurements

Every four days, a sample of the homogeneous suspensions of cyanobacterial culture was taken from each Erlenmeyer flask and analyzed for growth, chlorophyll a, chlorophyll b, and total carotenoids. Optical density at 750 nm was used to calculate cell growth every four days during the incubation period. Other than that, chlorophyll (a, b) and carotenoids were measured in accordance with Wellburn (1994). Volumes of 3 ml of each culture medium's homogenized cell suspension were centrifuged at 3800 rcf for 10 min (Centurion Scientific Ltd, C2 series Benchtop Centrifuges). After centrifugation at the same speed, the pellets were washed twice with 3 ml of distilled water. The supernatant was removed, and the cells were suspended in 3 mL of pure methanol (99.9%) in the dark at 4 °C for 24 h (until colorless pellets). After centrifugation, the aliquots were read at three different wavelengths (447, 653, and 666 nm) using a Jasco V-630 Spectrophotometer. Wellburn (1994) provided the equation for calculating chlorophylls and carotenoids.

$$\text{Chlorophyll a } (\mu g/ml) = 15.66 \times (A666) - 7.34 \times (A653)$$

$$\text{Chlorophyll b } (\mu g/ml) = 27.05 \times (A653) - 11.21 \times (A666)$$

$$\text{Total carotenoids } (\mu g/ml) = 1000 \times (A470 - 2.86 \times \text{Chl a} - 129.2 \times \text{Chl b})/221$$

Statistical analyses

The data from this study were statistically analyzed using IBM SPSS software (version 25) (SPPS I 2013). To verify if there were statistically significant differences in the growth of *L. fusiformis* TL03 under different concentrations of sodium chloride, the data of each parameter were subjected to One-Way ANOVA and Tukey's HSD (Honest Significant Difference) tests using SPSS with a 95% confidence interval.

Results

Morphological characteristics of the isolated strain

Based on the microscopic examination of the strain isolated from the current study, we recorded several characteristics and morphological features, among which a blue-green color, filamentous form, and unbranched and free-floating trichomes. The filaments are motile, gliding along their axis. The filaments are divided by the cross-wall of the cell into multiple cylindrical cells arranged in trichomes in an open helix with a regular helical coil, gradually narrowing slightly towards the ends (Fig. 3A). Morphological modification from loosely coiled trichomes to tightly reversed coils was observed. The isolated strain showed various degrees of coiling, from loosey coiled S-type (Fig. 3A) to intermediate C-type (Fig. 3B-C) to highly coiled H-type (Fig. 3D)

Cell cross-walls (Fig. 3E1), calyptras at the end of the trichome (Fig. 3E2), and gas vacuoles (Fig. 3F1) were visible under a light microscope.

The results of the measurements of 610 filament morphology are recorded in Table 1 and accompanied by photographs of the morphological features of this cyanobacterium (Fig. 3). The results indicate that the length, diameter, and pitch of the trichome range from 210 to 2027 µm (Fig. 3G-H), 20 to 60 µm (Fig. 3I1-J1), and 30 to 137 µm (Fig. 3I2-J2), respectively The diameters and lengths of the trichome's cells range from 7 to 9 µm (Fig.3K1-L1) and 4 to 6 µm (Fig. 3M1-N1), respectively.

Molecular characteristics of the isolated strain

The molecular studies based on the 16S rRNA gene allowed us to identify the newly isolated strain as *Limnospira fusiformis* TL03 with the accession number MZ970329. The partial sequence (1,411 bp length) of the *Limnospira fusiformis* TL03 16S ribosomal RNA gene was compared to all partial sequences presented in NCBI. MegaBlast searches revealed that the isolated strain matched with 100% similarity to *Limnospira fusiformis* SAG 85.79 (accession number: KM019968.1) (Figure 4).

Effects of different NaCl concentrations on the growth of the isolated *L. fusiformis* TL03

Figure 5 depicts the growth of *L. fusiformis* TL03 from the start to the end of the experiment for all treatments. The *L. fusiformis* TL03 strain demonstrated a high level of salt tolerance. The general trend of the curve shows that the highest optical density values for all treatments occurred on the 20th day, whereas the concentration of NaCl had a significant effect on cell growth across all treatment groups ($P < 0.05$). The maximum optical densities (1.580 ± 0.014 and 1.560 ± 0.003) were obtained in both cultures containing 15 g/l and 5 g/l NaCl, respectively, seven times greater than the initial optical density. In contrast, the lowest optical densities (1.314 ± 0.006 and 1.197 ± 0.003) were recorded in cultures containing 45 and 60 g/l NaCl, respectively.

These results show that when the concentration of sodium chloride increases, the growth of *L. fusiformis* TL03 slows down. We also remarked

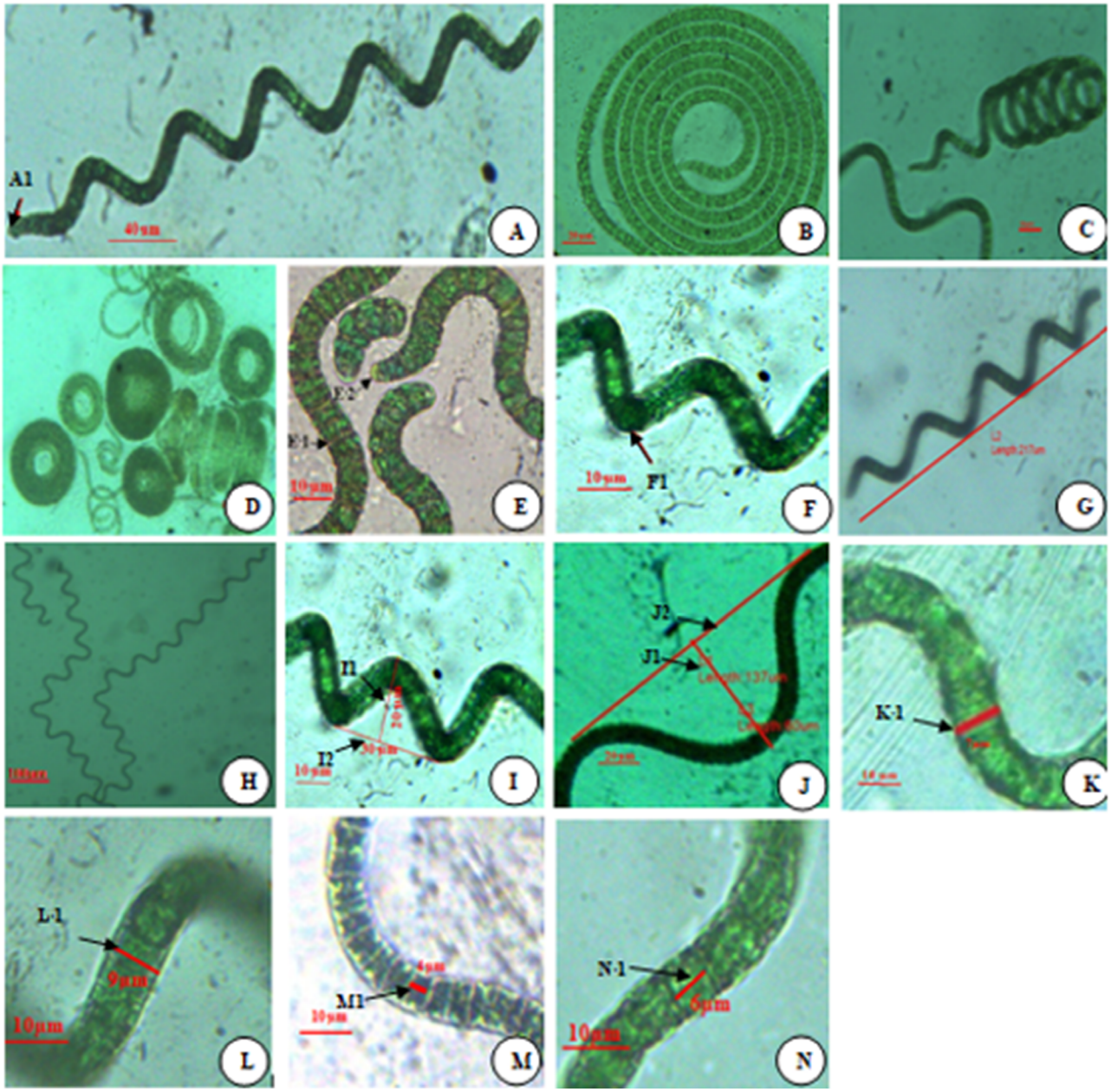

Figure 3. A-N Microscopic view of various morphological features of the isolated *Limnospira fusiformis* TL03 strain. (A.1) Trichome type C with attenuation at the end (100 X). (B-C) Tightly coiled trichome (400 X). (D) Tightly coils H shape (100X). (E.1) Cell cross wall. (E2 2) Caliptra at trichome end (1000 X). (F1) Gas vacuole (1000 X). (G-H) Smallest and tallest trichome with loose coils (100 X). (I1) Small coil diameter (400 X). (I2). Small pitch (400 X). (J1) Large coil diameter (400 X). (J2). Large pitch (400 X). (K1-L1). Cell diameter. (M1. N1) (1000 X) Cell length (1000 X).

that *L. fusiformis* TL03 continued to grow even under NaCl starvation conditions. The results of chlorophyll a content in the *L. fusiformis* TL03 strain subjected to salt stress for 20 days showed a gradual increase among all NaCl concentrations tested (Fig. 6). By comparing the trend of the curves of the effect of salinity on growth and the concentration of chlorophyll, it becomes clear that they follow a similar trend. Growth and chlorophylls (a, b) were negatively affected at the highest salt concentrations. Nevertheless, the TL03 strain was still able to grow until the 20th

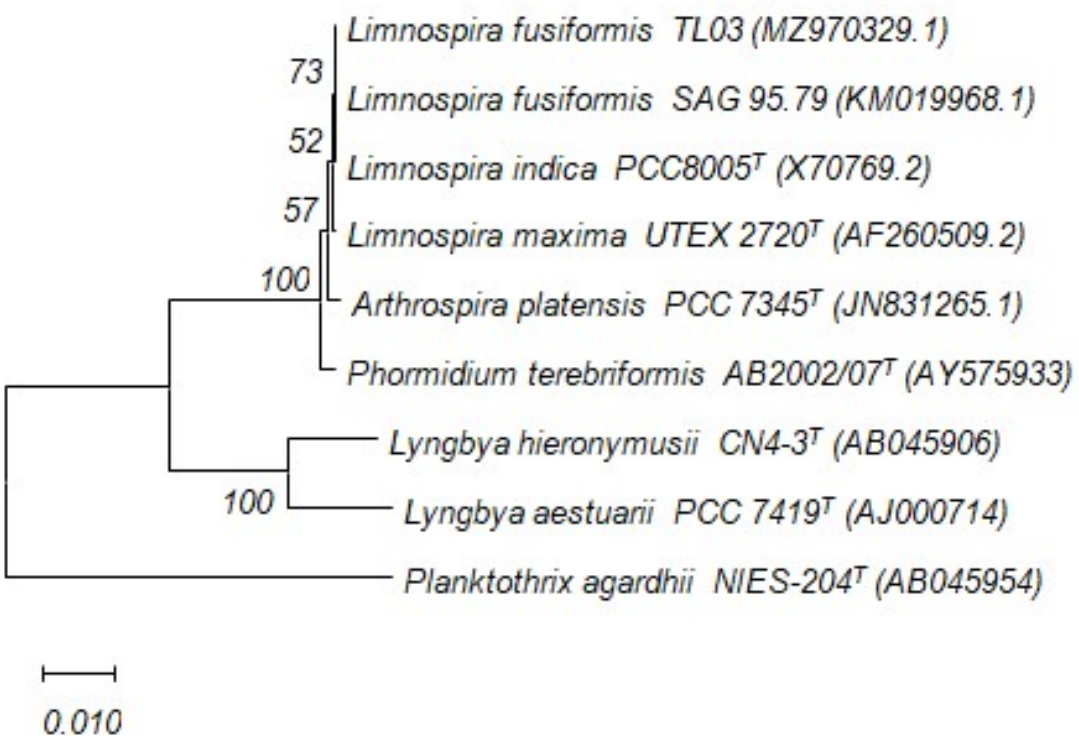

Figure 4. Phylogenetic tree based on 16S rRNA gene sequences showing the relationship between strain TL03 and related type-strains. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site (Bar, 0.01 nt substitution per nt position). The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The percentage of replicate trees (numbers at the nodes) in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. This analysis involved nine nucleotide sequences.

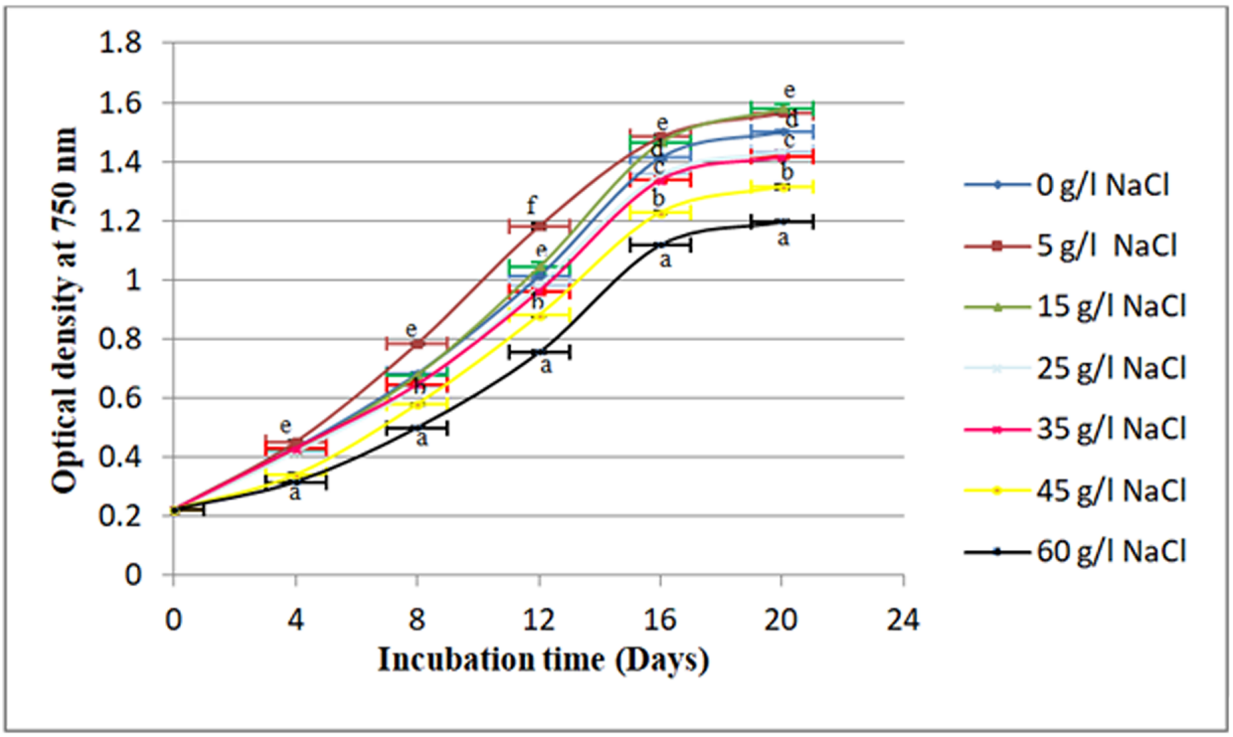

Figure 5. Effect of different NaCl concentrations on the growth of L. fusiformis TL03. Data represent mean values ± SEM (n = 4).

day, which was the end of the experiment. The highest concentrations of chlorophyll a (Fig. 6) and chlorophyll b (Fig. 7) in all tested cultures were observed at the end of the experiment (20th day). The maximum concentrations of chlorophyll a (19.42 ± 0.25 µg/ml) and chlorophyll b (3.52 ± 0.16 µg/ml) were obtained in BG11 containing 15 g/l NaCl. The lowest amounts of Chl *a* (12 ± 0.08 µg/ml) and Chl b (2.22 ± 0.01 µg/ml) were registered in BG11, which contained a high NaCl concentration (60 g/l).

The variation of total carotenoids in the *L. fusiformis* TL03 strain under different NaCl concentrations demonstrated a continuing increase in all treatment groups, reaching a maximum on the 20th day of the experiment, with a significant difference for all treatment media ($P < 0.05$) (Fig. 8). The medium without NaCl yielded the highest number of carotenoids, a 23-fold increase over the initial value. At the same time, carotenoid concentration ranged from the highest value of 1.88 ± 0.02 µg/ml in BG11 without NaCl to the lowest value of 1.10 ± 0.01 µg/ml under high salt stress (60 g/l). The total carotenoid concentration was lower in the TL03 strain cultured at 15 g/l NaCl (control) compared to those cultured at 25 and 35 g/l and was higher compared to those cultured in higher salinity culture media (45 and 60 g/l). On the fourth day of the experiment, the maximum concentrations of carotenoids (0.71 ± 0.02 and 0.71 ± 0.08 µg/ml) were observed in BG11 and BG11 containing 5 g/l of NaCl, respectively.

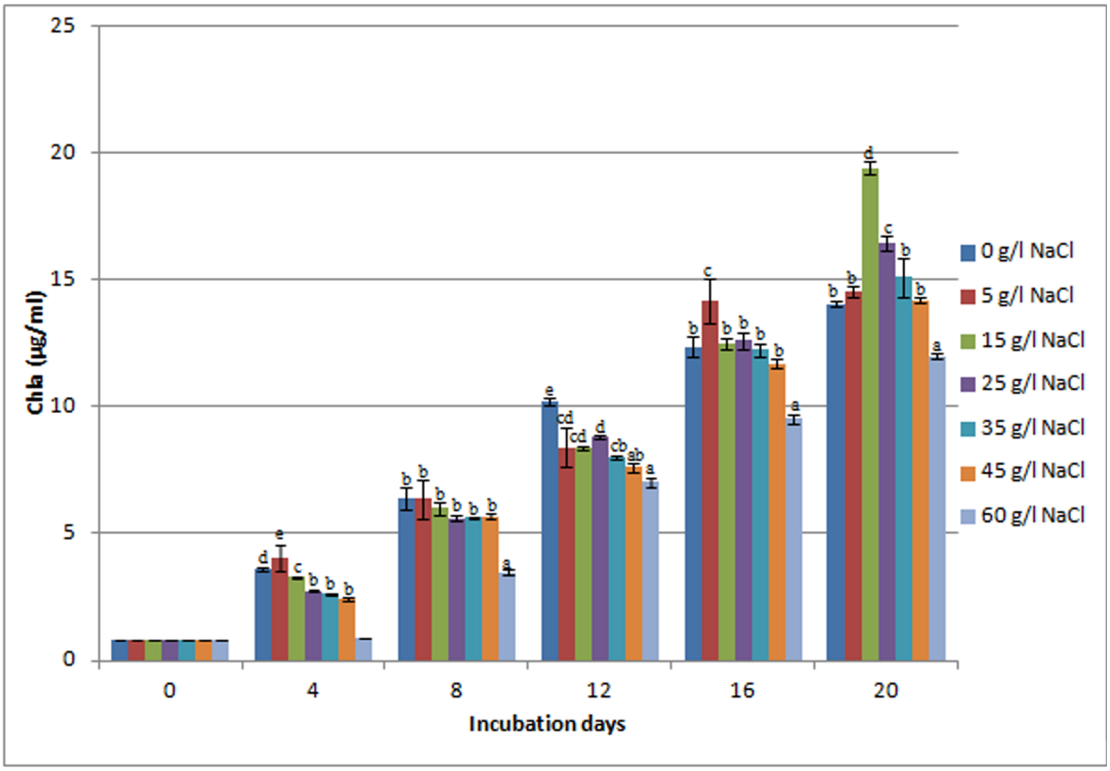

Figure 6. Chlorophyll a amount of *L. fusiformis* TL03 exposed to different NaCl concentrations. Different letters indicate a significant difference ($P < 0.5$). Error bar represents standard error (n = 4).

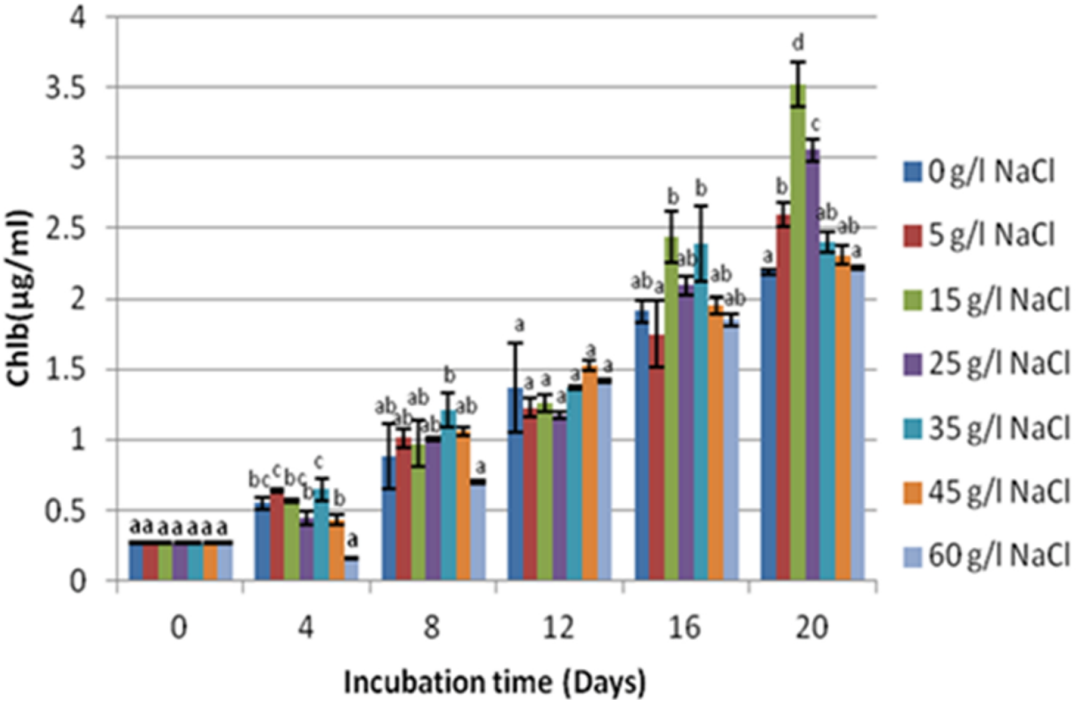

Figure 7. Chlorophyll b amount of *L. fusiformis* TL03 strain exposed to different NaCl concentrations. Different letters indicate a significant difference (P< 0.5). Error bar represents standard error (n = 4).

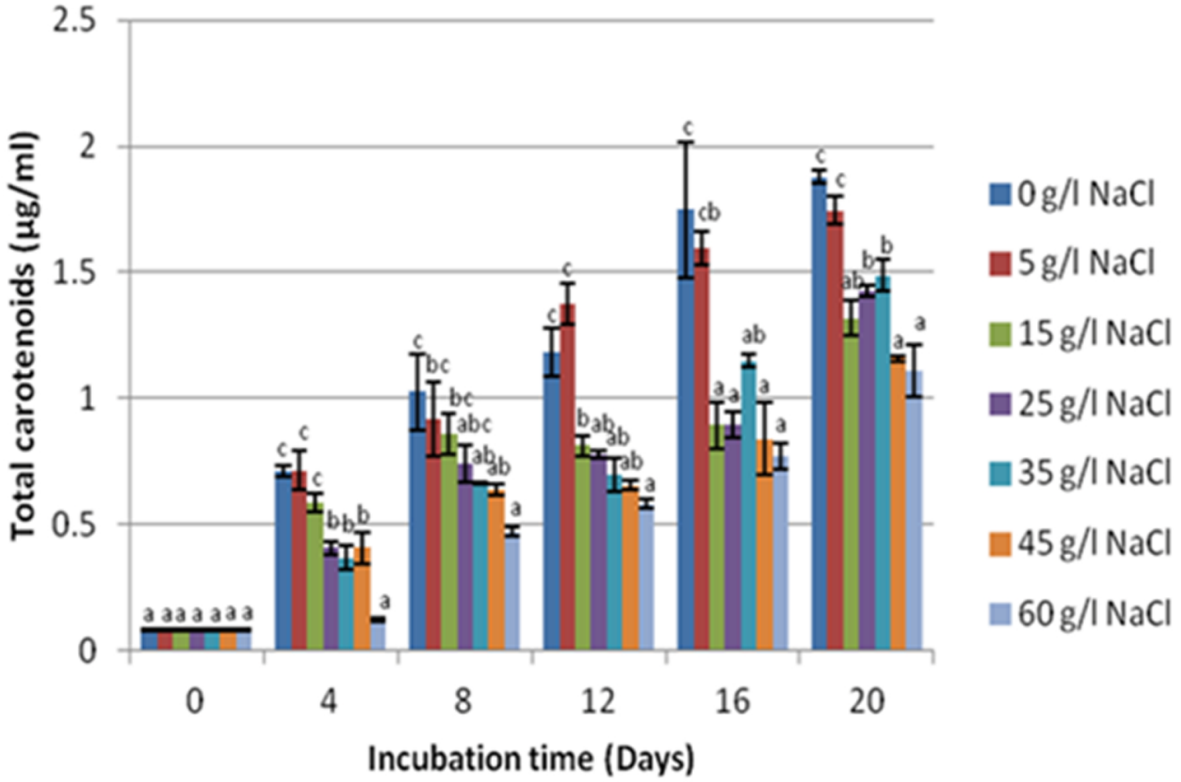

Figure 8. Total carotenoids amount of *L. fusiformis* TL03 strain exposed to different NaCl concentrations. Different letters indicate a significant difference (P< 0.5). Error bar represents standard error (n = 4).

Discussion

The presence of numerous cyanobacteria and microalgae, dominated by the *L. fusiformis* TL03 strain, was revealed by microscopic examination of the sample. This finding is consistent with the findings of Krienitz *et al*. (2005) and Sili *et al*. (2012), who found that in alkaline saline lakes in tropical and subtropical regions, the alkaliphilic filamentous cyanobacterium *Arthrospira* is frequently the dominant taxon and is responsible for permanent or seasonal blooms. In this study, we used the traditional method based on morphological and molecular characteristics to identify the genus of the isolated strain. The arrangement of its multicellular cylindrical trichomes in an open helix, usually of relatively large diameter, sometimes attenuated at the ends, is the main taxonomic criteria for the differentiation of both genus *Arthrospira* and *Limnospira species* (Sili *et al*., 2012).

A morphological study of the isolated strain revealed that there are several morphotypes that most likely belong to the same species. During successive cultures in liquid media, this isolated strain maintained a spiral shape with varying degrees of helicity. The results obtained by measuring trichome length, coil diameter, and pitch, as well as cell diameter and length (Table 2), differ slightly from those reported by Jung *et al*. (2021). Cell diameters of *Arthrospira platensis* grown in a flat-type bioreactor ranged from 5.25 ± 1.26 to 7.5 ± 2.9 µm, and coil diameters ranged from 20 to 60 µm.

Many studies have been conducted to describe the morphological characteristics of *A. fusiformis* and *A. maxima*. The diameter and length of the cells of the latter species range from 7 to 9 µm and 5 to 7 µm, respectively (Sili *et al*., 2012). They form open regular spirals with coil diameters ranging from 40 to 60 µm and pitches ranging from 70 to 80 µm. Coils have regular screw-like shapes at the ends that are slightly attenuated (Gardner, 1917; Fott & Karim, 1973; Komárek & Anagnostidis, 2005; Sili *et al*., 2012). Rout *et al*. (2015) isolated the *Arthrospira* strain NPS-011, which has the same cell trichome length and diameter as previously studied strains. This species has a variable helix pitch ranging from 80 to 150 µm and a helix diameter ranging from 30 to 70 µm. The results for filament length, coil diameter, pitch, cell diameter, and length for our isolate (Table 2) show that they are almost in agreement with those reported in several previous studies (Sanchez *et al*., 2003; Komárek & Anagnostidis, 2005; Rout *et al*., 2015; Cellamare *et al*., 2018; Roussel *et al*., 2023).

Sili *et al*. (2012) noted that the *Arthrospira* helix architecture (pitch and diameter) is highly dependent on growth and environmental conditions under laboratory and mass cultivation conditions. Dhiab *et al*. (2007) found that increasing the concentration of sodium chloride in the culture medium caused *Arthrospira platensis* to change its trichome shape from spiral to straight, as well as its physiological behavior. The salinity and anion content of the culture medium, according to Kebede (1997), influence the helicity degree of *Arthrospira fusiformis*. The authors observed that higher salinity culture media had the longest pitches, with very lax helices in media containing high concentrations of SO_4^{2-} compared to those containing the same concentrations of HCO_3^- and Cl^-.

Table 2. Range of dimensional variation of different measurements of morphology features of Limnospira fusiformis TL03

Morphology features	The number of measurements conducted	Dimension (µm)	
		Minimum	Maximum
Trichome length	610	210	2027
Coil diameter	3000	20	60
Pitch	3000	30	137
Cell diameter	3000	7	9
Cell height	3000	4	6

The phylogenetic trees matched the classifications based on the morphological characteristics of the isolates in this study very well. The data from the 16S rRNA gene sequence analysis confirmed that the isolated strain Tl03 (MZ215991) belongs to *Limnospira fusiformis*, with a 100% similarity to *L. fusiformis* SAG 85.79 (KM019968.1). Despite the high salinity of the culture medium (60 g/l), the *L. fusiformis* TL03 strain showed tolerance and continued growth in culture media with varying salinity rates. These findings are consistent with those reported by Reed & Stewart (1985), Kebede (1997), and Moisander *et al*. (2002), who found that *A. platensis* did not stop growing even at 88 g/l salinity. According to Dadheech *et al*. (2010), *Arthrospira* lives in alkaline waters with pH ranging from 8 to 11 and salinity ranging from 1.1 to 300 g/l.

When compared to other media, the *L. fusiformis* TL03 isolates grew the best in both media containing 5 and 15 g/l NaCl. The salinity of the BG11, which contains 15 g/l NaCl, is thought to be similar to that of our strain's original habitat (Telamine Lake). BG11 with 5 g/l NaCl has the same salinity concentration as when the strain was grown and adapted before being used in this experiment. In terms of optical density, chlorophylls (a, b) levels increased slowly in BG11 containing up to 15 g/l NaCl (control) during incubation compared to a lower salinity medium. These findings differ from those of several previous studies. According to Ayachi *et al*. (2007), *Spirulina platensis* (*A. platensis*) grew the fastest at a NaCl concentration of 1 g/l. Meanwhile, at 60 g/l NaCl, the first five days are latent, followed by a decreasing growth phase until the end of the experiment.

Phang (2002), on the other hand, described an immediate cessation of *A. platensis* growth when exposed to high NaCl concentrations. Slower growth usually follows this stage. Sudhir *et al*. (2005) discovered that a 47 g/l NaCl concentration significantly reduced *A. platensis*'s photosystem II (PS II)-mediated oxygen evolution activity. Sharma *et al*. (2014) reported that increasing the NaCl concentration reduced growth and completely inhibited *A. platensis* chlorophyll a biosynthesis. The total carotenoid content of *L. fusiformis* TL03 grown in a low-salinity medium was significantly higher than in the control medium but lower in higher-salinity media (45 and 60 g/l). This fact could be explained as follows: exposing *L. fusiformis* TL03 to hypersalinity and a slightly higher salinity compared to the *Arthrospira* origin environment induced carotenoids biosynthesis, but the biosynthesis was lower at a higher salinity rate.

This study reported the identification and isolation of a novel strain of cyanobacteria, L. fusiformis TL03, from Telamine Lake in northwest Algeria. This strain was first discovered by Fox (1996) in Tamanrasset Pond, located in the southern region of Algeria. Through the examination of morphological characteristics and comparing the 16S rRNA gene sequence of the isolated strain with other *Arthrospira* or *Spirulina* strains, we verified that the strain in question is indeed a member of the *Limnospira* genus. The strain was designated as *Limnospira fusiformis* TL03 and was submitted to the GenBank database with the accession number MZ215991.1.

The strain exhibited the ability to acclimate to varying levels of salinity, ranging from 0 to 60 g/l NaCl. This adaptability enables its cultivation using seawater, brackish water from lakes, and untapped subterranean saline water sources in Algeria. Additionally, it thrives in alkaline aquatic environments, such as alkaline lakes, where its exposure to contamination from microalgae and other microorganisms is minimized in outdoor cultivation systems such as ponds, basins, tanks, circular ponds, and raceway ponds. This strain exhibits elongated trichomes measuring up to 2027 μm in length, featuring spiral shapes that facilitate efficient harvesting through the filtration method. This method is particularly advantageous for commercial production due to its cost-effectiveness compared to alternative harvesting techniques. Due to its adaptation to the local climatic conditions, this strain can be cultivated in outdoor ponds.

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Authors' Contributions

Guenachi Belkacem, Korteby Mefti Hakima, and Lynda Lamary carried out the study's conception and design. Material preparation, data collection, statistics analysis, and morphological identification were performed by Guenachi Belkacem, Redhouane Benfares, and Khaled Abderrahmani. Genetic analysis by Guenachi Belkacem,, Khaled Boudjema, and Omrane Toumatia. The first draft of the manuscript was written by Guenachi Belkacem, and the English writing was checked by Azmane Bidin and Hafsa Yaiche Achour.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

Abed RM, Dobretsov S, Sudesh K. 2009. Applications of cyanobacteria in biotechnology. Journal of Applied Microbiology 106: 1-12. doi: 10.1111/j.1365-2672.2008.03918.x.

Aladaileh SH, Khafaga AF, Abd El-Hack ME *et al*. 2020. *Spirulina platensis* ameliorates the sub-chronic toxicities of lead in rabbits via anti-oxidative, anti-inflammatory, and immune stimulatory properties. Science of the Total Environment 701: 134879. doi: 10.1016/j.scitotenv.2019.134879.

Allen MM, Stanier RY. 1968. Growth and division of some unicellular blue-green algae. Microbiology 51: 199-202. doi: 10.1099/00221287-51-2-199.

Asulabh KS, Supriya G, Ramachandra TV. 2012. Effect of salinity concentrations on growth rate and lipid concentration in *Microcystis* sp., *Chlorococcum* sp., and *Chaetoceros* sp. In: National Conference on Conservation and Management of Wetland Ecosystems, 6-9 November 2012, Kottayam/Kerala. p 1-7.

Ayachi S, El Abed A, Dhifi W, Marzouk B. 2007. Chlorophylls, proteins, and fatty acids amounts of *Arthrospira platensis* growing under saline conditions. Pakistan Journal of Biological Sciences 10: 2286-2291. doi : 10.3923/pjbs.2007.2286.2291.

Babadzhanov AS, Abdusamatova N, Yusupova FM, Faizullaeva N, Mezhlumyan LG, Malikova MK. 2004. Chemical Composition of *Spirulina platensis* Cultivated in Uzbekistan. Chemistry of Natural Compounds 40: 276-279. doi: 10.1023/B:CONC.0000039141.98247.e8.

Benavente-Valdés JR, Aguilar C, Contreras-Esquivel JC, Méndez-Zavala A, Montañez J. 2016. Strategies to enhance the production of photosynthetic pigments and lipids in Chlorophyceae species. Biotechnology Reports 10: 117-125. doi: 10.1016/j.btre.2016.04.001.

Borowitzka MA. 1995. Microalgae as sources of pharmaceuticals and other biologically active compounds. Journal of Applied Phycology 7: 3-15. doi:10.1007/BF00003544.

Cellamare M, Duval C, Drelin Y *et al*. 2018. Characterization of phototrophic microorganisms and description of new cyanobacteria isolated from the saline-alkaline crater-lake Dziani Dzaha (Mayotte, Indian Ocean). FEMS Microbiology Ecology 94: fiy108. doi: 10.1093/femsec/fiy108.

Cho JA, Baek SY, Cheong SH, Kim MR. 2020. *Spirulina* enhances bone modeling in growing male rats by regulating growth-related hormones. Nutrients 12: 1187. doi: 10.3390/nu12041187.

Choi WY, Kang DH, Lee HY. 2013. Enhancement of immune activation activities of *Spirulina maxima* grown in deep-sea water. International Journal of Molecular Sciences 14: 12205-12221. doi: 10.3390/ijms140612205.

Costa JAV, Colla LM, Duarte Filho PF. 2004. Improving *Spirulina platensis* biomass yield using a fed-batch process. Bioresource Technology 92: 237-241. doi:10.1016/j.biortech.2003.09.013.

Costa NB, Kolman MA, Giani A. 2016. Cyanobacteria diversity in alkaline saline lakes in the Brazilian Pantanal wetland: A polyphasic approach. Journal of Plankton Research 38: 1389-1403. doi: 10.1093/plankt/fbw066.

D'Alessandro EB, Antoniosi Filho NR. 2016. Concepts and studies on lipid and pigments of microalgae: A review. Renewable and Sustainable Energy Reviews 58: 832-841. doi: 10.1016/j.rser.2015.12.162.

Da Silva SC, Fernandes IP, Barros L *et al*. 2019. Spray-dried *Spirulina platensis* as an effective ingredient to improve yogurt formulations: Testing different encapsulating solutions. Journal of Functional Foods 60: 103427. doi: 10.1016/j.jff.2019.103427.

Dadheech PK, Ballot A, Casper P *et al*. 2010. Phylogenetic relationship and divergence among planktonic strains of *Arthrospira* (Oscillatoriales, Cyanobacteria) of African, Asian and American origin deduced by 16S–23S ITS and phycocyanin operon sequences. Phycologia 49: 361-372. doi: 10.2216/09-71.1.

Dhiab RB, Ouada HB, Boussetta H, Franck F, Elabed A, Brouers M. 2007. Growth, fluorescence, photosynthetic O_2 production and pigment content of salt adapted cultures of *Arthrospira* (*Spirulina*) *platensis*. Journal of Applied Phycology 19: 293-301. doi: 10.1007/s10811-006-9113-z.

Falaise C. 2019. Valorisation des activités biologiques de la diatomée marine *Haslea ostrearia*. PhD Thesis, Universite Maine, French.

Felsenstein J. 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution 39: 783-791. doi: 10.2307/2408678.

Fott B, Karim AG. 1973. *Spirulina* plankton community in a lake in Jebel Marra, Sudan. Archiv für Protistenkunde 115: 408-418.

Fox RD. 1996. *Spirulina:* production & potential. Aix-en-Provence, Edisud.

Furmaniak MA, Misztak AE, Franczuk MD, Wilmotte A, Waleron M, Waleron KF. 2017. Edible cyanobacterial genus *Arthrospira*: Actual state of the art in cultivation methods, genetics, and application in medicine. Frontiers in Microbiology 8: 2541. doi: 10.3389/fmicb.2017.02541.

Gardner NL. 1917. New Pacific coast marine I. University of California Publications in Botany 6: 377-416.

Ghaeni M, Roomiani L. 2016. Review for application and medicine effects of *Spirulina*, microalgae. Journal of Advanced Agricultural Technologies 3: 114-117. doi: 10.18178/joaat.3.2.114–117.

Hadjadj M. 2018. Suivi des indicateurs de pollution dans une zone aride à semi-aride: Diagnostic des environs de Gdyel suite aux dépôts des déchets ménagers et rejet d'eau usée au niveau du lac Télamine. Magister Thesis, Université d'Oran, Algeria.

Hirooka S, Higuchi S, Uzuka A, Nozaki H, Miyagishima SY. 2014. Acidophilic green alga *Pseudochlorella* sp. YKT1 accumulates high amount of lipid droplets under a nitrogen-depleted condition at a low-pH. *PLoS One* 9: e107702. doi: 10.1371/journal.pone.0107702.

Jiménez C, Cossí o BR, Labella D, Niell FX. 2003. The feasibility of industrial production of *Spirulina* (*Arthrospira*) in Southern Spain. Aquaculture 217: 179-190. doi: 10.1016/s0044-8486(02)00118-7.

Jung CH, Braune S, Waldeck P, Küpper JH, Petrick I, Jung F. 2021. Morphology and Growth of *Arthrospira platensis* during Cultivation in a Flat-Type Bioreactor. Life 11: 536. doi: 10.3390/life11060536.

Jung F, Krüger-Genge A, Waldeck P, Küpper JH 2019. *Spirulina platensis*, a super food. Journal of Cellular Biotechnology 5: 43-54. doi: 10.3233/JCB-189012.

Kaggwa MN, Burian A, Oduor SO, Schagerl M. 2013. Ecomorphological variability of *Arthrospira fusiformis* (Cyanoprokaryota) in African soda lakes. Microbiology Open 2: 881-891. doi: 10.1002/mbo3.125.

Kebede E. 1997. Response of *Spirulina platensis* (*Arthrospira fusiformis*) from Lake Chitu, Ethiopia, to salinity stress from sodium salts. Journal of Applied Phycology 9: 551-558. doi: 10.1023/A:1007949021786.

Komárek J, Anagnostidis K. 2005. Cyanoprokaryota. 2. Teil: Oscillatoriales. In: Büdel B, Gärtner G, Krienitz L, Schagerl M (eds.). Süßwasserflora von Mitteleuropa, Bd. 19/2. München, Elsevier GmbH. p 341-353.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution 35: 1547-1549. doi: 10.1093/molbev/msy096.

Koyandea AK, Chewa KW, Rambabub K, Taoc Y, Chud DT, Showa PL. 2019. Microalgae: A potential alternative to health supplementation for humans. Food Science and Human Wellness 8: 16-24. doi: 10.1016/j.fshw.2019.03.001.

Krienitz L, Ballot A, Casper P, Kotut K, Pflugmacher S, Wiegand C. 2005. Toxic cyanobacteria and massive deaths of flamingos: What we can learn from *Arthrospira* mass–cultivation. Phycologia 44: 59-59.

Lane D. 1991. 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds.). Nucleic acids techniques in bacterial systematics. New York, Wiley. p. 115-147.

Lau E, Nash CZ, Vogler DR, Cullings KW. 2005. Molecular diversity of cyanobacteria inhabiting coniform structures and surrounding mat in a Yellowstone hot spring. Astrobiology 5: 83-92. doi: 10.1089/ast.2005.5.83.

Memije-Lazaro IN, Blas-Valdivia V, Franco-Colín M, Cano-Europa E. 2018. *Arthrospira maxima* (*Spirulina*) and C-phycocyanin prevent the progression of chronic kidney disease and its cardiovascular complications. Journal of Functional Foods 43: 37-43. doi: 10.1016/j.jff.2018.01.013.

Moisander PH, McClinton E, Paerl HW. 2002. Salinity effects on growth, photosynthetic parameters, and nitrogenase activity in estuarine planktonic cyanobacteria. Microbial Ecology 43: 432-442. doi: 10.1007/s00248-001-1044-2.

Mukhopadhyay CD. 2015. Engineering *Spirulina* for Enhanced Medicinal Application. In: Algal Biorefinery: An Integrated Approach. New York, Springer. p. 235-252. doi: 10.1007/978-3-319-22813-6_11.

Nowicka-Krawczyk P, Mühlsteinová R, Hauer T. 2019. Detailed characterization of the *Arthrospira* type species separating commercially grown taxa into the new genus *Limnospira* (Cyanobacteria). Scientific Reports 9: 1-11. doi: 10.1038/s41598-018-36831-0.

Nübel U, Garcia-Pichel F, Muyzer G. 1997. PCR primers to amplify 16S rRNA genes from cyanobacteria. Applied and Environmental Microbiology 63: 3327-3332. doi: 10.1128/aem.63.8.3327-3332.1997.

Phang SM. 2002. *Arthrospira* Stizenb. ex Gomont. Plant Resources of South–East Asia 15: 88-94.

Pulz O, Gross W. 2004. Valuable products from biotechnology of microalgae. Applied Microbiology and Biotechnology 65: 635-648. doi: 10.1007/s00253-004-1647-x.

Rastogi RP, Sinha RP. 2009. Biotechnological and industrial significance of cyanobacterial secondary metabolites. Biotechnology Advances 27: 521-539. doi: 10.1016/j.biotechadv.2009.04.009.

Reed RH, Stewart WDP. 1985. Osmotic adjustment and organic solute accumulation in unicellular cyanobacteria from freshwater and marine habitats. Marine Biology 88: 1-9. doi: 10.1007/bf00393037.

Roussel T, Halary S, Duval C *et al*. 2023. Monospecific renaming within the cyanobacterial genus *Limnospira* (*Spirulina*) and consequences for food authorization. Journal of Applied Microbiology 134: 1-15. doi: 10.1093/jambio/lxad159.

Rout NP, Khandual S, Gutierrez-Mora A, Ibarra-Montoya JL, Vega-Valero G. 2015. Divergence in three newly identified *Arthrospira* species from Mexico. World Journal of Microbiology and Biotechnology 31: 1157-1165. doi: 10.1007/s11274-015-1865-7.

Sanchez-Baracaldo P, Hayes PK, Blank CE. 2005. Morphological and habitat evolution in the Cyanobacteria using a compartmentalization approach. Geobiology 3: 145-165. doi: 10.1111/j.1472-4669.2005.00050.x.

Sanchez M, Bernal-Castillo J, Rozo C, Rodríguez I. 2003. *Spirulina* (*Arthrospira*): An edible microorganism: A review. Universitas Scientiarum 8: 7-24.

Sharma G, Kumar M, Ali MI, Jasuja ND. 2014. Effect of carbon content, salinity and pH on *Spirulina platensis* for phycocyanin, allophycocyanin and phycoerythrin accumulation. Microbial and Biochemical Technology 6: 202-206. doi: 10.4172/1948-5948.1000144.

Sili C, Torzillo G, Vonshak A. 2012. *Arthrospira* (*Spirulina*). In: Ecology of Cyanobacteria II. Dordrecht, Springer. p. 677-705. doi: 10.1007/978-94-007-3855-3_25.

Sotiroudis TG, Sotiroudis GT. 2013. Health aspects of *Spirulina* (*Arthrospira*) microalga food supplement. Journal of the Serbian Chemical Society 78: 395-405. doi: 10.2298/JSC121020152S.

SPSS I. 2013. IBM SPSS statistics for windows. New York/Armonk, IBM SPSS.

Sudhir PR, Pogoryelov D, Kovács L, Garab G, Murthy SD. 2005. The effects of salt stress on photosynthetic electron transport and thylakoid membrane proteins in the cyanobacterium *Spirulina platensis*. BMB Reports 38: 481-485. doi: 10.5483/bmbrep.2005.38.4.481.

Sudhir P, Murthy SDS. 2004. Effects of salt stress on basic processes of photosynthesis. Photosynthetica 42: 481-486. doi: 10.1007/S11099-005-0001-6.

Sydney EB, Schafranski K, Barretti BRV *et al*. 2019. Biomolecules from extremophile microalgae: From genetics to bioprocessing of a new candidate for large-scale production. Process Biochemistry 87: 37-44. doi: 10.1016/j.procbio.2019.09.012.

Tamura K, Nei M, Kumar S. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proceedings of the National Academy of Sciences 101: 11030-11035. doi: 10.1073/pnas.0404206101.

Thajuddin N, Subramanian G. 2005. Cyanobacterial biodiversity and potential applications in biotechnology. Current Science 89: 47-57.

Varshney P, Mikulic P, Vonshak A, Beardall J, Wangikar PP. 2015. Extremophilic micro-algae and their potential contribution in biotechnology. Bioresource Technology 184: 363-372. doi: 10.1016/j.biortech.2014.11.040.

Villaró S, Morillas-España A, Acién G, Lafarga T. 2022. Optimisation of operational conditions during the production of *Arthrospira platensis* using pilot-scale raceway reactors, protein extraction, and assessment of their techno-functional properties. Foods 11: 2341. doi: 10.3390/foods11152341.

Volkmann H, Imianovsky U, Oliveira JL, Sant'Anna ES. 2008. Cultivation of *Arthrospira* (*Spirulina*) *platensis* in desalinator wastewater and salinated synthetic medium: Protein content and amino-acid profile. Brazilian Journal of Microbiology 39: 98-101. doi: 10.1590/S1517-838220080001000022.

Wellburn AR. 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. Journal of Plant Physiology 144: 307-313. doi: 10.1016/S0176-1617(11)81192-2.

Wu Q, Liu L, Miron A, Klímová B, Wan D, Kuča K. 2016. The antioxidant, immunomodulatory, and anti-inflammatory activities of *Spirulina*: An overview. Archives of Toxicology 90: 1817-1840. doi: 10.1007/s00204-016-1744-5.