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Dedication (Amani)

الحمد لله حبا و شكرا و امتنانا ما كنت لافعل هذا لولا فضل الله فالحمد لله على البدا و الختام

ها انا اليوم اهدي نجاحي الى كل من سعى معي لاتمام هذه المسيرة :

الى الذي دعمني بلا حدود و اعطاني بلا مقابل الى الذي بذل جهد السنين من اجل ان اعتلي سلالم النجاح
الى من احمل اسمه بكل فخر الى من حصد الاشواك عن دربي ليمهد لي طريق العلم

(ابي الغالي).

الى من اضائت في ليالي العتمة طريقي الى من افنت عمرها في سبيل ان احقق طموحي و ابلغ اعلى
المراتب الى من سهرت و ساندت الى من ضلت دعواتها تضم اسمي

(امي الحبيبة).

الى خيرة ايامي و صفوتها الى ضلعي الثابت و امان ايامي الى من يعلو حبهم فوق كل حب الى نوري
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.الحمد لله الذي بنعمته تتم الصالحات، والصلاة والسلام على سيدنا محمد وعلى آله وصحبه أجمعين

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أتقدم إليكم بأسمى آيات الشكر والتقدير على كل ما قدمتموه لي من حب ورعاية والدعم اللامحدود خلال مسيرتي العلمية والحياتية. لقد كنتم السند الحقيقي والدافع الأكبر لي في كل خطوة خطوتها، وبدون دعمكم وتشجيعكم لما تمكنت من تحقيق .هذا الإنجاز

.يا والدَيّ العزيزين، شكراً لصبركم وتضحياتكم، ولحبكم الذي يملأ قلبي قوة وأملًا. أنتم من زرع في نفسي روح المثابرة والاجتهاد، وبفضل دعائكم المستمر كان النجاح حليفًا لي

.وإلى جدتي الحبيبة، التي كانت دومًا مصدر الحنان والحكمة، شكراً لدفء كلماتك وتشجيعك الذي أضاء لي الطريق في أصعب اللحظات. وجودك في حياتي هو نعمة لا تقدر بثمن

.أدعو الله أن يبارك في أعماركم، ويجعل هذا العمل ثمرة جهودكم ودعائكم، وأن يديم علينا المحبة والود والنجاح في كل مراحل حياتنا

واتوجه أيضا بكلمات يعتصرها الشوق والوفاء الى روح جدي الغالي الذي كان رمزا للمعرفة ولكن شاءت الاقدار ان يغادر دنيانا قبل ان تكتحل عيناه برؤية هذا الإنجاز فلولا غرسك الطيب لما اينعت هذه الثمار رحمك الله رحمة واسعة وجعل هذا العمل في ميزان حسناتك

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كما أعبر عن خالص تقديري وامتناني لمشرفتي (مطروح الرميصاء) ، التي بفضل توجيهاتها السديدة، وصبرها اللامحدود، ونصائحها القيمة، أصبح بحثي أكثر عمقًا وإتقانًا. لقد كان دعمها الأكاديمي والمعنوي حجر الأساس في نجاح .هذا العمل

.ولا يفوتني أن أشكر جيهان التي ساعدتني في عملي المخبري فكانت شريكة حقيقية في الحياة العملية

في ختام هذا الفصل المعرفي المليء بالتحديات أجد من الواجب ان أقف لحظة امتنان لنفسي لما بذلته من جهد وما تحملته من صبر

اشكر نفسي التي لم تنكفئ امام العوائق ولم تستسلم امام الانهاك بل تماسكت كلما تعثرت ونهضت كلما خذلتها الظروف وظلت ساعية بان النور يستحق العناء وان للمعرفة ضريبة لا يدفعها الا من امن بقيمتها وهكذا مع اسدال الستار على هذه المرحلة العلمية لا أرى ختاما يليق سوى لحظة تأمل و اعتراف. لقد كانت المرحلة أكثر من مجرد من بحث أكاديمي كانت مرآة للذات وامتحان للإرادة و مجال للنمو الإنساني و العقلي و ن كان النجاح ان يقاس بما تعلمته عن انفسنا في طريق .الوصول

وختاما امضي لله أولا الذي بلطفه تتم النعم وبتوفيقه تتحقق الغايات فلولا عونه وستره لما وفقت في إتمام هذا الجهد و لا اجتزت ما اعترض طريقي من عقبات اساله جل في علاه ان يجعل هذا العمل خالصا لوجهه الكريم نافعا للناس مباركا في اثره وان يرزقني دوما التواضع في العلم و صدقا في المسعى.

Aknowlegment

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Abstract:

Marine macroalgae and their associated microorganisms are increasingly recognized as valuable sources of natural bioactive compounds. This study investigates the antibacterial potential of various algal specimens and the microbial communities naturally associated with them, collected from diverse aquatic environments in northeastern Algeria. The work combines morphological analysis, microbiological isolation, and antimicrobial assays to evaluate their biological activity.

Algal samples were cultivated and analyzed under controlled laboratory conditions. Bacterial strains were isolated from the surface of algae and tested for their ability to inhibit selected pathogenic microorganisms. In addition, the direct antibacterial effect of the algal material itself was assessed using standardized *in vitro* techniques.

The results demonstrated that both the algae and their associated bacteria possess significant antibacterial properties. Several isolates produced measurable zones of inhibition, while algal extracts also exhibited inhibitory effects against common human pathogens. These findings confirm the presence of bioactive substances and suggest a potential synergistic interaction between algae and their microbiota. Environmental factors such as salinity and nutrient conditions appeared to influence the expression of these bioactivities.

This study provides new insight into the antimicrobial potential of algae–bacteria associations and reinforces the value of marine biodiversity as a source for natural therapeutic agents. It also highlights the ecological and biotechnological importance of studying aquatic microbial communities in underexplored regions.

Key word: Marine macroalgae, associated bacteria, antibacterial properties, salinity, bioactivities.

Résumé:

Les macroalgues marines et les microorganismes qui leur sont associés sont de plus en plus reconnus comme des sources précieuses de composés bioactifs naturels. Cette étude évalue le potentiel antibactérien de divers spécimens d'algues ainsi que des communautés microbiennes qui leur sont naturellement associées, collectés dans différents environnements aquatiques du nord-est de l'Algérie. Le travail combine l'analyse morphologique, l'isolement microbiologique et des tests d'activité antimicrobienne pour évaluer leur activité biologique.

Les échantillons d'algues ont été cultivés et analysés dans des conditions de laboratoire contrôlées. Des souches bactériennes ont été isolées à partir de la surface des algues et testées pour leur capacité à inhiber des micro-organismes pathogènes sélectionnés. Parallèlement, l'effet antibactérien direct du matériel algal a été évalué à l'aide de techniques in vitro standardisées.

Les résultats ont démontré que les algues et les bactéries qui leur sont associées possèdent des propriétés antibactériennes significatives. Plusieurs isolats ont produit des zones d'inhibition mesurables, tandis que les extraits d'algues ont également montré des effets inhibiteurs contre des pathogènes humains courants. Ces résultats confirment la présence de substances bioactives et suggèrent une interaction synergique potentielle entre les algues et leur microbiote. Des facteurs environnementaux tels que la salinité et les conditions nutritionnelles semblent influencer l'expression de ces bioactivités.

Cette étude offre un nouvel éclairage sur le potentiel antimicrobien des associations algues–bactéries et renforce la valeur de la biodiversité marine comme source d'agents thérapeutiques naturels. Elle met également en évidence l'importance écologique et biotechnologique de l'étude des communautés microbiennes aquatiques dans des régions encore peu explorées.

Mots clés : macroalgues marines, bactéries associées, propriétés antibactériennes, salinité, bioactivités.

الملخص :

تُعدّ الطحالب البحرية والكائنات الدقيقة المرتبطة بها من المصادر الواعدة للمركّبات النشطة حيويًا ذات الأهمية البيوتكنولوجية والطبية. تهدف هذه الدراسة إلى تقييم القدرة المضادة للبكتيريا لعينة من الطحالب ومجتمعاتها الميكروبية الطبيعية، التي جُمعت من بيئات مائية متنوعة في شمال شرق الجزائر. اعتمد البحث على التحليل المورفولوجي، والعزل الميكروبيولوجي، واختبارات النشاط المضاد للميكروبات لتقييم الفعالية البيولوجية.

تمت زراعة عينات الطحالب وتحليلها في ظروف مخبرية خاضعة للرقابة. كما تم عزل سلالات بكتيرية من على سطح الطحالب واختبار قدرتها على تثبيط نمو بعض البكتيريا الممرِضة. إضافة إلى ذلك، تم تقييم التأثير المضاد للبكتيريا للمادة الطحلبية نفسها باستخدام تقنيات مخبرية موحدة.

أظهرت النتائج أن الطحالب والكائنات الدقيقة المرتبطة بها تتمتعان بخصائص مضادة للبكتيريا مهمة. حيث أظهر عدد من العزلات مناطق تثبيط واضحة، كما أظهرت مستخلصات الطحالب تأثيرًا مثبطًا ضد بعض مسببات الأمراض الشائعة. تؤكد هذه النتائج وجود مركبات نشطة حيويًا، وتشير إلى احتمال وجود تفاعل بين الطحالب وميكروبيوتها. كما أن العوامل البيئية مثل الملوحة والظروف الغذائية يبدو أنها تؤثر على تعبير هذه الأنشطة الحيوية.

تقدم هذه الدراسة رؤى جديدة حول الإمكانيات المضادة للميكروبات في التفاعلات بين الطحالب والبكتيريا، وتعزز من أهمية التنوع البيولوجي البحري كمصدر للعلاجات الطبيعية. كما تسلط الضوء على الأهمية البيئية والبيوتكنولوجية لدراسة المجتمعات الميكروبية المائية في المناطق التي لم تُدرس بشكل كافٍ.

الكلمات المفتاحية: الطحالب البحرية الكبيرة، والبكتيريا المرتبطة بها، والخصائص المضادة للبكتيريا، والملوحة، والنشاطات الحيوية.

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ABBREVIATION	Meaning
ADH	Argenine Dihydrolase
AMY	Amygdalin fermentation
API	Analytic profile index
ARA	Arabinose fermentation
BG11	Bleu-Green medium 11
BN	Nutrient broth
CIT	Citrate utilization test
EMA	Enriched sea water
EPS	Exopolysaccharides
FFA	Free fatty acids
GEL	Gelatinase test
GLU	Glucose fermentation
H2S	Hydrogen sulfide production
IND	Indole test
INO	Inositol fermentation
LDC	Lysine Decarboxylase
MAN	Mannitol fermentation
MEL	Melibiose fermentation
MH	Muller-Hinton medium
ODC	Ornithine Decarboxylase
ONPG	Ortho-nitrophenyl- β-galactoside
RHA	Rhamnose fermentation
SAC	Sucrose fermentation
SOR	Sorbitol fermentation
TDA	Tryptophan Deaminase test
URE	Urease test
VP	Voges-Proskauer test

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General Introduction

Marine ecosystems are among the most diverse and productive environments on Earth, with macroalgae (seaweeds) playing a fundamental role in ecological balance, nutrient cycling, and the foundation of aquatic food webs. Marine macroalgae are not only primary producers but also hosts to a complex community of microorganisms, including bacteria, that contribute significantly to their health, development, and chemical defense. These microbial associations can influence algal morphology, resilience, and even the production of bioactive compounds with biotechnological and pharmaceutical interest.

This study aims to explore the biological potential of marine macroalgae and their associated bacteria, particularly their antibacterial activity. In an era of increasing antibiotic resistance, the search for new natural antimicrobial agents is more critical than ever. Seaweeds and their microbial partners represent a largely untapped source of novel compounds that may help address this global challenge.

The topic of this research the evaluation of biological activities from algae and their coexisting bacteria was chosen due to the growing interest in marine resources as alternatives to synthetic chemicals and antibiotics. Algae are known for producing a wide array of secondary metabolites, and their symbiotic bacteria may also contribute to their bioactive profiles. Investigating this partnership is both scientifically valuable and potentially applicable in pharmaceutical, environmental, and agricultural fields.

Despite the known benefits of macroalgae and their microbiomes, there remains a lack of comprehensive understanding of their joint antimicrobial potential. Most existing studies focus on either the algae or the bacteria in isolation, without considering their interactions. Additionally, the microbial biodiversity of Algerian coastal environments, particularly in regions like Annaba and El Tarf, is underexplored.

This research is built around the following core objectives:

- To isolate and identify bacteria associated with various algal samples from selected Algerian coastal sites.
- To evaluate the antibacterial activity of both the algae and their associated bacterial strains against selected pathogenic microorganisms.
- To assess environmental parameters (e.g., salinity, pH, conductivity) influencing algal and microbial characteristics.
- To contribute to the understanding of algae–bacteria interactions and their potential use in biotechnology and medicine.

The study is guided by the following hypotheses:

- Marine macroalgae and their coexisting bacteria possess significant antibacterial properties.
- Environmental conditions influence the nature and strength of the bioactivities observed.
- There exists a functional symbiosis between algae and bacteria that enhances their collective antimicrobial potential.

The present work is divided into several chapters. The first chapter provides a literature review on macroalgae, their classification, ecological roles, and interactions with microorganisms. The second chapter addresses the concept of allelopathy in aquatic environments and its relevance to algal biology. The third section presents the materials and

methods used for sample collection, microbial isolation, and activity testing. Finally, the results are discussed in light of the initial hypotheses, followed by a general conclusion summarizing key findings and suggesting future research directions.

In light of these considerations, the present research seeks to answer the following key question:

Can marine macroalgae and their associated bacteria serve as reliable sources of antibacterial agents, and how are these bioactivities influenced by environmental factors and microbial interactions?

Literature Review

Chapter I

Diversity and ecological roles of macroalgae and their associated microbiome

1. Generalities:

Algae are photosynthetic organisms that exhibit a diverse range of forms, from unicellular species to large multicellular varieties. Marine algae encompass various types, typically divided into two primary categories: microalgae and macroalgae. Microalgae, like phytoplankton, live suspended in the water column, while macroalgae, commonly referred to as seaweed, includes large multicellular marine algae that can thrive independently of a supporting structure. Some species can reach lengths of up to 60 meters. These non-vascular plants serve as the primary producers in oceanic ecosystems and belong to the kingdom Protista rather than Plantae. Large seaweeds can rise from the ocean floor, forming extensive underwater forests, and they are capable of adapting to various environments, including small tidal rock pools along the shore and depths several kilometers offshore, where they receive adequate light for photosynthesis. Marine algae are classified into three distinct groups based on their pigmentation, which absorbs different wavelengths of light and imparts their characteristic green, brown, or red colors, along with variations in anatomy, morphology, and biochemical composition. The total number of algae species globally is estimated to be around 50,000, constituting approximately 10% of the total plant population. Marine algae encompass a diverse range of species among marine organisms. A recent analysis suggests that approximately 72,500 algal species have been documented worldwide**.** (Indira Gandhi National Open University, n.d.)

2. Macroalgae classification:

Macroalgae are classified into three categories:

- red (Rhodophyta)
- green (Chlorophyta)
- brown (Ochrophyta)

While these groups are evolutionarily distinct, they are interconnected through endosymbiotic events that led to the formation of plastids. In the Mediterranean Sea, there are approximately 660 species of red algae, 180 species of green algae, and 280 species of brown algae. Due to the absence of roots, only a limited number of species, such as *Caulerpa spp*., can thrive on sedimentary bottoms.

- The red coloration of Rhodophyta is primarily due to the abundance of pigments like phycoerythrin and phycocyanin, which overshadow other pigments, including chlorophyll a, beta-carotene, and various xanthophylls.
- The green color of Chlorophyta results from chlorophyll a and b, which mask accessory pigments such as beta-carotene and different characteristic xanthophylls.
- The brown color of Ochrophyta arises from the predominance of the xanthophyll pigment fucoxanthin, which conceals other pigments, such as chlorophyll a and c, beta-carotene, and xanthophylls. Long-lived brown macroalgae from the genus *Cystoseira* play a crucial role in the Mediterranean benthic ecosystem, as their three-dimensional structures provide habitat and shelter for smaller algae, invertebrates, and fish. Currently, the degradation of benthic ecosystems is viewed as one of the most

significant threats to the macroalgal biodiversity of the Mediterranean. (Indira Gandhi National Open University, n.d.)

3. Distribution of Algae in Different Environmental Areas:

Algae are widely distributed in different environments, ranging from aquatic systems to terrestrial soils, depending on different factors:

3.1.Aquatic algae:

Most algae thrive in aquatic environments, and they quickly dry out and perish in the absence of water. The type of water body varies based on salt concentration, resulting in categories such as freshwater, brackish water, seawater, brine-salt lakes, and salt pans. Furthermore, these habitats are increasingly subjected to various pollutants, including excess organic matter, heavy metals, pesticides, and industrial effluents, which are produced and discharged by human activity. This contamination significantly impacts algae and other organisms living in the water.

3.1.1. Fresh water habitats:

In gently flowing rivers with rocky shorelines, filamentous algae such as *Spirogyra*, *Oedogonium*, and *Cladophora* can often be found forming large floating mats that are typically attached to submerged boulders. The surfaces of these underwater rocks also display various types of adherent epiphytic algae, including diatoms, desmids. The composition of the algal flora in these areas varies seasonally, influenced by factors like water turbidity, flow rates, and other environmental conditions. In lakes, different regions host distinct algal communities. A variety of epiphytic algae, including *Chaetophora*, *Coleochaete*, and desmids, coexist with unicellular and colonial algae such as *Chlamydomonas*, *Volvox*, *Pandorina*, *Scenedesmus*, and *Euglena*, as well as diatoms like *Microcystis* and *Anabaena*. These algae are usually small and exhibit phototactic behavior, moving up and down in response to light conditions floating during the day and sinking at night. When water conditions are rich in nutrients and optimal temperature and sunlight are present, certain algal species like *Microcystis* and *Euglena* can multiply rapidly, dominating other algal types and causing harmful algal blooms. These blooms pose risks to aquatic life, as they can deplete oxygen levels in the water during the night.

3.1.2. Marin habitat:

The ocean is home to the largest variety of algae, collectively referred to as seaweed. Despite India's extensive coastline, rich marine flora is primarily found in the rocky regions of states such as Gujarat, Tamil Nadu, and Andhra Pradesh, as well as in the Andaman and Lakshadweep Islands. The seacoast experiences periodic flooding and exposure to sunlight due to tidal movements. The area between the high tide and low tide marks is known as the intertidal zone. Seaweeds that thrive in this zone endure alternating periods of drying and wetting, and they are securely anchored to the underlying rocks using holdfasts. Occasionally, they may become detached and drift in open waters, as seen in the Sargasso Sea. On the coasts of India, particularly in the Gulf of Mannar (Tamil Nadu), various seaweed species can be collected manually, including *Gracilaria edulis* (red alga), *Gelidiella acerosa* (red alga), *G. folifera, G. crassa, Hypnea musciformis* (red alga), *H. valentiae, H. pannosa, Sargassum*

wightii (brown algae), and *Turbinaria* (brown algae), many of which hold commercial significance. Benthic algae, which make up the seaweed attached to the ocean floor in deeper waters, are always submerged and never exposed to air. Their distribution is influenced by the depth of the sea and the amount of light that can penetrate, with seaweed beds found at depths of 100-200 meters predominantly containing red algae. This is because only these algae can utilize the blue wavelengths of light absorbed by their red pigment, phycoerythrin. (Indira Gandhi National Open University, n.d.)

3.2.Soil algae:

In soil, several hundred algal species are classified into three major groups: green algae, yellow-green algae (*Xanthophyta*), and diatoms (*Bacillarophyta*). Some algae are motile and can be found a few centimeters below the surface, although earthworm activity and rainwater infiltration may relocate them deeper into the soil. Algal populations typically range from 10,000 to 100,000 cells per gram of soil. Being photosynthetic, algae play a crucial role in enriching the soil with organic matter. Additionally, they secrete polysaccharides that promote soil particle aggregation. Green algae tend to thrive in moist, non-flooded, acidic soils, whereas diatoms prefer well-drained soils rich in organic matter. Upon death or desiccation, these algae, along with other soil microorganisms, form a surface crust that helps prevent wind erosion and contributes further organic matter to the soil.

4. Function and Important Species of algae:

Marine macroalgae are valuable sources of highly regulated bioactive compounds and offer a novel avenue for pharmaceutical production. They contain bioactive components that can inhibit the growth of various Gram-positive and Gram-negative bacterial pathogens. Numerous compounds extracted from these microalgae have demonstrated a range of biological activities, including antibacterial, antioxidant, anti-inflammatory, anticoagulant, antiviral, anti-apoptotic, and probiotic effects. Additionally, macroalgae are recognized as significant sources of dietary fiber, essential nutrients, lipids, omega-3 fatty acids, essential amino acids, polysaccharides, and various vitamins. Many bioactive substances derived from aquatic organisms have been experimentally investigated to thoroughly assess the biological impacts of newly developed pharmaceuticals. Seaweeds are particularly rich in compounds such as polysaccharides, tannins, flavonoids, phenolic acids, and bromophenols, which contribute to numerous biological processes and exhibit varying solubility and polarity characteristics. (Rashad et al., 2020)

5. Seaweed associated microbiome:

The structure and composition of the surface-associated microbiome (SAM) evolve over time and are influenced by both the internal state of the host (whether healthy or stressed) and external environmental factors, which may vary between locations. Gaining a better understanding of SAM composition and dynamics can help uncover the mechanisms behind seagrass responses to environmental changes across various spatial and temporal scales, ultimately supporting effective ecological and industrial management.

Microbial communities shift over different timeframes from daily fluctuations to changes occurring over the course of a year often in response to seasonal variations. This seasonal succession is driven by biotic factors, such as the growth and maturation cycle of the host and microbial interactions (both on the seaweed surface and in surrounding seawater), and abiotic factors like water levels and rising summer temperatures. However, the effects of temperature shifts on seaweeds and their associated microbes remain debated. For instance, *Laminaria hyperborea* experiences increased mud accumulation in the summer, which is thought to support the growth of specific associated microbial communities. (Ren et al., 2022)

6. The chemical relationships that arise between certain bacteria and algae:

Chemical interactions play a crucial role in the relationships between bacteria and algae, with macroalgal surfaces hosting secondary metabolites and extracellular exopolymers that can be broken down by associated bacteria. These bacteria can degrade algal sugars like alginate, cellulose, and mannitol, contributing to the decay of algal fronds and fostering specific macroalgal-bacterial interactions. Research indicates that macroalgae are rich in bioactive compounds that exhibit antifouling activity against colonizing organisms, serving both as defense mechanisms and as triggers for specific interactions. Algae lacking their own chemical defenses depend on the secondary metabolites from their associated bacteria, as fouling organisms negatively impact growth and reproduction, leading to evolutionary pressure that favors the development of protective mechanisms against biofilms (Goecke et al., 2010)

6.1.Antibacterial Activities of Bacteria Associated with Macroalgae:

Metabolites released from macroalgae have been shown to directly affect planktonic bacteria, with studies indicating that species like *Ulva clathrata* can inhibit the fish and mussel pathogen *Vibrio anguillarum* without reducing the overall population of heterotrophic bacteria. This inhibitory effect may be due to unknown chemical substances released by *U.clathrata* or produced by associated microorganisms. Additionally, research has demonstrated that polycultures with red macroalgae, such as *Gracilaria textorii*, can control the population of various Vibrio species. Notably, after introducing V. parahaemolyticus into cultures of *Grateloupia turuturu,* the bacterium experienced inhibited growth and ultimately disappeared.

Marine macroalgae often host bacteria like Bacillus and *Pseudoalteromonas spp*., which produce biologically active compounds. For instance, three strains of *Pseudoalteromonas* isolated from *Ulva lactuca* were able to inhibit the growth of multiple bacteria and fungi. One strain, P. tunicata, prevents biofouling by releasing at least five specific compounds, including a large antibacterial protein and violacein, which inhibits protozoan grazing. This diverse chemical production is crucial for the survival of *P. tunicata* in competitive marine environments. While antibiotic-producing bacteria are significant in marine surface communities, further research is needed to fully understand how these bacteria protect their hosts and the specific compounds they produce under natural conditions. (Goecke et al., 2010)

6.2.Chemical Defenses of Macroalgae against Microorganisms:

Macroalgae, which lack traditional immune systems, rely on antimicrobial defense mechanisms based on the production of bioactive secondary metabolites such as fatty acids, phenols, and terpenes. These compounds serve to deter microbial colonization, reduce epibiosis, inhibit decomposition, and increase resistance to diseases. While antifouling and antibacterial activities of macroalgal extracts have been known since 1917, their ecological functions are still not fully understood. Some algae release antifouling substances into the seawater, while others retain antigrazing compounds within their tissues. Recent studies have shown that these defenses can be induced by microbial threats; for example, the red algae *Laurencia majuscula* produces selective antibacterial compounds, and *Kappaphycus alvarezii* shows increased levels of halogenated metabolites during disease outbreaks. Additionally, *Laurencia obtusa* exhibits vesicle transport responses to bacterial biofilms, suggesting a dynamic interaction between macroalgae and microbial communities aimed at preventing fouling and infection. (Goecke et al., 2010)

6.3.Oxidative Burst: The Antibacterial Defense Mechanism of Macroalgae:

Macroalgae utilize oxidative burst as a non-specific antibacterial defense mechanism, similar to higher plants, characterized by the rapid activation of reactive oxygen species that eliminate pathogens. This response is triggered by cell recognition of signal molecules from invading organisms, with common elicitors including oligosaccharides, glycoproteins, and glycopeptides. Recent studies have identified additional compounds like methyl jasmonate and free fatty acids that provoke oxidative bursts in species such as *Laminaria digitata*. Research indicates that degradation of cell wall polysaccharides by pathogen-released enzymes can elicit these defenses, particularly in oligo alginate-rich brown algae. Furthermore, red macroalgae like *Solieria chordalis* demonstrate a constitutive release of hydrogen peroxide to thwart bacterial biofilm formation. The presence of bacterial components as exogenous elicitors in brown macroalgae has been documented, suggesting defense pathways in marine macroalgae resemble those in animals and land plants. The oxidative burst also triggers secondary defense responses, leading to the production of volatile halogenated compounds and increased brominating activity. (Goecke et al., 2010)

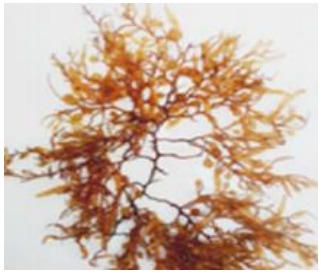
7. Different types of bacteria that coexist with algae:

Phylogenetic studies of epiphytic bacteria have shed light on the intricate bacterial communities associated with macroalgae. The bacterial communities that inhabit marine macroalgae differ in both the number and composition of species compared to those found in seawater. In many instances, the epiphytic bacterial communities exhibit a high level of specificity, with certain microbes consistently identified as epiphytes. It has been suggested that the physiological and biochemical characteristics of macroalgae play a critical role in determining the composition of the microbial communities that adhere to them. Various species of marine algae, even when growing under similar environmental conditions, host distinct bacterial communities that vary in both abundance and composition. (Goecke et al., 2010).

Furthermore, algal-bacterial interactions encompass a wide range of potential symbiotic relationships. Algae, along with heterotrophic bacteria and archaea, serve as primary producers and decomposers, respectively, forming the foundational structure and functionality of the ecosystem. While the main role of heterotrophic bacteria is decomposition, it is increasingly recognized that some bacterial species also contribute to promoting algal growth, thereby establishing mutualistic interaction. (Ramanan et al., 2016).

Table 01: Macroalgae as a Source of Novel Bacterial Species

Macroalgae	**Bacterial species**
Chlorophyta: **1/*Acrosiphonia sonderi*:**  (https://exigus.webnode.hu/spirulina/)	➢ *Algibacter lectus* ➢ *Formosa agariphila* ➢ *Mesonia algae* ➢ *Pibocella ponti* ➢ *Winogradskyella epiphytica* ➢ *Zobellia russellii*
2/*Ulva fenestrata Ruprecht*:  **(https://www.inaturalist.org/taxa/54644-Ulva)**	➢ *Algibacter lectus* ➢ *Arenibacter certesii* ➢ *Arenibacter palladensis* ➢ *Maribacter ulvicola.* ➢ *Pseudozobellia thermophila* ➢ *Roseivirga ehrenbergii*
3/Ulva lactuca:  (https://www.ima.gov.tt/2022/08/03/ulva-lactuca/)	➢ *Ulvibacter litoralis* ➢ *Pseudoalteromonas ulvae*

Ochrophyta: ***1/Sargassum Natans:***  (https://www.researchgate.net/figure/The-biomasses-covered-in-this-study-are-sargassum-natans)	➢ *Leucothrix sargassi* ➢ *Altermonas* ➢ *Flavobacterales* ➢ *Roseobacter*
Rhodophyta: ***1/Asparagopsis Taxiformis :***  (https://marinebiodiversity.org.bd/species/asparagopsis-taxiformis/)	➢ *Pseudoalteromonas* ➢ *Halomonas vibirio* ➢ *Cyanobacteria* ➢ *Bacteroidetes* ➢ *Actinobacteria*

8. The importance of the relationships that algae form with specific types of bacteria:

The relationship between bacteria and algae is largely symbiotic, providing mutual benefits that are essential for their growth and survival.

8.1.**Mutualism:**

Where algae provide organic compounds for bacteria, and bacteria supply essential nutrients, protect against toxins, and contribute to the overall growth and health of algal species. This intricate interaction is vital for ecosystem stability and productivity.

- **Nutrient Exchange:** Algae and bacteria engage in a beneficial relationship where both organisms contribute to nutrient cycling. Algae produce organic compounds and oxygen through photosynthesis, which bacteria utilize for their growth.
- **Organic Matter Mineralization:** In turn, bacteria mineralize organic substrates, releasing carbon dioxide, minerals, and growth factors that are beneficial for algae. This reciprocal interaction promotes the overall health of both organisms.

- **Nitrogen Fixation:** Marine epiphytic bacteria serve as a significant nitrogen source for algae. Specific cyanobacteria are known to supply fixed nitrogen to certain algal species, such as *Codium*. Notable nitrogen-fixing bacteria, such as *Dichothrix fucicola* and *Azotobacter sp*., have been found associated with various macroalgae, enhancing their nitrogen supply.
- **Facilitation of Invasive Species:** The presence of these nitrogen-fixing bacteria may also contribute to the successful invasion of harmful macroalgae, like *Caulerpa taxifolia*, in nutrient-poor environments by providing the necessary nutrients for growth.
- **Protection against Toxic Substances:** Bacteria also play a protective role for macroalgae by detoxifying harmful substances, including heavy metals. They can transform these toxins into less harmful forms through processes such as precipitation and transformation, thereby safeguarding algal health.
- **Support for Growth:** Bacteria are essential in promoting the growth of macroalgae by producing growth factors, including phytohormones and biostimulators that are crucial for cell growth and development.
- **Specific Examples:** The bacterium *Pseudoalteromonas porphyrae* is specifically mentioned as promoting the growth of *Laminaria japonica*, showcasing the direct influence of certain bacterial species on algal health.
- **Hormone Production:** Research indicates that marine bacteria associated with macroalgae produce higher quantities of cytokinin-type and auxin-type hormones compared to bacteria found in planktonic environments. This suggests that the presence of macroalgae enhances bacterial productivity in terms of growth factors.
- **Conversion of Tryptophan:** Bacteria associated with *Ulva spp*. can convert tryptophan into indole-3-acetic acid (IAA), a key phytohormone that influences plant growth. This biochemical interaction underscores the role of bacteria in modifying nutrient availability for algae.
- **Gall Formation and Bacterial Influence**: In *Prionitis lanceolata*, the formation of galls abnormal growths has been linked to bacteria from the *Roseobacter* group, which increases IAA production in these galls compared to the surrounding algal tissue. This illustrates how bacteria can influence algal morphology and growth patterns.
- **Potential Coevolution:** Although the specific physiological roles of bacteria in promoting macroalgal health are not completely understood, the interactions suggest a potential coevolution between the two groups, indicating a long-standing relationship that benefits both parties. Also, bacteria influence the morphology and life cycle of macroalgae, particularly marine foliaceous green macroalgae like *Ulva spp*.
- **Influence on Morphology and Life Cycle:** Bacteria are crucial for maintaining the typical structure and morphology of macroalgae. For example, *Ulva spp*. exhibit structural changes when cultured in the absence of bacteria, indicating that bacterial presence is essential for their proper growth and development.
- **Similar Effects in Other Algae:** Changes in morphology due to the absence of bacteria have also been observed in red macroalgae such as *Dasya pedicellata* and *Polysiphonia urceolata*, further emphasizing the importance of bacterial interactions across different algal species.

- **Restoration of Morphology:** The morphology of these macroalgae can be restored by adding specific marine bacteria or their culture filtrates. Particularly, bacteria from the *Bacteroidetes phylum,* including *Cytophaga* and *Flavobacterium spp*., are noted for their effectiveness in this regard.
- **Morphogenic Effects:** Morphogenic effects have been documented with various bacterial genera, including *Caulobacter, Vibrio, Pseudomonas, Deleya, Escherichia*, and certain Gram-positive bacteria. This indicates a broad range of bacteria contributing to algal morphology.
- **Mutual Dependence:** The text suggests that these bacteria lose their morphogenic capabilities when cultured alone for several generations but can regain them when co-cultivated with Ulva in synthetic mineral media. This highlights a mutual dependence on the metabolites produced by both the algae and the bacteria, indicating a synergistic relationship that benefits both organisms. (Ramanan et al., 2016)

8.2.**Parasitism:**

A parasite is an organism that lives on or within another organism (the host), obtaining nutrients at the host's expense. This definition sets the context for understanding the dynamics between parasites and algae.

- **Hosts for Parasites:** Phytoplankton and seaweeds, which rely on photosynthesis for their energy, serve as suitable hosts for various parasites, including plankton parasites that can rapidly evolve and reproduce within host cells.
- **Opportunistic Microorganisms:** Opportunistic microorganisms, such as certain protists, infect algae to exploit their metabolites. This parasitic relationship negatively impacts the fitness of the algae and alters their microbiome.
- **Bacteria and Viruses as Pathogens:** In addition to protozoan parasites, bacteria and viruses are identified as significant marine pathogens that can parasitize algae, further complicating the interactions within marine ecosystems.
- **Oomycete Infections:** Oomycete species, which can be either obligate or generalist parasites, are capable of infecting various host algae. They have distinct cell walls compared to fungi and may hijack the biosynthetic functions of their algal hosts for their own growth.
- **Host-Jumping Capability:** Generalist parasites, such as the *perkinsid Parvilucifera*, can survive across multiple host species by evolving through host-jumping, which contributes to the dynamics and resilience of marine ecosystems

The relationship between parasites and algae plays a significant role in shaping aquatic ecosystems:

- **Regulation of Algal Populations:**

Selective Pressure: Parasites can selectively target weaker or more susceptible algal individuals, which helps maintain the overall health of algal populations. By removing fewer fit individuals, parasites can prevent overcrowding and promote genetic diversity, leading to more resilient algal communities.

- **Enhancement of Ecosystem Health:**

Healthy Herd Selection: The concept of healthy herd selection suggests that while parasitism may reduce the number of certain algal individuals, it does not necessarily decrease overall algal biomass. Instead, it can enhance the health and functionality of the entire algal population, contributing to a more stable ecosystem.

- **Nutrient Cycling:**

Facilitation of Organic Matter Transfer: Parasites can facilitate the transfer of organic matter from larger algal hosts to smaller organisms, such as zooplankton, through processes like the "mycoloop" and the fungal shunt. This transfer enhances nutrient cycling and remineralization in the aquatic environment, making nutrients available to other organisms.

- **Influence on Food Web Dynamics:**

Increased Trophic Chain Length: The presence of parasites can lead to changes in the structure of plankton communities and increase the length of the trophic chain. This can enhance the complexity and stability of food webs, providing more niches and resources for various organisms.

- **Impact on Biodiversity:**

Promoting Biodiversity: By influencing which algal species thrive, parasites contribute to the overall biodiversity of the aquatic ecosystem. A diverse algal community can support a wider range of herbivores and higher trophic levels, thereby enhancing ecosystem stability.

- **Resistance to Environmental Changes:**

Adaptation and Resilience: The interactions between algae and their parasites can drive evolutionary adaptations, allowing algal populations to become more resilient to environmental stresses, such as climate change, pollution, or nutrient loading.

- **Potential Negative Effects:**

Pathogen Spread: While parasites can have beneficial effects, they can also pose risks. If pathogenic bacteria proliferate excessively, they may lead to harmful algal blooms or negatively impact fish and other aquatic organisms, disrupting the ecosystem balance. (Ramanan et al., 2016)

8.3.**Commensalism:**

Commensalism is a relationship where one partner benefits while the other is neither helped nor harmed, distinct from mutualism.

- **Underexplored Relationship:** While algal-bacterial associations are often classified as mutualistic or parasitic, commensalism has been less studied.
- **Environmental Influences:** The structure of algal-bacterial communities and their interactions are significantly influenced by environmental factors, particularly nutrient availability (such as phosphorus).

- **Recent Findings:** Some studies suggest that certain bacteria may act as commensals within the algal sheath, highlighting the potential significance of commensalism in algal-bacterial relationships shaped by environmental conditions. (Ramanan et al., 2016)

9. Ecology application:

Seaweeds serve as vital ecological engineers, contributing significantly to primary production and offering food and shelter to aquatic organisms. Their symbiotic relationship with epiphytic bacteria enhances seaweed growth, stress resilience, and biomass yield, which can be used in biofuels, fertilizers, and animal feeds. These seaweed microorganism holobionts also support the bioremediation of organic pollutants and heavy metals. While microbial interactions with microalgae are well-studied, research on seaweed-associated microorganisms remains limited despite their high potential in biodegrading hydrocarbons and chemical pollutants. Seaweed cell walls, rich in compounds like alginate and fucoidan, enable efficient heavy metal biosorption, with species such as *Sargassum filipendula* and *Osmundea pinnatifida* showing promising results. Though the exact microbial mechanisms of metal stress resistance are still being explored, microbial consortia appear more effective than single strains in enhancing both algal resilience and wastewater treatment, offering benefits for environmental sustainability and agriculture. (Ren et al., 2022).

10. The application of seaweed and their related microorganisms in organic pollutant remediation:

Many studies have focused on the use of algae–bacteria consortia for the bioremediation of metals and the degradation of organic pollutants. This research has shown that algal–bacterial interactions can effectively degrade harmful organophosphate insecticides like monocrotophos, quinalphos, and methyl parathion, as well as other toxic pesticides such as DDT and atrazine. Additionally, algae and bacteria play a significant role in treating various organic pollutants, including black oil and phenol. Recent findings have highlighted the potential of these interactions in degrading thiocyanate wastewater, showcasing their utility in addressing toxic substrates.

Algae require certain metals in small amounts for growth and metabolism, acting as micronutrients. When deprived of these essential metals, algae display specific stress responses. However, excessive metal levels can be toxic, prompting algal–bacterial communities to work together to detoxify and assimilate these metals from contaminated environments. This accumulation occurs through various mechanisms, including physical adsorption and ion exchange, while some metals are actively taken up for metabolic processes or defensive strategies. The growth of microalgae also leads to the release of metal chelators and pH increases, which can precipitate heavy metals absorbed by associated bacteria. (Ramanan et al., 2016)

The applications of seaweeds in bioremediation of polluted water and soil, as well as in environmental monitoring, are vital in mitigating the impacts of industrialization, urbanization, and agriculture on natural ecosystems. Toxic metals and organic compounds from various industrial activities pose significant environmental hazards, as they are persistent, non-degradable, and bioaccumulative. Contaminated soil and water bodies, particularly due to pesticides and heavy metals, require effective remediation strategies to ensure environmental sustainability.

To address contamination, several remediation technologies have been developed, including biosorption with low-cost adsorbents and phytoremediation techniques. Seaweeds, or marine macroalgae, from different phyla, are emerging as promising candidates for these technologies due to their ecological significance and rapid growth. They contribute oxygen to marine ecosystems and are key components of the food chain, acting as primary producers. As effective tools in reducing water and soil contamination, seaweeds can play a crucial role in agricultural sustainability and environmental biotechnology (Michalak, 2020).

11.Co-friendly and low-cost remediation method:

Humanity is increasingly aware of the urgent need to protect the environment due to the harmful effects of pollution. Industrial sectors particularly manufacturing and mining significantly contribute to environmental degradation through the use of toxic substances and pollutant-generating technologies. This situation is further intensified by global population growth and the rising demand for goods and services, which lead to excessive consumption and exploitation of natural resources. In response, a major shift is underway toward cleaner production methods that use environmentally friendly technologies, aiming to reduce ecological impacts while still supporting economic growth. Among these innovations, biotechnology offers promising tools to combat pollution. One key approach is bioremediation, which uses living organisms such as plants, bacteria, and fungi to degrade or transform hazardous contaminants in the environment. Bioremediation has proven to be a more cost-effective and sustainable alternative to traditional chemical treatments.

By implementing bioremediation strategies, industries can effectively reduce environmental damage while supporting long-term goals for sustainable development. (Acuña et al., 2020)

Recent advancements have led to the creation of eco-friendly adsorbents made from natural and renewable materials, focusing on sustainability, environmental safety, and reusability. These green adsorbents are derived from agricultural waste, bio-based polymers, clays, zeolites, and recycled materials, offering low toxicity and biodegradability. Their development not only reduces dependency on non-renewable resources but also contributes to lower carbon emissions and waste valorization. The goal is to efficiently treat both organic and inorganic pollutants, making them a promising tool for sustainable waste management and pollution control.

In parallel, marine eutrophication a widespread issue can be mitigated through seaweed cultivation in coastal waters. Seaweeds absorb excess nutrients like nitrogen and phosphorus, thus controlling harmful algal blooms and improving water quality. Successful farming depends on factors such as light, temperature, nutrients, salinity, depth, and water movement.

Seaweeds not only sequester carbon and release oxygen but also reduce the need for fertilizers and pesticides. They can be reused in agriculture or converted into valuable bioproducts, aligning environmental and economic benefits. In countries like China, large-scale cultivation of species such as *Ulva*, *Cladophora, Gracilaria*, and *Porphyra* has proven effective in removing high nutrient loads from eutrophic waters. These fast-growing algae with rich nutrient content support sustainable aquaculture and contribute to healthier marine ecosystems. (Ren et al., 2022)

12.Environmental Remediation of Toxic Heavy Metals:

Bioremediation of toxic metals from wastewater by algae can be carried out through biosorption (using non-living organisms) or bioaccumulation (using living cells) The former is known to be much faster and effective than the latter where higher concentrations of pollutants in wastewater can cause the death of living organisms. Therefore, biosorption, as a subcategory of adsorption is more often used for the removal of contaminants from wastewater than bioaccumulation. Biosorption is also recognized as one of the tools of environmental biotechnology. This process is used when other technologies such as chemical precipitation, coagulation, membrane separation, electrochemical reduction fail e incomplete metal removal especially when the concentration of metal ions is low and ranges from 1 to100 mg/L or are too expensive Biosorbents of metal ions used in this process should be widely available, renewable, cost-effective and environment friendly. (Goswami et al., 2021).

Seaweeds have very good biosorption properties, which result from the presence of macromolecules (e.g., polysaccharides, proteins) in the algal cell wall which offer different functional groups such as amino, hydroxyl, carboxyl and sulfate, capable of binding metal ions Macroalgae can efficiently remove different pollutants, such as dyes from paper, textile and printing industry, nitrogen, phosphorous and phenolic compounds (e.g., from aquaculture), and heavy metals from various sources. Ion exchange is indicated as a principal mechanism for biosorption of metal ions by dry macroalgal biomass e light metal ions from seawater such as calcium, magnesium, sodium, potassium naturally bound to the algal functional groups are exchanged with metal ions from wastewater. A similar analysis was performed for metal ions, which are the most often selected for biosorption by seaweeds. (Ren et al., 2022)

13.Biosorption by algal biochar:

Biosorption by seaweed biochar involves utilizing waste algal biomass either as a biosorbent directly or converting it into biochar through pyrolysis, a thermochemical process that produces carbon-rich "biological charcoal." Seaweed biochar is recognized for its applications in carbon sequestration, soil improvement, nutrient retention, and wastewater treatment, making it a sustainable alternative in clean technology. Biochar derived from seaweeds cultivated in unpolluted waters is free from excessive toxic elements, enhancing its suitability for soil amendment. The production of biochar from various waste seaweed biomass, generated during oil or agar extraction, promotes better waste management and increases the sustainability of macroalgae cultivation.

Research shows that seaweed biochar is effective in removing heavy metal ions due to its high content of alkali and alkaline earth metals, which contribute to its cation exchange capacity. The pyrolysis temperature significantly influences the biosorption properties, with higher temperatures yielding better removal efficiencies for pollutants like Cd (II), Cu (II), and Zn (II). Additionally, algal biochar has demonstrated superior sorption capacities compared to raw algal biomass, making it a promising candidate for wastewater treatment systems and a cost-effective alternative to commercial activated carbon. (Michalak, 2020)

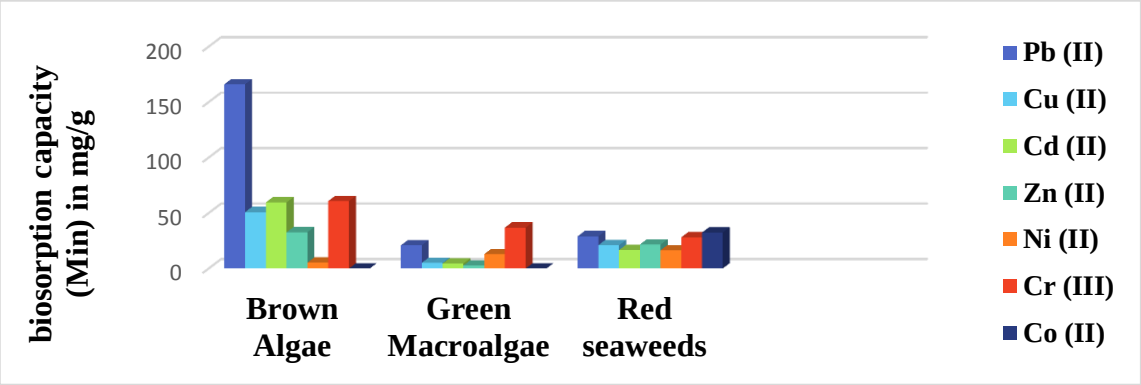


Figure 01: The biosoption capacities (Min) of various seaweed species for the removal heavy metal.

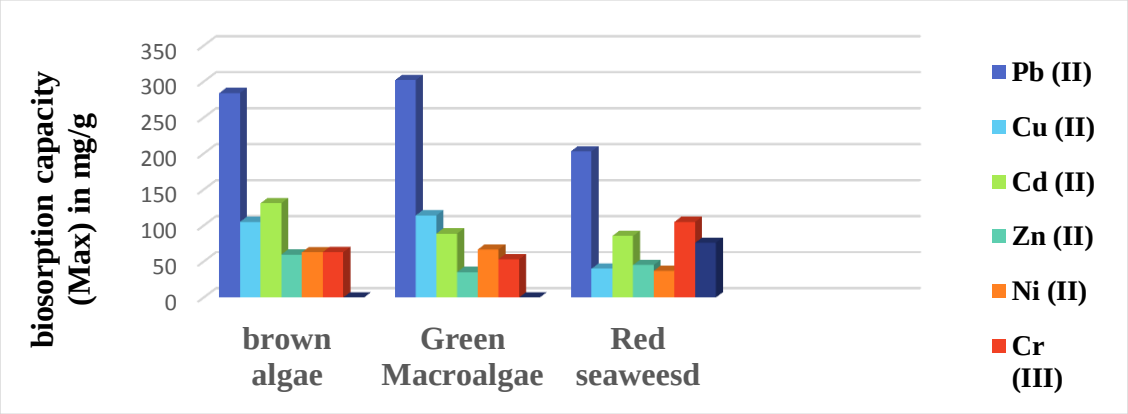


Figure 02: The biosoption capacities (Max) of various seaweed species for the removal heavy metal ions

Chapter II

Allelopathy in aquatic and marine environments

1. Defining Allelopathy:

Allelopathy is a biological phenomenon where an organism produces biomolecules that influence the growth, survival, and reproduction of other organisms. Introduced by Molisch in 1937, the term describes how one plant can affect another, rooted in the Greek words "allelos" (mutual) and "pathos" (suffering). Historically observed by Theophrastus around 3000 BC, allelopathy was initially viewed negatively, but its definition has evolved to encompass both harmful and beneficial effects resulting from the release of compounds into the environment. The International Allelopathy Society defines it as the study of secondary metabolites from plants, algae, bacteria, and fungi that impact agricultural and biological systems. These compounds, termed allelochemicals, can either support or hinder the growth of target organisms.

The discussion of allelopathy also involves the terms "allelochemistry" and "bio-communicator," which refer to the chemical exchanges among living organisms. These exchanges can govern various behaviors, including defense mechanisms and social interactions. There is ongoing debate about the distinction between allelopathy and competition for resources, as differentiating the two in natural systems is challenging. (Chaïb et al., 2021).

Allelopathy in aquatic and marine environments:

The eco-physiological process of allelopathy, while recognized by scientists, remains controversial in aquatic systems due to the complexities of demonstrating its effects in the field. Methodological challenges in studying chemical interactions among species in freshwater and marine ecosystems contribute to the underestimation of allelopathy in marine plant competition. This dynamic and complex process involves the analysis of chemical and biological variables, making detailed investigations essential. Despite its potential significance, ecological models often overlook allelochemistry, which is crucial for understanding interspecies interactions. Researchers view allelopathic interactions as either competition for resources or as defense mechanisms. Algal allelopathy can manifest in various ways, including inhibiting the growth of competing algae, self-toxicity, and affecting other microorganisms and higher plants.

Microalgal allelopathy can negatively impact predator performance, especially during toxic algal blooms, suggesting that allelochemical production plays a vital role in algal competition. Some microalgae, such as *Chrysochromulina* and *Prymnesium* species, utilize phagotrophy to enhance their growth by ingesting particles like bacteria and other microalgae. Although there is no evidence that phagotrophy is directly triggered by allelopathy, the latter contributes to environmental stability by promoting coexistence among competitive phytoplankton species. The release of allelochemicals, which typically target cell membranes, can lead to decreased biomass of affected species. However, low concentrations may induce defense responses in target species. The sensitivity of these species to allelopathic substances is influenced by various factors, including the concentration of the chemicals and the ecological state of the organism.

The resistance and detoxification of target species to allelochemicals are largely influenced by their physiological state and characteristics. While these organisms may experience sub lethal damage,

allowing them to survive with a reduced growth rate compared to conditions without competing species, they can still thrive. Osmotrophy, the ingestion of dissolved organic compounds, can occur alongside allelopathy from microalgae. When zooplankton predating on microalgae are lysed, they provide nutrients that benefit the microalgae. Allelopathic species can utilize both inorganic and organic nitrogen and phosphate from the environment, enhancing their growth rates, particularly through osmotrophy mechanisms that allow for continued positive growth even under stress. (Chaïb et al., 2021).

2. Biotic and abiotic factors affecting the production and accumulation of Allelochemicals:

Abiotic factors encompass all the physical and chemical elements of an ecosystem that impact a specific biocenosis, reflecting the influence of non-living components on living organisms. In contrast, biotic factors are associated with the activities of living beings, affecting the distribution of plant and animal species within a particular biotope. Both abiotic and biotic factors play significant roles in toxicity and the production of allelopathic substances by algae.

2.1.Abiotic factors:

Abiotic stressors such as nutrient deficiencies (particularly nitrogen and phosphorus), low temperatures, and high pH levels (around 9.0) enhance allelochemical production in algae. In eutrophic environments, microalgae can survive by fixing nitrogen or using bicarbonate as a carbon source. However, allelopathic activity decreases under high light, elevated temperatures, nutrient-rich conditions, and low pH (~6.0). Studies on *Prymnesium parvum* show that while nutrient enrichment can trigger blooms and toxicity, adding nitrogen and phosphorus may reduce toxicity by promoting competitor algae. Additionally, nutrient preferences and pH variations can influence both the production and effectiveness of allelochemicals in various algal species.

2.2.Biotic factors:

Biotic influences such as the growth phase and population density of allelopathic algae affect toxin production. The strongest allelopathic effects occur during the exponential growth phase and decline in stationary or senescent phases. For example, *Chrysochromulina polylepis* loses toxicity after prolonged stationary growth. Cell concentration also matters, with high densities of certain algae triggering toxic effects through mechanisms like quorum sensing (QS). QS enables density-based microbial communication and affects algicidal behavior, as observed with *Ponticoccus sp*. from *Prorocentrum donghaiense.* The inhibition of QS reduces toxicity without impacting growth. These dynamics highlight the importance of ecological context in regulating allelopathic interactions.

2.3.Allelochemicals in macroalgae:

Macroalgae in aquatic environments produce a wide range of bioactive compounds, including allelochemicals, which play key roles in community structure and defense mechanisms. These substances offer competitive advantages by inhibiting the growth and survival of rival species, suggesting potential for antimicrobial and herbicidal applications. Although toxins like domoic and okadaic acids were once thought to be responsible for such effects, recent studies suggest that environmental pH may play a more significant role.

Despite the identification of over 550 secondary metabolites from cyanobacteria, their chemical nature and ecological roles remain poorly understood, indicating a need for further research. (Chaïb et al., 2021).

2.3.1. Free fatty acids:

Seaweeds are emerging as a valuable source of omega-3 polyunsaturated fatty acids (PUFAs), despite their low total lipid content. They offer several advantages over fish oils, such as better odor and easier refinement. Since fish acquire PUFAs by consuming algae, seaweeds represent a direct and sustainable alternative for PUFA production. Each macroalgal phylum Chlorophyta, Phaeophyta, and Rhodophyta has a distinct fatty acid profile, influenced by environmental conditions like geography and season. For example, green algae like *Caulerpa* and *Ulva* contain high levels of palmitic acid and rare C16-PUFAs, highlighting their nutritional and biotechnological potential. (Sohrabipour et al., 2019).

2.3.2. Alkaloids:

Marine macroalgae produce unique alkaloids, particularly indole and halogenated derivatives not typically found in terrestrial plants. These compounds, concentrated in red and green algae, include brominated and chlorinated structures with potential therapeutic uses. While marine alkaloids are less common than their terrestrial counterparts, about 44 have been identified from algae. Historically, alkaloid research focused on land plants, but marine-derived compounds like hordenine have gained interest since their discovery. Marine alkaloids are classified into three main groups:

- Phenylethylamine alkaloids.
- Indole and halogenated indole alkaloids.
- Other alkaloids. Some alkaloids previously attributed to marine algae have been misidentified, leading to clarification in their classification and therapeutic applications. (Goecke et al., 2010)

2.3.3. Lactones:

Macroalgae associated with *Roseobacter* clade bacteria release compounds such as N-acylhomoserine lactones (AHLs) and N-acyl-alanine methyl esters, which can inhibit algal growth even at low concentrations. These compounds are key players in algae–bacteria interactions, operating through quorum sensing mechanisms that regulate microalgal behavior and microbial dynamics. Another important lactone, cyanobacterin, produced by *Scytonema* cyanobacteria, inhibits photosynthesis in a broad range of microalgae. AHLs also act as signaling molecules that influence bacterial growth, enzyme activity, and microalgal responses such as self-aggregation and the production of bio-macromolecules like proteins and fatty acids. These molecular interactions are particularly relevant in eutrophicated waters, where they may help manage harmful algal blooms and support bioremediation strategies for water quality protection. (Goecke et al., 2010)

2.3.4. Aldehyde:

The taste and quality of farmed macroalgae are strongly influenced by the physicochemical properties and storage conditions of the water. Compounds such as organic acids (lactic, succinic, propionic) enhance the umami flavor, while lipid oxidation and the presence of free fatty acids (e.g., α-linolenic and linoleic acid) can cause a bitter taste. Light exposure during storage accelerates lipid oxidation, with pigments like chlorophyll and phycoerythrin acting as

photosensitizers. This leads to the formation of aldehydes, which are key indicators of oxidative stress and vary by algal species depending on their lipid content. For example, *Botryococcus* and *Rhodomonas* have high aldehyde levels, while *Tetraselmis* and *Nannochloropsis* show lower levels. Other processes like Strecker degradation also contribute to aldehyde formation in algae. (Güven et al., 2010).

2.3.5. Peptides:

Algal proteins serve as a source of bioactive peptides, also known as cryptides, which can exert direct physiological effects once they are released from their inactive parent proteins. Similar to endogenous peptide hormones, such as those derived from proopiomelanocortin, insulin, and angiotensin, bioactive peptides are generated from parent polypeptide sequences through a series of controlled and specific proteolytic cleavages. In some instances, multiple sequences with hormone-like activity can be derived from the same parent polypeptide via differential cleavage. Although many bioactive peptides with potential health benefits, such as anti-hypertensive properties, have been isolated from both macroalgae and microalgae, there remain technical challenges related to the production and commercialization of these bioactive peptides that need to be addressed. (Güven et al., 2010).

2.3.6. Extracellular metabolites (exopolysaccharides, extracellular proteins and lipids):

Exopolysaccharides (EPS) are high molecular weight biopolymers secreted by microalgae during growth, either attached to the cell wall or released into the environment. Various microalgae, especially red microalgae and cyanobacteria, produce a diverse range of EPS with different structures. Although the specific roles of EPS in microalgae physiology and interactions remain partially unclear, they are believed to help protect cells from environmental stressors and may modulate the effects of allelochemicals in response to toxins from species like Microcystis aeruginosa.

Also, microalgae release extracellular enzymes, such as alkaline phosphatases and proteases, which can affect microbial growth and ecosystem dynamics. For example, cyanobacteria like Oscillatoria sp. produce proteins that inhibit green algae growth, potentially acting as algicides. The extracellular production of lipophilic substances, like free fatty acids (FFA) by *Ochromonas danica*, is being investigated for innovative bioprocesses, particularly in biodiesel production. Cultivating *Ochromonas* under different conditions reveals significant variations in production, suggesting that integrating all production steps into a single bioprocess could optimize costs and enhance yields. (Chaïb et al., 2021).

Practical Part

MATERIAL AND METHODS
(Experiments and analysis section)

I. Material:

1. **Glassware and Containers:**
   - Test tubes
   - Beakers
   - Petri dishes
   - Slides and cover slips
   - Eppendorf tubes
2. **Pipetting and Measuring Tools:**
   - Micropipettes
   - Blue pipette tips
   - Yellow pipette tips
   - Syringes
3. **Sterilization and Heating:**
   - Bunsen burner
   - Autoclave container
   - Aluminum foil
4. **Microbiological Tools:**
   - Inoculating loop (platinum loop)
   - Swabs
   - Test tube racks
   - Toothpicks
   - Whatman paper discs
   - Mortars
   - Spatula
   - Forceps (metal tongs)
5. **Reagents and Liquids:**
   - Distilled water
   - Glycerol
   - Gram staining dyes
6. **Culture Media:**

   a) Solid culture media
   - Chromagar
   - Chapman agar
   - GN agar
   - ISP2 agar
   - Mueller-Hinton agar (MH)

   b) Liquid culture media
   - BN broth
   - BG11 medium
   - Enriched seawater
7. **Equipment (Apparatus):**
   - Incubator
   - Refrigerator
   - Vortex mixer
   - Optical microscope
   - Shaker (Agitator)
   - Laminar flow hood (Microbiological hood)

- pH meter
- Balance (laboratory scale)
- Sterilizer

8. **Other:**
   - API 20 E

II. Methods:

1. Study framework:

Our work focused on the isolation and identification of bacteria associated with algae, as well as to assess the biological potential of the algae themselves through various analyses, and evaluates the potential of both bacteria and algae to produce bioactive compounds.

The samples were collected from:

- Beaches in the Wilaya of Annaba
- Beaches and lakes in the Wilaya of El Tarf

The biological analyses were carried out at the Microbiology Laboratory of the Biology Department at Cheikh Larbi Tébessa University, Tébessa, between February 17 and May 14, 2025.

Our study included:

- The observation and morphological characterization of algae
- The isolation of bacteria from algal samples
- The preservation of bacterial strains
- The screening for antibacterial activity of both bacteria and algae
- The salinity tolerance test of algae
- The biochemical identification of the isolated bacterial strains

2. Sampling:

Algal specimens were collected manually during daylight at low tide, using sterile gloves and forceps, and immediately placed in sterile containers.

Samples were transported to the laboratory under cooled conditions and processed within 24 hours to preserve microbial integrity.

- Annaba and El Tarf were chosen for their rich algal biodiversity and diverse aquatic environments (marine and freshwater). These regions allow comparison between natural and human-impacted sites, while also being easily accessible for sample collection.



Figure 03: Sampling

The table below presents the different algae samples collected:

Table 02: Algal samples collected

Samples	Location	Date	Name
Annaba			
	**Caroube Beach**	**15 february 2025 09 :40**	**V**
	**Caroube Beach**	**15 february 2025 10 :15**	**R**
	**Caroube Beach**	**15 february 2025 09 :30**	**Vf**

	Caroube Beach	15 february 2025 09 :45	M
	Caroube Beach	15 february 2025 09 :00	J
	Ain Achir Beach	15 February 2025 11 :30	B
Samples	**Location**	**Date**	**Name**
TAREF			
	Lake Tonga	26 February 2025 09 :19	F
	Lake Tonga	26 February 2025 09 :28	P

	**El-djebel Beach**	**26 February 2025 09 :52**	**Y**
	**El-djebel Beach**	**26 February 2025 09 :55**	**L**
	**AL-Awaint Beach**	**26 February 2025 10 :17**	**G**

3. Sample Preparation:

The algae samples were prepared under sterile conditions to avoid any contamination. The procedure was carried out as follows:

3.1. Sample grinding and Suspension:

The algae samples were ground in sterile mortars near a Bunsen burner to ensure aseptic conditions, then diluted using sterile distilled water to obtain a homogeneous suspension.



Figure 04: Sample grinding

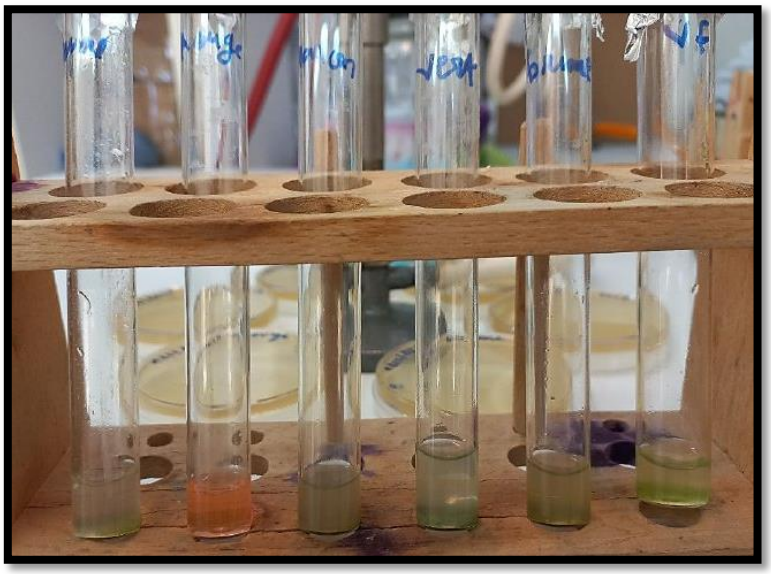


Figure 05: Algual suspension

3.2. Inoculation into Liquid Culture Media:

Each algae suspension was inoculated into four different liquid culture media:

- BG11
- Supplemented seawater (enriched with nutrients)
- Nutrient broth (NB)
- Unsupplemented seawater

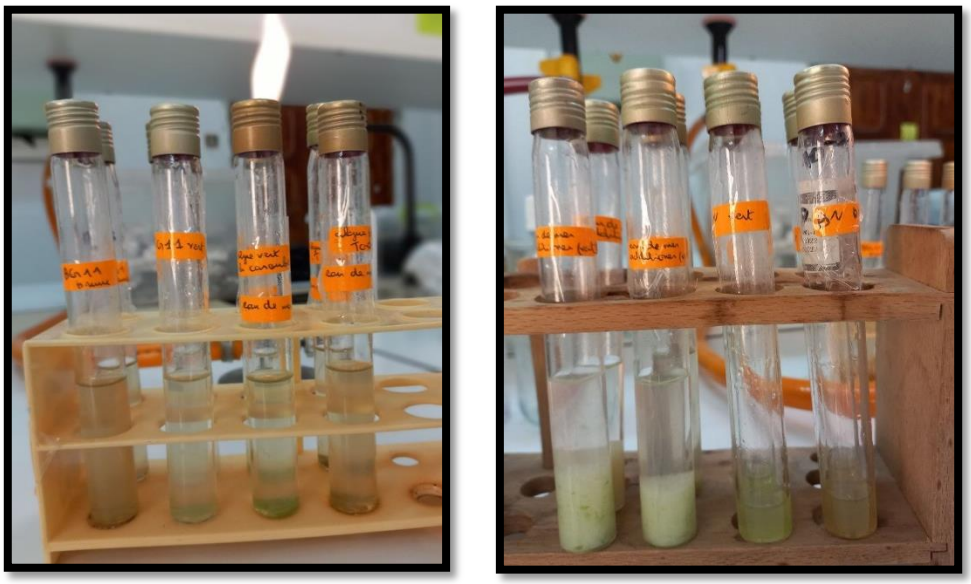


Figure 06: Inoculation into Liquid Culture Medias

4. Fresh Mount Observation:

After incubating the algal suspensions in the different liquid culture media, a fresh mount observation was carried out to examine the presence, diversity, and morphological features of algae and microalgae.

- Using a sterile platinum loop, a drop of culture was carefully taken from each tube.
- The drop was placed on a clean slide and gently covered with a coverslip.
- No staining was used in order to preserve the natural structure and pigmentation of the algal cells.
- The slides were observed under a light microscope at 40x magnification using white light.

5. Salinity test:

In order to assess the salt content of the collected algae, a salinity test was carried out on the raw samples. This parameter is important for understanding the natural environment of the algae as well as its potential influence on microbial composition and biological activity.

5.1.Sample Preparation:

- Each algal sample was manually ground in a sterile mortar until a homogeneous paste was obtained.
- The resulting paste was transferred into a clean beaker containing sterile distilled water.
- The mixture was placed on a magnetic stirrer and stirred for 2 hours to ensure proper release of salts present in the algae.
- After stirring, the preparation was left to rest for 10 minutes to allow solid particles to settle

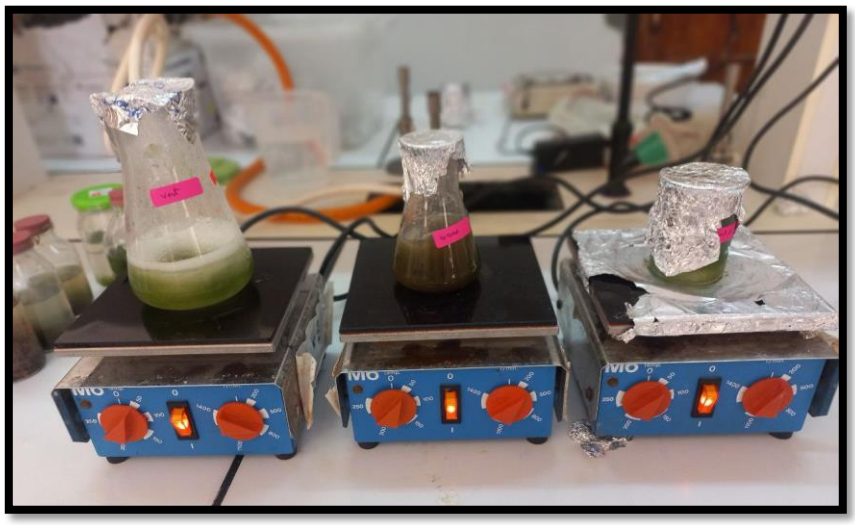


Figure 07: Sample preparation for salinity test

5.2.Salinity Measurement:

- The salinity of the liquid phase was measured using a conductivity meter.
- The device was calibrated according to the manufacturer's instructions before use.
- Only the clear liquid phase at the surface was used for the measurement.
- A clean probe was immersed in the decanted liquid, and the salinity reading was directly recorded.



Figure 08: Salinity measurement

6. Culturing:

To isolate microorganisms associated with the algae samples after their enrichment in liquid media, culturing was carried out on various specific solid media. This step aimed to allow differential growth of bacteria and other associated microorganisms.

6.1. Selection of Enriched Suspensions:

For each algae sample, only one enriched suspension was selected from the two replicates in the following liquid media: BG11, supplemented seawater, and nutrient broth (NB).

These suspensions were chosen based on turbidity and visible microbial growth, indicating successful enrichment.

6.2. Solid Media Used:

The enriched suspensions were inoculated onto the following solid media, each serving a specific purpose:

- GN (Glucose Nutrient Agar): A general-purpose medium that supports the growth of a wide range of bacteria.
- Chromagar: A differential medium that allows distinction of certain bacterial species based on colony color.

- Chapman (Mannitol Salt Agar): Selective for staphylococci, particularly Staphylococcus aureus.
- ISP2 (International Streptomyces Project Medium 2): Designed for the isolation of actinomycetes.
- MacConkey: A selective and differential medium for Enterobacteriaceae (Gram-negative bacteria).

All media were prepared under sterile conditions **(annex)** and poured into sterile Petri dishes.

6.3. Inoculation using the grid method:

- The inoculation was carried out under sterile conditions near a Bunsen burner.
- Using a sterile platinum loop, a drop of the enriched suspension was taken for each sample.
- This drop was spread over the surface of the solid media using the quadrant streaking method, allowing for the progressive dilution of microorganisms in order to obtain isolated colonies.
- All Petri dishes were incubated in an inverted position (lid facing down) in an incubator at 37°C.
- Incubation times varied depending on the medium:
  GN, Chromagar, Chapman, MacConkey: incubated for 24 to 48 hours.
  ISP2: incubated for 7 days due to the slow growth of actinomycetes.



Figure 09: Culturing

7. Gram Staining:

After culturing the algal suspensions on solid media and obtaining isolated colonies, a Gram stain was performed to determine the nature of the bacterial cell walls (Gram-positive or Gram-negative) as well as their cellular morphology (shape, arrangement).

7.1. Preparation of the Bacterial Smear:

- A small portion of an isolated colony was taken using a sterile loop.
- This portion was spread onto a clean slide with a drop of sterile distilled water to form a thin and homogeneous smear.
- The slide was then air-dried and fixed by briefly passing it over a Bunsen burner flame.

7.2. Steps of the Gram Staining Procedure:

1) The technique used follows the classic four-step method:
2) Primary Staining: The slide is covered with crystal violet for 1 minute, then rinsed with water.
3) Mordanting: Application of iodine (Lugol's solution) for 1 minute, followed by rinsing.
4) Decolorization: Rapid rinse with alcohol or acetone-alcohol for 10 to 20 seconds, then immediately rinsed with water.
5) Counterstaining: Addition of safranin for 30 to 60 seconds, followed by rinsing with water and air drying.



Figure 10: Gram staining

7.3.Microscopic Observation:

The stained slides were observed under a light microscope using the 100x oil immersion objective.

8. Purification of Mixed Colonies:

Following the Gram staining, some microscopic preparations revealed the presence of mixed bacterial cultures, characterized by the cohabitation of several types of bacteria differing in shape or staining color (Gram-positive or Gram-negative). Purification was then carried out to isolate each strain individually.

- Microscopic analysis allowed for the identification of slides showing morphological heterogeneity, indicating the presence of multiple bacterial species.
- The colonies corresponding to these mixtures were selected directly from the initial solid culture media.
- Using a sterile loop, each colony suspected of being involved in a mixture was purificated individually onto a Petri dish containing the specific medium.
- The inoculation was performed using the quadrant streaking method to allow for the isolation of well-separated colonies.
- The purificated Petri dishes were incubated at 37°C for 24 to 48 hours.
- After incubation, the uniformity of the colony morphology was visually checked. A second Gram stain was performed to confirm the purity of the cultures.

9. Conservation of Pure Strains:

To ensure the viability and stability of the pure bacterial strains isolated from the algae samples, two conservation methods were implemented: one for short-term storage on slanted solid media, and the other for long-term storage by freezing in a nutrient broth/glycerol mixture. All procedures were carried out under strict sterile conditions to prevent contamination.

9.1. Prior Enrichment in Nutrient Broth:

Before any conservation, the isolated pure strains were first reactivated and enriched in a liquid nutrient medium:

- An isolated colony was taken with a sterile loop and inoculated into a tube containing sterile nutrient broth (NB).
- The tube was incubated at 37°C for 24 hours to allow for active growth.
- This enriched liquid culture was then used for both short-term and long-term conservation.



Figure 11: Strains enrichment in NB

9.2. Short-Term Conservation:

9.2.1. Preparation of Slant Tubes:

- Sterile test tubes were filled with: Chapman agar for Gram-positive strains and MacConkey agar for Gram-negative strains
- The tubes were then slanted at approximately 45° to create an oblique culture surface.



Figure 12: Short-Term Conservation

9.2.2. Inoculation:

Using a sterile platinum loop, a drop of the enriched NB culture was deposited on the surface of the slanted agar in a zigzag motion from the bottom to the top of the tube, carefully avoiding piercing the medium.

9.2.3. Incubation and Storage:

- Each tube was precisely labeled (strain code, date, medium).

- Tubes were incubated at 37°C for 24 to 48 hours.
- After incubation, they were stored at 4°C for up to 3 weeks

9.3. Long-Term Conservation:

9.3.1. Preparation of Tubes:

- Sterile Eppendorf tubes were filled with sterile glycerol and the enriched NB culture
- The mixture was gently homogenized by inversion.
- Three replicates were prepared for each strain.

9.3.2. Storage:

- Each tube was carefully labeled (strain code, date, medium).
- The tubes were stored at –20°C for long-term preservation, allowing for strain recovery several months later without loss of viability.

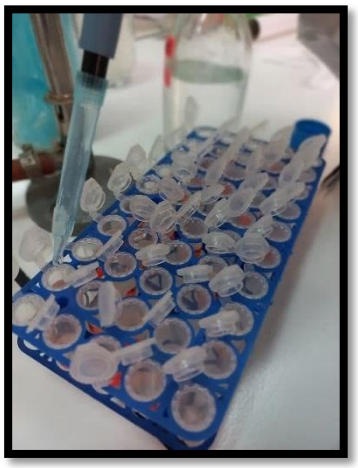


Figure 13: Long-Term Conservation

10. Evaluation of the Antibacterial Activity:

10.1. Evaluation of the antibacterial activity of the isolated strains:

To evaluate the antibacterial potential of the microbial strains isolated from the algae samples, an *in vitro* inhibition test was performed against a panel of pathogenic indicator bacteria. This test aimed to detect the potential production of antimicrobial substances by the marine isolates. Five pathogenic bacterial strains were selected as target strains:

- *Klebsiella* sp.10676
- *Escherichia coli* 10
- *Staphylococcus aureus* ATCC25923
- *Enterococcus aerogenosa* 10646
- *Proteus vulgaris*

These strains were maintained on solid nutrient media and then reactivated in physiological saline for the test.



Figure 14: Antibacterial activity preparations

10.1.1. Inoculation of indicator bacteria:

- Each indicator strain was resuspended in sterile saline until a uniform visual turbidity was obtained.
- The MH plates were inoculated using a sterile swab, dipped in the suspension, then uniformly spread across the surface by sweeping in three directions to obtain a homogeneous bacterial lawn.

10.1.2. Application of the tested (producing) strains:

In each MH plate seeded with an indicator bacterium, two types of applications were performed:

- Two wells were made in the agar using a sterile tip (approximately 6 mm in diameter).
- Two sterile paper discs (antibiogram type) were placed on the surface of the medium.
- Then:
- An enriched culture (24 h in NB) of the isolated (potentially producing) strain was used.
- Using a sterile micropipette, a drop of this culture was placed in each well (~20 µL) and imbibed onto each disc (~10 µL).
- All procedures were carried out under strict aseptic conditions in a fume hood.
- The plates were incubated at 37°C for 24 hours in a normal position.



Figure 15: Application of the tested strains

10.2. Evaluation of the antibacterial activity of algae:

To assess the potential antibacterial activity of the algae themselves, a separate test was conducted using algae against the same panel of pathogenic indicator bacteria. This approach aimed to determine whether the algae contained bioactive compounds capable of inhibiting bacterial growth.

10.2.1. Preparation of the algae powder:

- The algae samples were air-dried in a clean, moisture-free area until completely dehydrated.

- Once dry, the algae were finely ground using a sterile mortar until a homogeneous powder was obtained.
- This powder was stored in a hermetically sealed sterile container, away from moisture and light, until use.

10.2.2. Preparation of the MH medium enriched with algae powder:

- For each preparation, mL of sterile liquid Mueller-Hinton (MH) was mixed with quantity of dried algae powder.
- The mixture was homogenized manually or with a stirrer.
- The mixture was immediately poured into sterile Petri dishes.
- The dishes were left at room temperature until the medium completely solidified.



Figure 16: MH medium enriched with algae powder

10.2.3. Inoculation of indicator bacteria:

- A fresh colony of each indicator strain previously used was picked using a sterile loop.
- It was placed in the center of each dish (one strain per dish).
- For each strain, two replicates were performed.
- The dishes were incubated at 37°C for 24 hours.

10.3. Double-layer overlay method:

In addition to the previous tests, the double-layer overlay method was used to evaluate the antibacterial effect of the algae powder in an environment simulating more homogeneous diffusion.

Protocol:

- A first layer of sterile Mueller-Hinton (MH) agar was poured into Petri dishes and then allowed to solidify.
- A fresh colony of each indicator strain was picked using a sterile loop and placed on the agar.
- Then, a second layer of sterile liquid MH, previously mixed with g of dry algae powder per mL, was poured on top.
- The dishes were left at room temperature until the top layer had completely solidified.
- Finally, the dishes were incubated at 37°C for 24 hours.



Figure 17: Double-layer overlay method

Also:

- The raw algal suspensions were directly tested against pathogenic indicator bacteria.

11. Identification of Strains Using the API 20 E Gallery:

Bacterial strains that exhibited marked antibacterial activity in previous tests were selected for precise biochemical identification using the commercial API 20 E system (bioMérieux), which is designed for the identification of *Enterobacteriaceae* and other Gram-negative bacteria.

11.1. Preparation of the Bacterial Suspension:

- A fresh colony of each selected strain was collected from a solid medium (BN or GN) using a sterile loop.
- The colony was transferred into 5 mL of sterile water in a test tube.
- The suspension was homogenized until a turbidity equivalent to McFarland standard 0.5 to 0.6 was achieved, in accordance with the manufacturer's recommendations.
- This suspension was used to inoculate the API gallery.

11.2. Implementation of the API 20 E Gallery:

The API 20 E strip contains 20 microtubes filled with dehydrated substrates for various biochemical tests.

Using a sterile micropipette, each well was inoculated with the bacterial suspension:

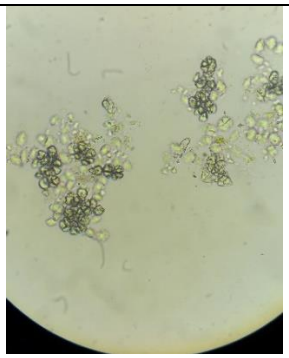
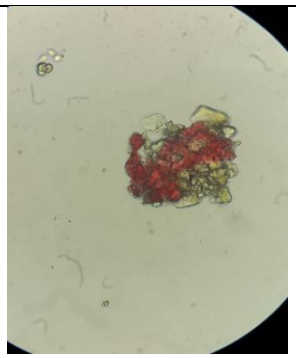
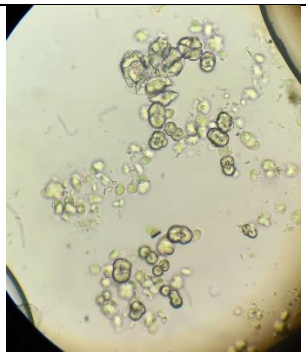
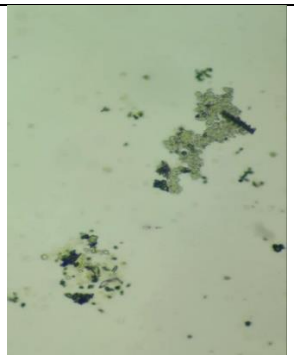
- Most of the microtubes were completely filled and saturated.
- Tests requiring anaerobic conditions were covered with mineral oil (ADH, LDC, ODC, H□S, URE).
- The strip was placed in a humidified incubation tray to prevent evaporation.
- Incubation was carried out at 37°C for 24 hours.
- After incubation, a color change in each microtube was observed, indicating a positive or negative result depending on the enzymatic reaction.
- Some tests (e.g., TDA, IND, VP) required the addition of specific reagents before reading.
- A 7-digit numerical profile was obtained based on the results of the 20 tests.
- This code was entered into the APIweb database (or identification table), allowing the probable genus and species of the tested strain to be determined.

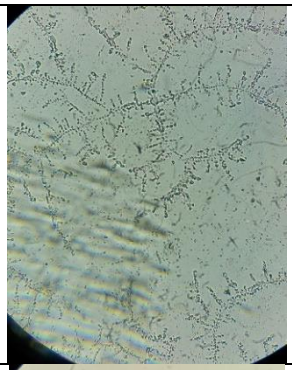
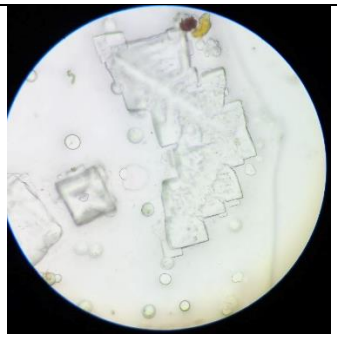
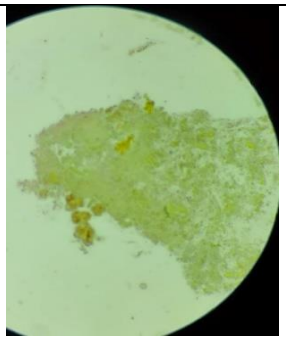
RESULTS AND DISCUSSIONS

1. Fresh Mount Observation:

Fresh mount microscopy was carried out to observe the general morphology of the algal samples cultivated in different media. This observation allowed a quick visual assessment of the samples' structure and diversity under the microscope. The results are presented in the following table:

Table 03: Fresh mount observation of algae samples.

Sample name	Fresh mount observation	Sample name	Fresh mount observation
BN.V		BG11.B	
BG11.J		BN. R	
EMA. Vf		EMA.L	

EMA.V		EMA.Y	
EMA.F		EMA.M	
EMA.L		EMA.P	

The observation of fresh algal suspensions allowed for morphological characterization of the samples and confirmed their affiliation with different genera.

The differences observed between the culture media used (NB, BG11), as well as the specific codes assigned to the samples, reflect variations in growth and cellular morphology, likely due to differences in the nutritional composition of the media and culture conditions (pH, light, temperature).

- Samples such as NB. V and EMA.Y revealed well-developed filaments, typical of cyanobacteria such as *Oscillatoria* or *Phormidium*, which are easily identifiable by their elongated, segmented, and sometimes motile morphology. These structures indicate a dominance of filamentous cyanobacteria in these cultures, likely favored by suitable culture conditions (medium composition, lighting, pH, etc.).
- Similarly, NB. R exhibited a compact reddish mass, which could suggest the presence of red algae (*Rhodophyta*) or a high accumulation of accessory pigments such as phycoerythrin.
- Samples EMA.Vf and BG11.B showed spherical cell aggregates, characteristic of unicellular green algae such as *Chlorella* or *Scenedesmus*. These genera are often

associated with rapid growth in culture and strong tolerance to environmental variation, suggesting favorable adaptation to the EMA medium.

- Moreover, EMA.F and EMA.L exhibited regular geometric structures, indicating the presence of diatoms (*Bacillariophyceae*), which are easily recognized by their siliceous frustules. Their presence, along with other morphological types, confirms the heterogeneity of the cultivated algal communities, which could result from either mixed natural isolates or a non-strict selection process during culture setup.
- Some samples, such as EMA.P, showed an amorphous greenish structure with no distinct cellular shapes, suggesting a dense biomass or a culture in the senescence phase. This may reflect an advanced growth stage or cellular degradation due to suboptimal culture conditions.
- When comparing results across the different media (NB, BG11, EMA), it appears that BG11, particularly in samples BG11.B and BG11.J, promotes clear morphological expression, likely due to its formulation tailored for the growth of cyanobacteria and certain green microalgae.

Although elementary, these fresh-state observations provide valuable insights into morphological diversity, physiological state, and overall culture quality. They serve as a foundation for guiding further analyses.

2. Salinity test:

The results obtained are presented below:

2.1. salinity levels:

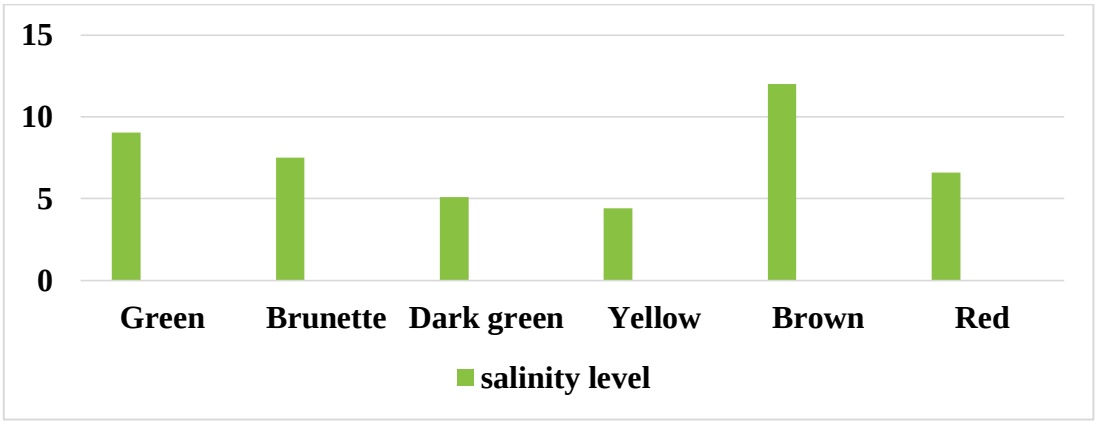


Figure 18: Different salinity ration for each algae sample taken from Annaba

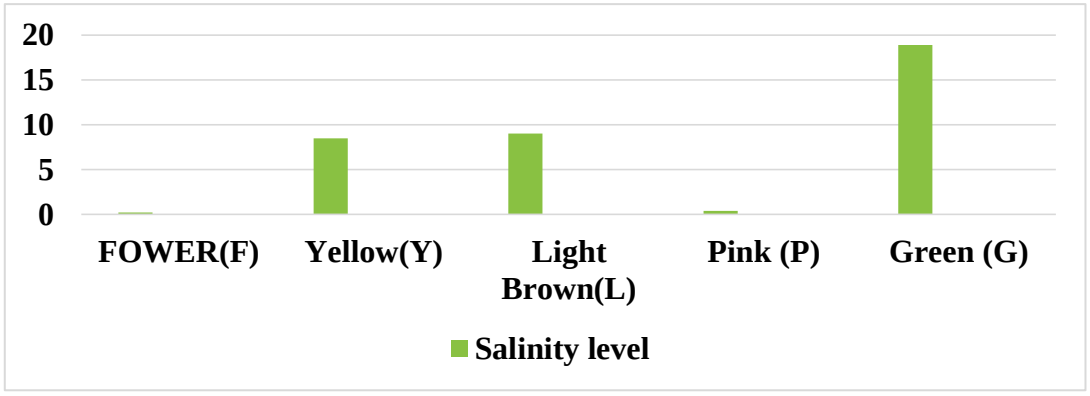


Figure 19: Different salinity ration for each algae sample taken from Taref

Salinity levels in algae samples vary significantly based on their collection locations, leading to a classification into two categories: freshwater and brackish water algae. Freshwater algae exhibit salinity levels up to 0.5 g/l, with samples F and P showing levels of 0.2 g/l and 0.4 g/l, respectively. These algae are vital primary producers in their ecosystems, converting sunlight into energy through photosynthesis and serving as a food source for various aquatic organisms. Optimal conditions in freshwater, including ideal temperatures (20°C to 30°C) and adequate light, promote algae reproduction through the production of resilient reproductive structures like spores and cysts, which can be dispersed by rain and contribute to biodiversity in aquatic habitats.

In contrast, brackish water algae thrive in salinity levels ranging from 0.5 g/l to 30 g/l, with samples collected from Al-Taraf showing higher concentrations (Y: 8.5 g/l, L: 9 g/l, G: 18.9 g/l) compared to those from Annaba. These algae exhibit unique adaptations to survive fluctuating salinity, including osmotic regulation through cellular adaptations and metabolic changes that enhance their resilience to osmotic stress. Morphological adaptations, such as alterations in thallus structure, also play a role in nutrient absorption. Additionally, specialized ion channels and aquaporins help regulate internal ion concentrations and water balance, ensuring efficient photosynthesis and overall growth in low-salinity environments.

2.2. TDS:

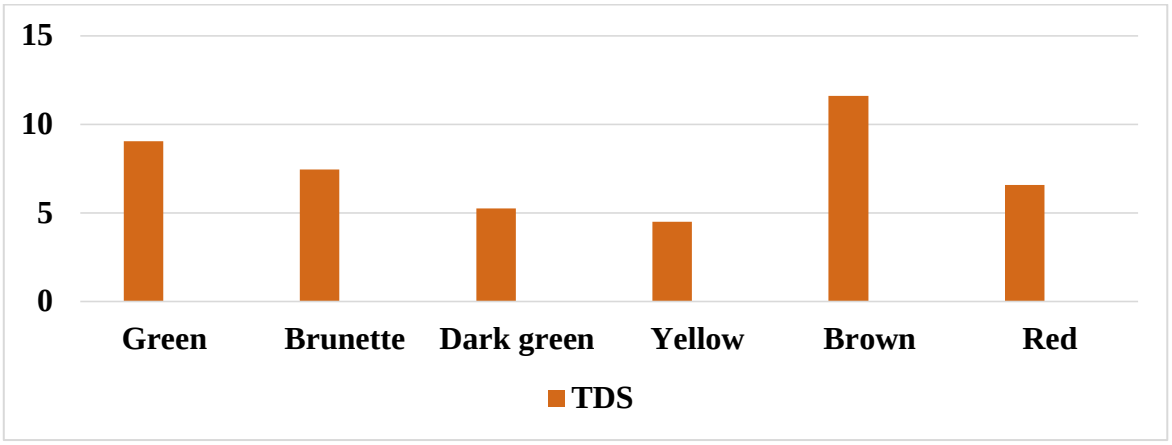


Figure 20: Total dissolved solide (mg/l) rate for each algae taken from Annaba

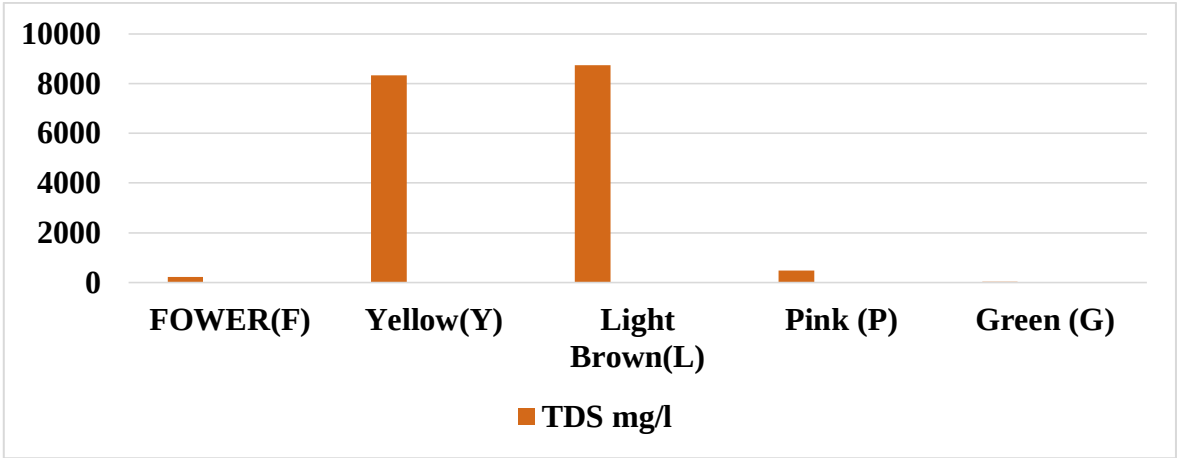


Figure 21: Total dissolved solide (mg/l) rate for each algae sample taken from Taref

The observation of total dissolved solids in water samples reveals averages between 1,000 and 30,000, indicating brackish water conditions. This high range is influenced by natural processes such as rock weathering, where erosion releases essential minerals like potassium, calcium, and sodium, enriching the water and fostering algae growth. However, excessive nutrient levels can lead to algal blooms, resulting in oxygen depletion and negatively impacting aquatic life. Additionally, allelopathic interactions among algae, through the release of chemicals or toxins, can suppress competing species, reducing biodiversity. Variations in organic material levels also contribute to stress and diseases in algae, further affecting their productivity and health.

2.3. PH:

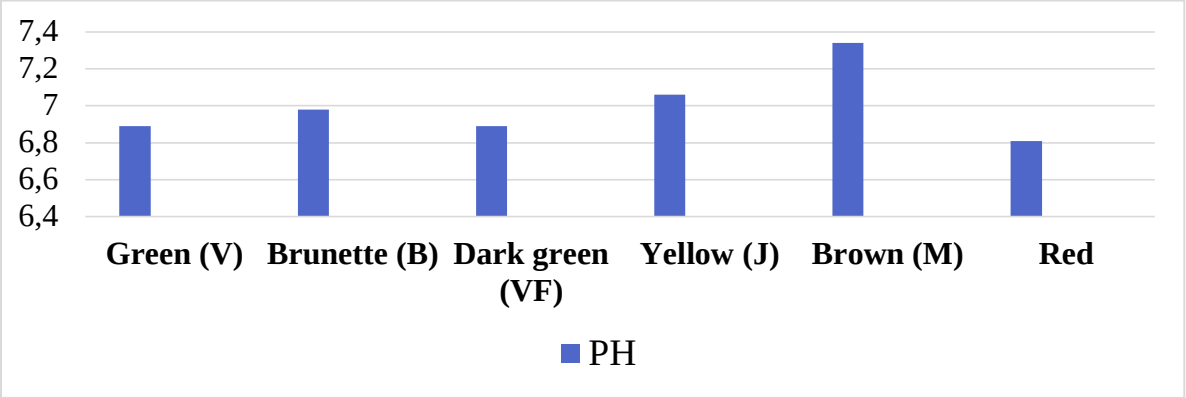


Figure 22: PH value of different type of algae living in Annaba

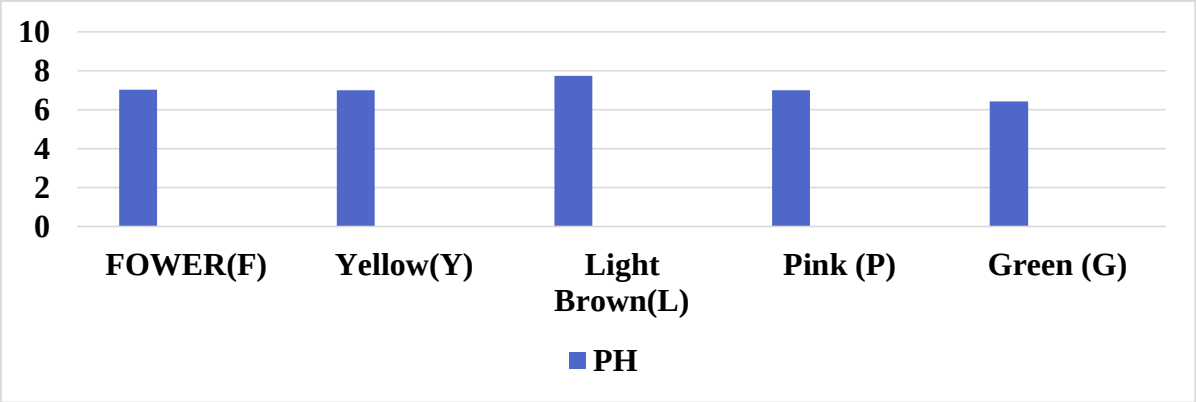


Figure 23: PH value of different type of algae living in Taref

Moderate pH levels, typically between 6.5 and 8.5, promote the growth of various algae, as seen in samples from Annaba and El Tarf with pH values ranging from 6.89 to 7.73. This range facilitates the dissolution of essential nutrients like nitrogen and phosphorus, enhancing their availability for absorption. It also optimizes enzyme activity for photosynthesis, enabling efficient energy conversion from sunlight. Additionally, moderate pH stabilizes conditions in lakes and ponds, reduces fluctuations, and supports nutrient cycling through the positive interactions between algae and microorganisms, including bacteria, which diminish competition in extreme pH environments.

2.4. Conductivity:

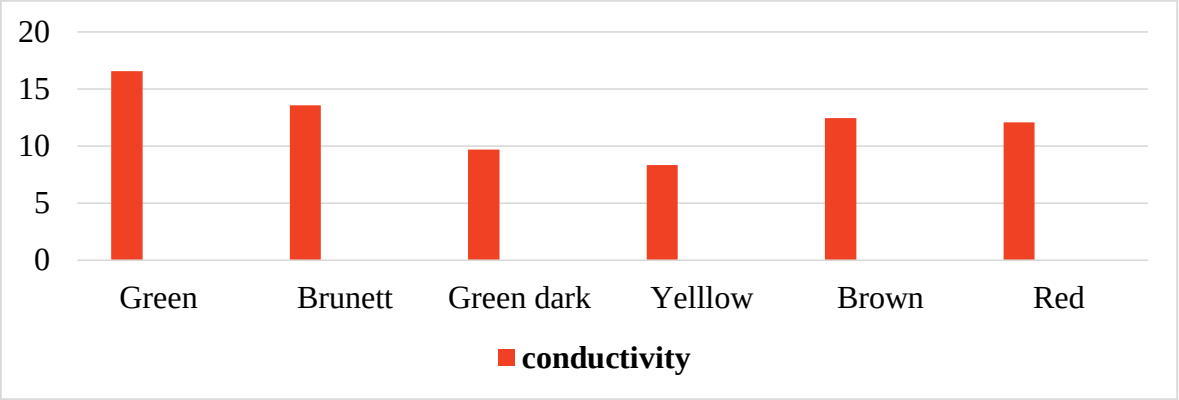


Figure 24: Measurement of algae conductivity (ms/cm) taken from Annaba

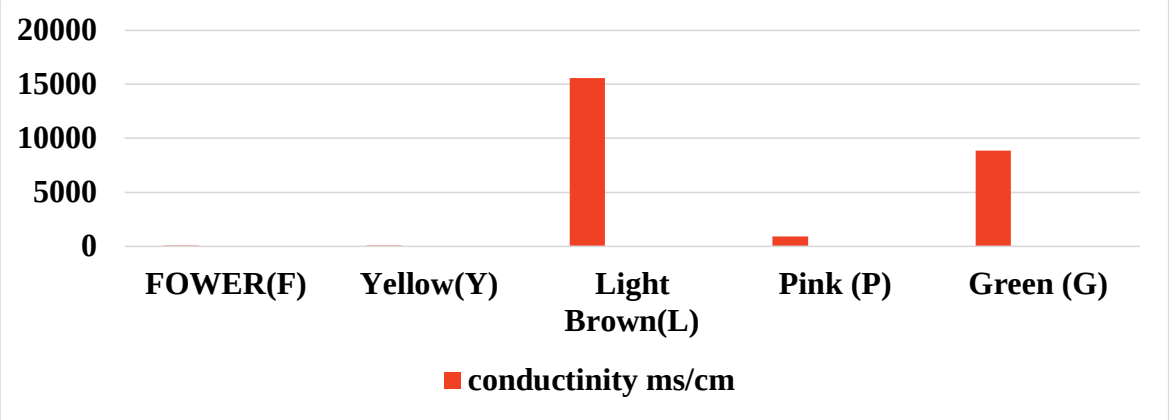


Figure 25: Measurement of algae conductivity (ms/cm) taken from Taref

The bar graphs illustrate varying conductivity levels in algae, revealing a lowest value of 0.409 ms/cm for F and a peak of approximately 16550 ms/cm for V. The increase in conductivity for other algae types (13590, 12450, 12100, 9700, and 8300 ms/cm) is attributed to pollution from high salt concentrations, which exceed the natural seawater value of 60 ms/cm. Elevated conductivity can suggest either low-salinity or high-salinity environments, impacting algal growth and community formation. This may lead to osmotic stress on algal cells, hindering their growth and metabolic functions.

Conversely, conductivity values of 15.15 ms/cm, 15.58 ms/cm, and 20.1 ms/cm indicate flourishing algae in low-salinity environments, which are conducive to their growth. The lowest value of 0.409 ms/cm suggests the presence of algae in freshwater, characterized by minimal salt and mineral levels, typically below 1. Such conditions support healthy reproduction and growth of algae, allowing them to thrive and maintain a balanced ecosystem with other organisms.

2.5. O2 ppm+O2%:

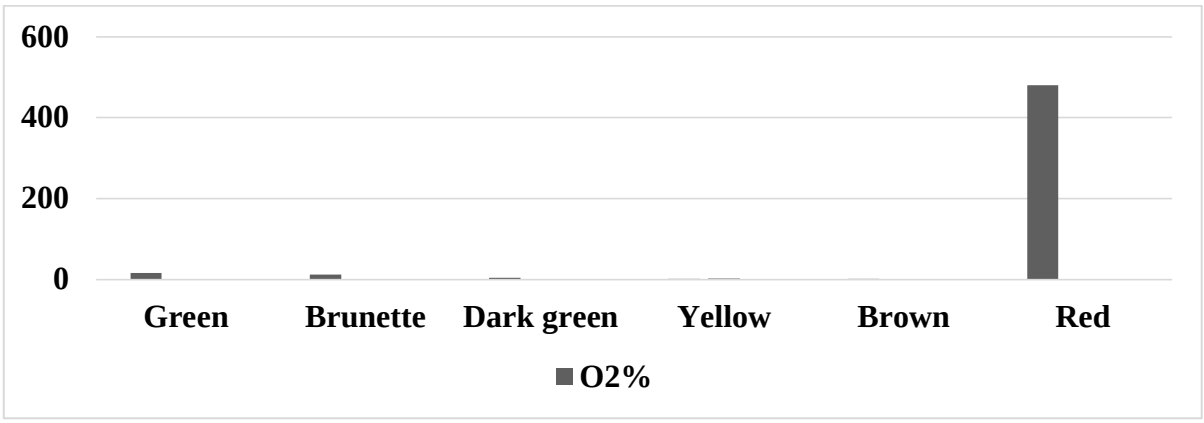


Figure 26: Dissolved O2% in algae solution (Annaba)

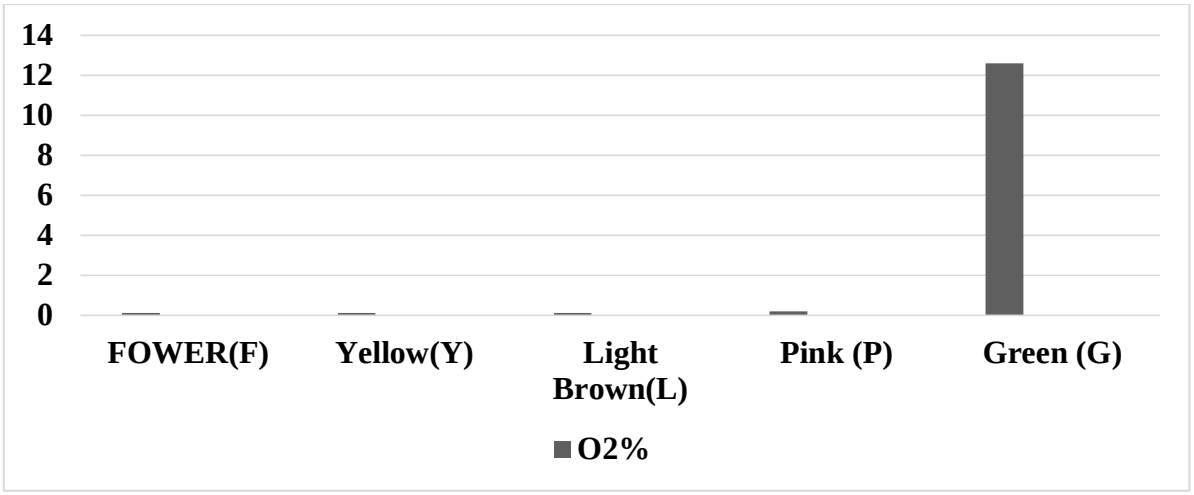


Figure 27: Dissolved O2% in algae solution (Taref)

The data indicates a significant lack of oxygen in certain types of algae (F, Y, L, P) and near absence in others (G, V, B, VF), typically below 5 parts per million. This deficiency can be attributed to several factors, including insufficient light that reduces photosynthetic activity, stress from temperature fluctuations, salinity changes, and pollution, all of which hinder oxygen production. Additionally, cell death from nutrient deficiency leads to decay, which decreases oxygen levels. Organic material decomposition further consumes dissolved oxygen, exacerbating low levels.

Conversely, one sample (R: 36.58) shows abundant oxygen production, suggesting active photosynthesis under optimal lighting and nutrient conditions. Brown algae (M: 8.27) also demonstrate good oxygen levels due to aeration from water movement, such as in coastal areas with wave action. Symbiotic relationships with bacteria enhance growth and photosynthesis, contributing to increased oxygen production. Notably, samples from El Tarf exhibit a sharp decrease in oxygen percentages, not exceeding 16.2, for reasons similar to those affecting other samples. The value of 480.7 (R), exceeding 100, indicates supersaturation in stagnant or slow-moving water bodies where oxygen bubbles accumulate.

2.6. Resistivity (Ω.cm):

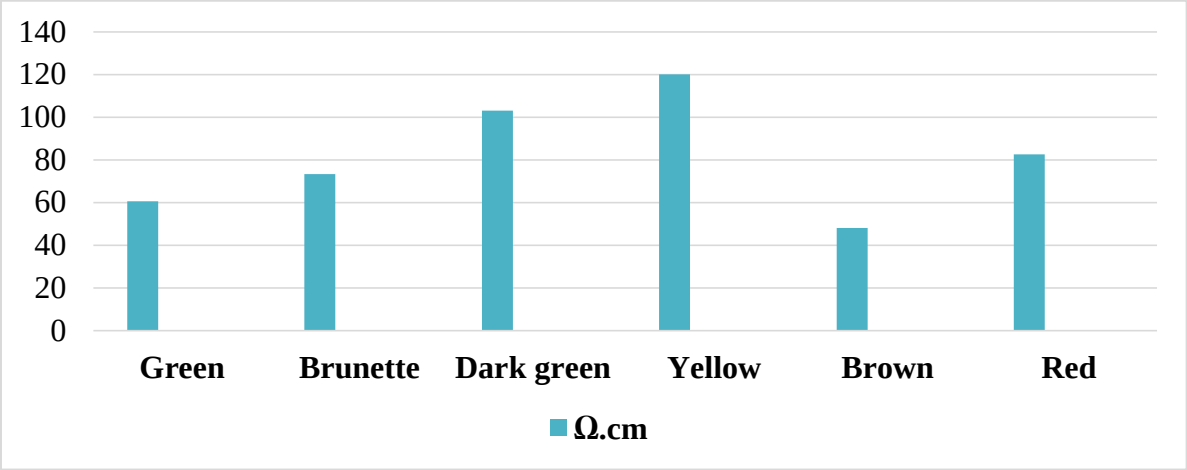


Figure 28: Electrical conductivity values of algae (Ω.cm) (Annaba)

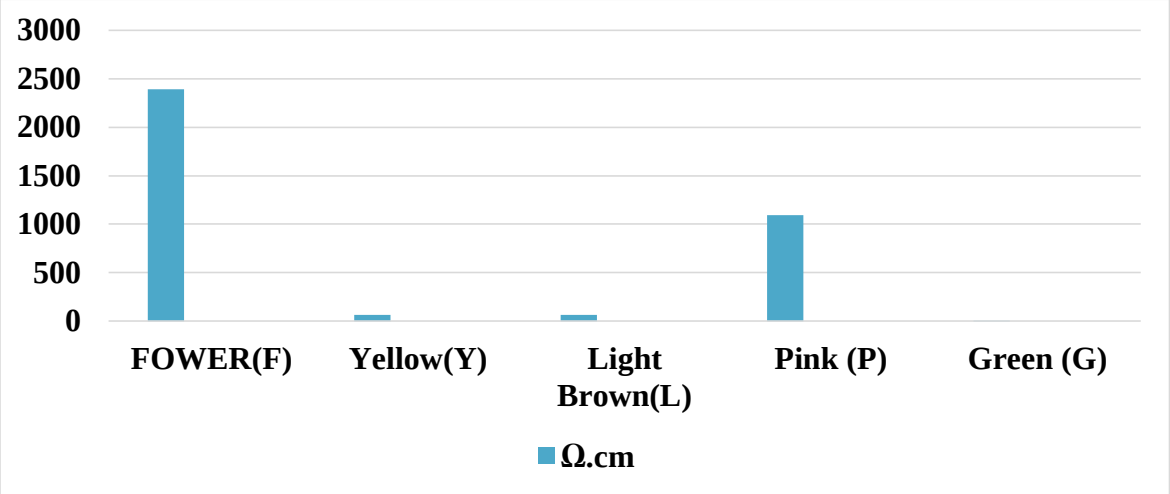


Figure 29: Electrical conductivity values of algae (Ω.cm) (Taref)

The data indicates a significant lack of oxygen in certain types of algae (F, Y, L, P) and near absence in others (G, V, B, VF), typically below 5 parts per million. This deficiency can be attributed to several factors, including insufficient light that reduces photosynthetic activity, stress from temperature fluctuations, salinity changes, and pollution, all of which hinder oxygen production. Additionally, cell death from nutrient deficiency leads to decay, which decreases oxygen levels. Organic material decomposition further consumes dissolved oxygen, exacerbating low levels.

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2.7. MV:

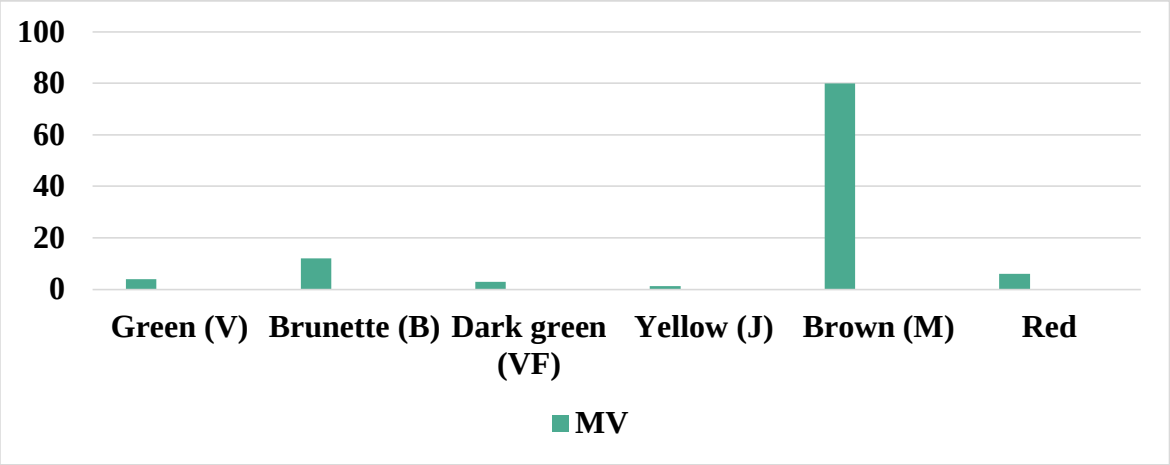


Figure 30: The various electronic potential of algae (MV) (Annaba)

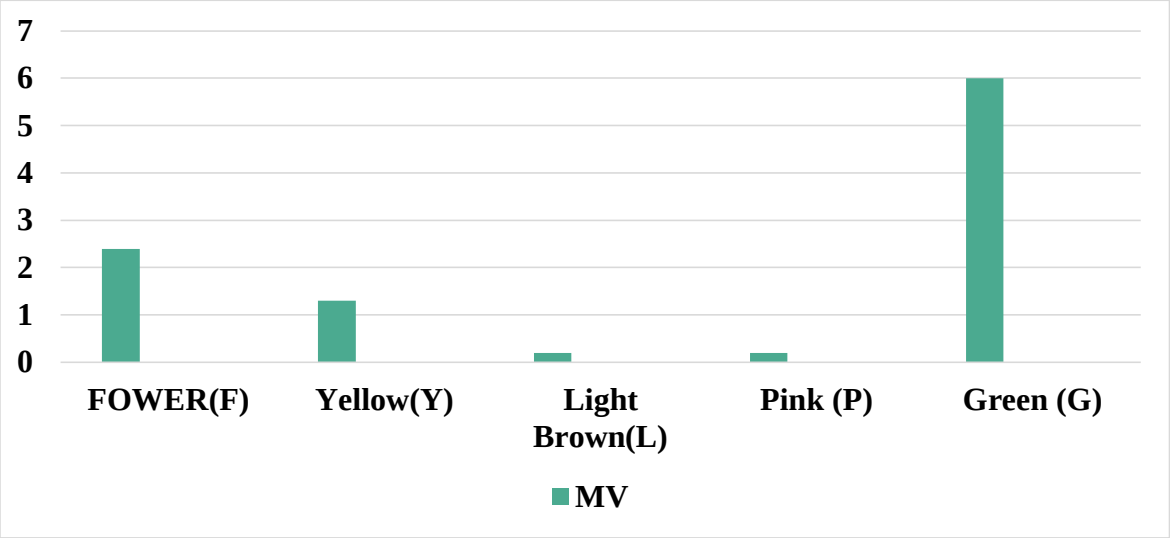


Figure 31: The various electronic potential of algae (MV) (Taref)

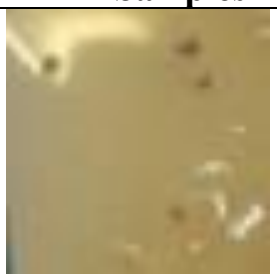
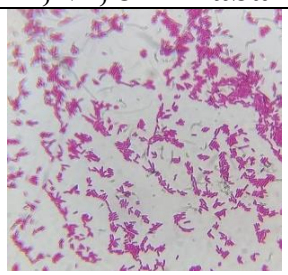
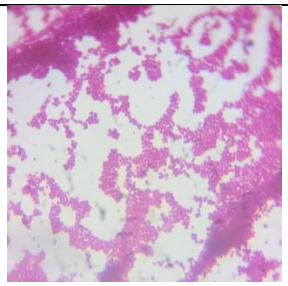
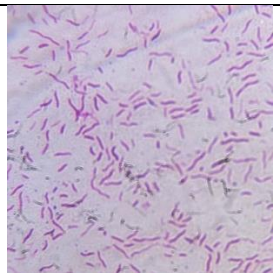
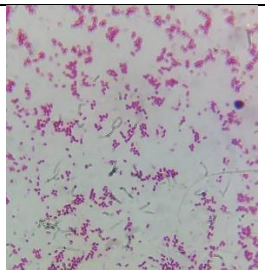
The data presented in the graphical columns related to electronic potentials reveals a significant decline in membrane potential across various samples (L: 0.2; P: 0.2; Y: 1.3; F: 2.39; VF: 3; R: 6). This decrease indicates the cells' inability to maintain essential ion gradients, which can lead to stress in algae due to nutrient deficiencies, pH changes, and unfavorable environmental conditions. Increased pressure may further weaken cellular functions, disrupting ion transport and membrane potential.

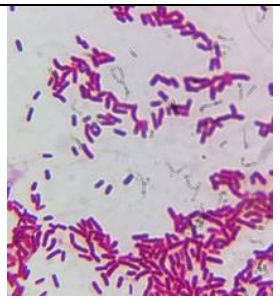
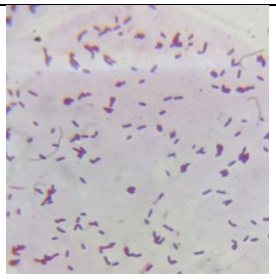
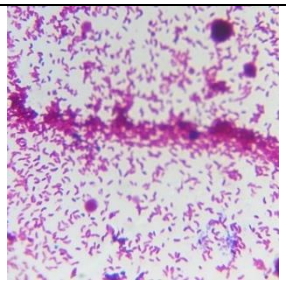
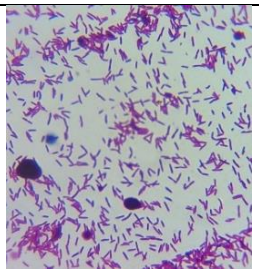
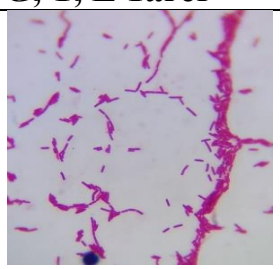
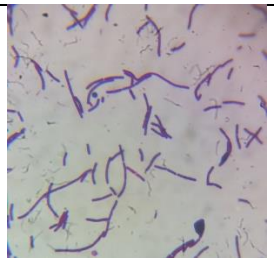
In contrast, the sample with a value of 12 for (B) reflects a healthier physiological state, suggesting enhanced activity in photosynthesis. When compared to the highest value of 80 recorded for sample (M), it indicates a robust membrane potential in algae, particularly in harsh environments. This strength is often attributed to stable ionic gradients, especially sodium (Na^+) and potassium (K^+), which are crucial for cellular functions such as signaling, osmotic regulation, and increased metabolic activity involving enzymes

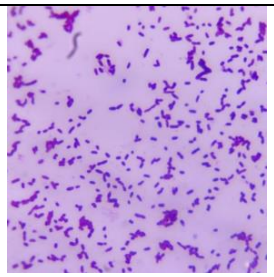
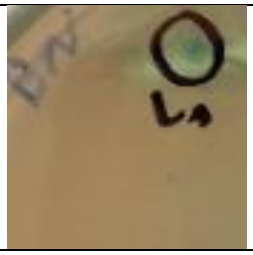
3. Bacteria culturing and Gram staining:

To assess the general characteristics of the bacterial isolates, each sample was cultured on appropriate nutrient media and subjected to Gram staining. These preliminary tests provided essential information on colony morphology, Gram reaction, and cellular shape, which help guide further identification steps. The results are summarized in the following table:

Table 04: Bacteria culturing and Gram staining results.

Strain code	Colonies	Gram stain	Colony color, shape, Gram
Samples B, V, R, M, Vf, J Annaba			
Chro.BN. V3-2 rep			Move Gram-negative bacillus
Chro.BG11. B1			Turquoise blue Gram-negative cocci
Chap.BN.B1 rep			Beige Gram-negative bacillus
Mac.BN. V2.1 rep			Pink Gram-negative diplococcus

Mac.BN.V1			Pink Gram-negative bacillus
Chro.BN.R1			Green Gram-positive bacillus
Chro.EM.M			Turquoise blue Gram-negative curved bacillus
Chro.EM.R			Beige Gram-positive bacillus
Samples P, F, G, Y, L Taref			
Chro.EMA. Y4			Green Gram-negative bacillus
Chro.BN. P1			Green Gram-positive chain bacillus

Chro.EMA. F2			Turquoise blue Gram-positive bacillus
Chro.EMA. G2			Green Gram-negative bacillus clusters
Chro.BN. L1			Green Gram-negative chain bacillus

The microbiological study conducted on the algal samples led to the isolation of several bacterial strains, characterized by their colony morphology, pigmentation, and Gram staining type.

This diversity reflects the rich microbial community associated with algal surfaces; an environment known to be a complex ecosystem that is highly conducive to bacterial colonization.

- Morphological Diversity of Colonies:

The isolated colonies displayed a variety of colors (blue-green, beige, pink, turquoise, green), reflecting significant metabolic diversity. Color variations are often associated with the production of bacterial pigments such as pyocyanins or prodigiosins, which may serve ecological functions (e.g., UV protection, microbial competition, etc.).

- Gram Staining Characteristics:

The results revealed a predominance of Gram-negative bacteria, although several Gram-positive strains were also identified. Gram-negative bacteria are generally more prevalent in marine environments, as their outer membrane rich in lipopolysaccharides provides enhanced resistance to extreme conditions such as salinity and chemical agents.

- Observed Bacterial Forms:

Bacilli (straight or curved): This was the predominant form. Gram-negative bacilli are characteristic of genera such as *Pseudomonas, Vibrio, or Shewanella*, which are commonly found in marine ecosystems.

Cocci (in clusters or diplococci): Less frequently observed, but their presence suggests potential functional diversity.

Chains of bacilli: These may indicate spore-forming species or bacteria with specific cell division patterns, such as certain Bacillus strains.

The observed diversity indicates that algae host a complex microbiota, which likely contributes to:

- Protecting algae against pathogens.
- Degrading organic compounds.
- Producing bioactive molecules.

4. Evaluation of the Antibacterial Activity:

The antibacterial activity of the strains isolated from algae was evaluated against different bacterial pathogens, and the results are illustrated below

4.1. Evaluation of the antibacterial activity of the isolated strains:

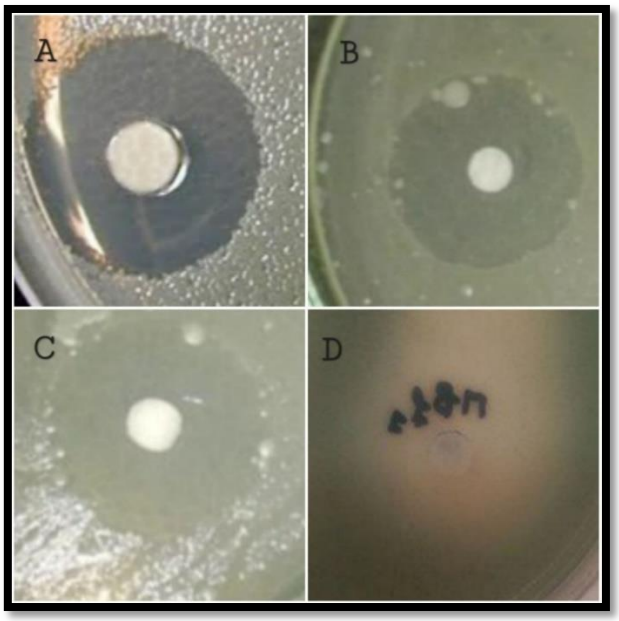


Figure 32: Antibacterial activity of the isolated strains (**A:** CEJ1 against *E. coli*, **B:** CBJ2 against *Klebsiella*, **C:** CBP1 against *Klebsiella*, **D:** MBF1 against *Enterobacter aerogenes*)

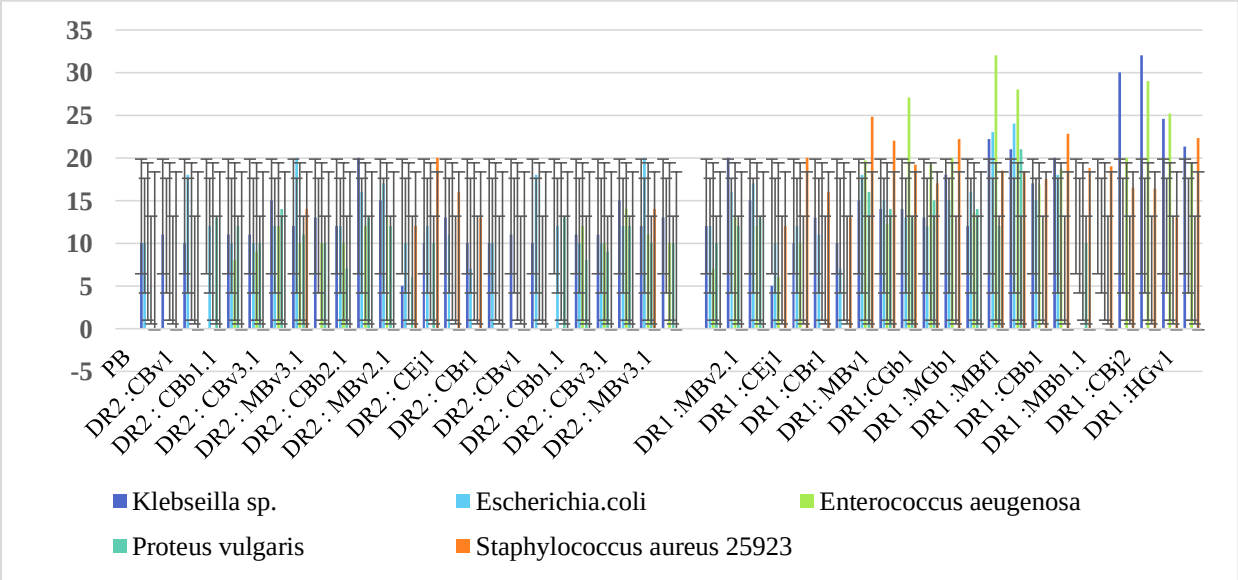


Figure 33: inhibition of the antibacterial activity of the isolated strains (Annaba)

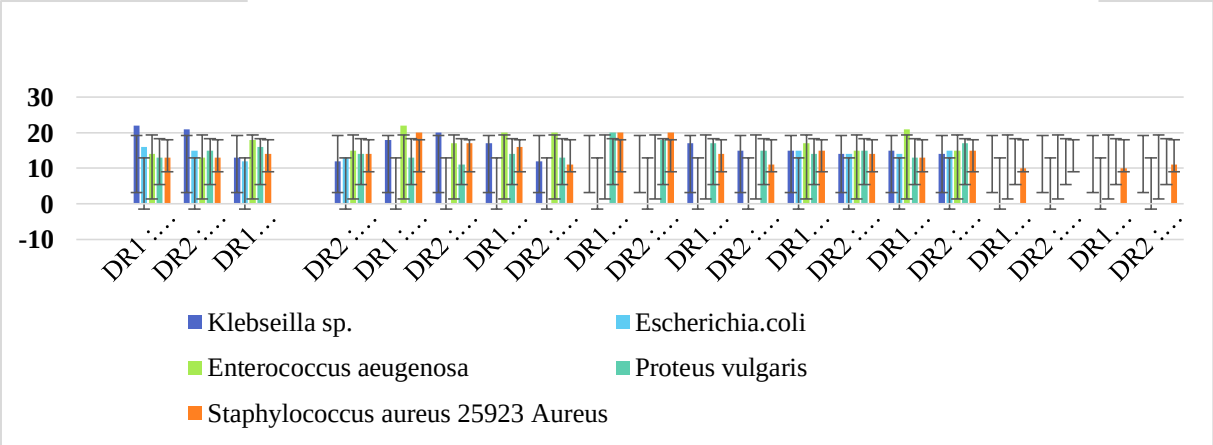


Figure 34: inhibition of the antibacterial activity of the isolated strains (Taref)

CEj1:

A note indicates an inhibition zone with diameters of 28 mm, 27 mm, 26.7 mm, and 25 mm around the discs containing the CEJ1 producing bacteria alongside *Escherichia coli*, *Staphylococcus aureus*, and *Proteus*, demonstrating a significant antibacterial effect explained as follows:

The CEJ1 bacteria exhibit strong antibacterial activity, evidenced by a significant 28 mm inhibition zone against pathogens like *Escherichia coli*, *Staphylococcus aureus*, and Proteus. This suggests that CEJ1 releases biologically active compounds that effectively inhibit or kill these bacteria. Such compounds are likely to include antibiotics that disrupt bacterial cell walls and antimicrobial peptides that interfere with essential cellular functions. The association of CEJ1 with algae indicates a natural relationship, as algae produce various biologically active substances to defend against pathogens, which in turn benefits the bacteria in their environment.

Moreover, the biochemical diversity of CEJ1 contributes to its antimicrobial properties, as it may produce secondary metabolites similar to those found in algae. The inhibition zone

observed with *Staphylococcus aureus*, measuring 30 mm, highlights the significant antibacterial effect of CEJ1, which is effective even against enterotoxins produced by the pathogen. In contrast, the inhibition observed against *Klebsiella* was 20 mm, suggesting that while higher concentrations in well diffusion methods can be effective, the disc diffusion method allows for a more gradual concentration gradient that may enhance the antimicrobial efficacy of CEJ1.

CEMb:

Our observations of inhibition zones indicate that the producing bacteria show no reaction to *Escherichia coli* and *Staphylococcus*, suggesting intrinsic resistance to compounds produced by CEMb. This resistance may stem from certain strains of Staphylococcus aureus and Escherichia coli, which are known to resist various antibiotics and antimicrobial agents. Additionally, an insufficient concentration of antibiotics produced by CEMb could hinder their effectiveness, while competitive relationships over nutrients between CEMb and these bacteria may allow both to coexist, further inhibiting CEMb growth.

Conversely, CEMb demonstrates significant inhibitory effects against *Klebsiella* (21mm) and *Enterococcus aeroginosa* (24mm) due to the secretion of substances like bacteriocins, antibiotics, or toxins that disrupt cellular activities. These compounds can damage cell walls by creating pores or interfere with the cytoplasmic membrane, leading to leakage of essential cellular contents and oxidative stress. The antibacterial agents may also stimulate the production of reactive oxygen species (ROS) within *Klebsiella* and *Enterococcus aeroginosa,* which can damage cellular components and result in cell death.

CBv3.1:

Through our observation of the inhibition zones (11mm,10mm,9mm,12 mm), we find that the bacteria exhibit significant resistance to agents *Klebseilla, E. coli, Enterococcus Aerogenosa, Proteus* This indicates that they target Gram-negative bacteria, producing antimicrobial that penetrate the cell wall or bind to the peptidoglycan layer. This process inhibits cell wall construction, leading to bacterial lysis, or it disrupts the influx of ions into the membrane, causing increased permeability and loss of essential cellular components, resulting in death.

In comparison to *Staphylococcus*, we find that it resists bacteria due to its ineffectiveness against Gram-positive strains, utilizing enzymatic degradation of the antibiotic by producing another antibiotic, or by reducing the affinity and susceptibility of its target site. This can occur either through acquiring external DNA or through changes in its own genes.

CBJ2:

CBj2 demonstrates effective resistance against certain bacteria, showing inhibition zones of 29mm for *Enterococcus aerogenosa*, 32mm for *Klebsiella*, and 16.4mm for Staphylococcus aureus, while it fails to inhibit Proteus and *E. coli*. This effectiveness may stem from CBJ2 bio-production of antimicrobial compounds that specifically target pathways in Gram-positive bacteria, such as Staphylococcus, and certain Gram-negative bacteria like Klebsiella. Conversely, *Proteus* and *E. coli* exhibit intrinsic resistance mechanisms, including efflux pumps and protective outer membranes, which limit the impact of these agents. Their outer membrane serves as a barrier, preventing many antimicrobial agents from penetrating effectively. Additionally, differences in cell wall structure contribute to this specificity; Gram-

positive bacteria possess a thick peptidoglycan layer that interacts more favorably with CBJ2 compounds compared to the more complex structures of Gram-negative bacteria like *Proteus* and *E. coli*.

CBL1:

When we observe the inhibition zones, we find that CBL1 resisted (*Escherichia coli; Klebsiella, Enterococcus Aerogenosa; Proteus; Staphylococcus aureus*), but it showed greater resistance to *Klebsiella* (22mm) and *Escherichia coli* (16mm). This is due to its production of bacteriocin, which exerts antibacterial effects by forming pores in the membranes of the targeted bacteria. This disrupts membrane integrity, leading to cell lysis and ultimately cell death. Additionally, bacteriocin can alter membrane potential, potentially causing depolarization, which affects essential processes like energy production. Furthermore, bacteriocin can inhibit cell wall synthesis by interfering with the formation of peptidoglycan, a key component of bacterial cell walls.

CBp1:

When we observe the results, we notice inhibition areas that show the resistance of CBp1 against (*Escherichia coli, Klebsiella, Enterococcus Aerogenosa, Proteus, Staphylococcus aureus*), but it is greatest against both *Proteus* (16mm) and *Pseudomonas aeruginosa* (18 mm). This indicates that it has secreted antimicrobial substances to protect itself or produced bacteriocins, where:

Many bacteriocins are bivalent, meaning they contain both hydrophilic and hydrophobic regions. This allows them to embed in the cell membrane of the target bacteria and form pores. Once these pores are formed, they create holes in the membrane, leading to the loss of essential ions and small molecules, ultimately resulting in cell death.

4.2. Evaluation of the antibacterial activity of the algae:

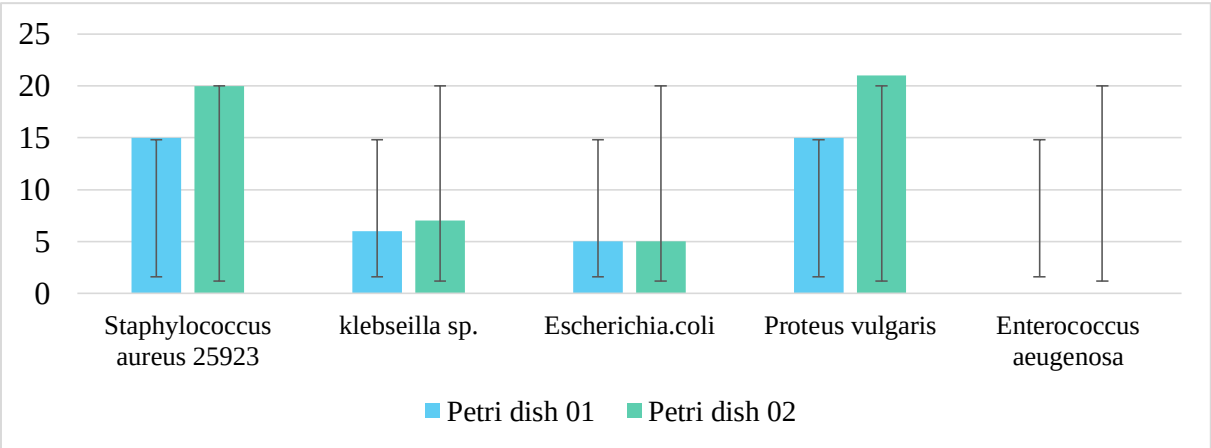


Figure 35: Inhibition zone of algae with 5 bacteria

When we observe the results shown in the statement, we note that the algae resisted *Staphylococcus aureus* and *Escherichia coli*, and *Klebsiella*, however, the greatest resistance was seen with *Proteus*, with an inhibition zone of more than 20 mm. This indicates the production of antimicrobial peptides, which are small proteins that can have a direct toxic effect on bacteria by disrupting their cell membranes or inhibiting metabolic processes. Additionally, they release secondary receptors, including phenolic acids and flavonoids,

which have antimicrobial properties by damaging bacterial cell membranes, leading to cell death.

Moreover, they can secrete carrageenan, which works to:

- **Nutrient depletion:** By forming a gel matrix, carrageenan may absorb nutrients from the environment, limiting their availability for bacterial growth.
- **Targeting peptidoglycan:** In Gram-positive bacteria, carrageenan may interfere with the synthesis or stability of the peptidoglycan layer, which is essential for maintaining cell wall integrity.

However, the algae do not affect *Enterococcus aerogenes*, as there is no inhibition zone observed. This explains its innate resistance, which protects it from certain antimicrobial agents and includes:

- **Structural defenses:** *Enterococcus aerogenes* is characterized by a thick outer membrane containing lipopolysaccharides (LPS), which acts as a barrier against many antimicrobial agents. This membrane prevents the penetration of compounds produced by the algae.
- **Active transport:** These bacteria possess multiple efflux pumps that can effectively expel a range of toxic substances, including antimicrobial compounds. By pumping out these compounds before they can exert their effects, they effectively resist inhibition.
- **Nutrient utilization:** They are metabolically versatile and can utilize a wide range of substrates. This ability to adapt may help them survive in environments containing algal powders, minimizing the impact of any antimicrobial compounds.
- **Inhibit biofilm formation:** It can prevent bacteria from adhering to surfaces, thus inhibiting biofilm formation, which is crucial for many pathogenic bacteria.

5. Identification of Strains Using the API 20 E Gallery:

To confirm the biochemical identity of the bacterial strains, the API 20E identification system was used. This standardized gallery allows for the characterization of Gram-negative bacteria based on a series of miniaturized biochemical tests. The image below shows the appearance of the galleries after incubation:



Figure 36: Identification of CEF2 strain using API 20E



Figure 37: Identification of Strains Using the API 20 E Gallery

The table below summarizes the positive and negative results obtained from the biochemical tests of the API 20E identification system for each bacterial strain:

Table 05: API 20E gallery results

Strains / Caractere	CBp1	CBf1	CEf2	CEMm	CBl1	CBf4	CEj1	MBVf1	CEr1	CBf5	CBm2	CBj2	CEl3
ONPG	+	+	+	+	+	+	–	–	+	+	–	–	+
ADH	–	+	+	–	–	–	+	+	+	–	+	+	–
LDC	–	–	–	–	–	–	–	–	–	–	–	–	–
ODC	–	–	–	–	–	–	–	–	–	–	–	–	–
CIT	–	+	+	–	–	–	+	+	+	–	+	+	–
H2S	–	–	–	–	–	–	–	–	–	–	–	–	–
URE	–	–	–	–	–	–	–	–	–	–	–	–	–
TDA	–	–	–	–	–	–	–	–	–	–	–	–	–

IND	+	+	–	+	+	+	–	–	–	+	–	–	+
VP	–	–	+	–	–	–	+	+	+	+	+	+	–
GEL	–	–	+	–	–	–	+	+	+	–	+	+	–
GLU	+	+	+	+	+	+	+	+	+	+	+	+	+
MAN	+	+	+	+	+	+	+	+	+	+	–	+	+
INO	–	–	–	–	–	–	–	–	–	–	–	+	–
SOR	+	+	–	+	+	+	–	–	–	+	–	–	+
RHA	+	+	+	+	+	+	–	–	+	+	–	–	+
SAC	+	+	–	+	+	+	–	–	–	+	–	–	+
MEL	+	+	+	+	+	+	+	+	+	+	+	+	+
AMY	+	+	–	+	+	+	–	–	–	+	–	–	+
ARA	+	+	+	+	+	+	–	–	+	+	–	–	+
CODE	104457	324457	320715	104457	104457	104457	220714	220714	320715	104557	220704	220734	104457

The API 20E system was used to identify Gram-negative bacteria isolated from marine algae. A total of thirteen isolates were analyzed, and the results showed high identification probabilities, indicating a reliable and diverse microbial community associated with the algal samples. The biochemical profiles led to the identification of 13 bacterial isolates belonging to the following genera:

- ***Aeromonas* spp:**

Five isolates (CEL3, CBVfl, MBVfl, CBF4, and CEJ1) were identified as *Aeromonas hydrophila* groups 1 or 2, with probabilities ranging from 97.1% to 100%. This genus is well known for its ubiquity in aquatic environments and its opportunistic pathogenicity in humans and animals. The repeated identification of Aeromonas species suggests a strong association between this genus and the algal surface microbiome, likely due to its ability to thrive in nutrient-rich marine environments.

- ***Pantoea* spp:**

Three isolates (CEMm, CBLI, and CBF5) were identified as Pantoea species with identification probabilities between 75.5% and 79.1%. Pantoea is typically associated with plants and soil, but its presence in marine algae might reflect contamination from terrestrial sources or adaptability to various ecological niches. Their identification is notable as some Pantoea strains have been reported to produce antimicrobial compounds, which could contribute to the observed inhibition zones in this study.

- ***Enterobacter* spp:**

Two isolates (CBJ2 and CER1) were identified as *Enterobacter cloacae* and *Enterobacter sakazakii* with high confidence (92.3% and 79.4%, respectively). These bacteria are known for their adaptability and are frequently encountered in hospital and environmental settings. Their presence in algae could suggest resilience in diverse habitats and potential roles in nutrient cycling or interaction with algal metabolites.

- **Other Identified Species:**

Leclercia adecarboxylata (CBP1) was identified with a moderate probability (55.1%), categorized as "very good identification." Although less common, this species is part of the Enterobacteriaceae family and occasionally isolated from aquatic sources.

Pseudomonas luteola (CBM2), identified with 100% certainty, is another aquatic-associated bacterium, known for its pigment production and potential biotechnological interest.

Biochemical Profile Highlights:

Summary of Metabolic Traits:

These isolates exhibited a range of enzymatic activities and sugar fermentation capabilities, highlighting two major groups:

- Broad-spectrum metabolizers capable of utilizing multiple sugars (e.g., glucose, mannitol, inositol, melibiose) and producing enzymes like β-galactosidase and tryptophanase.
- Metabolically specialized strains, such as CEJ1, CBM2, CBJ2, and MBVf1, which showed selective sugar fermentation but were positive for citrate utilization, gelatinase activity, and acetoin production (VP test), suggesting adaptation to specific environmental niches and a potential for secondary metabolite production.

The presence of proteolytic enzymes (e.g., gelatinase), mixed-acid or butanediol fermentation pathways, and broad sugar utilization reflects a functional versatility well suited to the marine algal environment. Importantly, some isolates with distinct metabolic traits also showed antibacterial activity, suggesting a link between metabolic capacity and bioactive compound production. These findings reinforce the idea that marine algae host diverse bacterial communities with biotechnological potential.

Table 06: Biochemical Profile of the Isolates

Test	Positive isolates	Negative isolates	interpretation
ONPG	CBP1,CBF4,CEMm,CBL1 ,CEF2,CER1,CBF5,CEL3	CEJ1, MBVf1, CBM2, CBJ2	Ability to hydrolyze ONPG, indicating lactose fermentation potential
ADH	EJ1, MBVf1, CBM2, CBJ2, CEF2, CER1	CBP1, CBF4, CEMm, CBL1, CBF5, CEL3	Arginine metabolism into ornithine and ammonia under nitrogen-limited conditions
LDC/ODC	None	All	Absence of amino acid decarboxylation pathways
CIT	CBF1, CEF2, CEJ1, MBVf1, CER1, CBM2, CBJ2	CBP1, CEMm, CBL1	Ability to use citrate as sole carbon source
H2S	None	All	Lack of sulfur compound reduction (e.g., thiosulfate reductase)
URE	None	All	Inability to hydrolyze urea into ammonia and CO□
TDA	None	All	No deamination of tryptophan into indolepyruvate (unlike Proteus spp.)
IND	CBP1, CBF4, CEMm, CBL1, CBF5, CEL3	CEF2, CEJ1, MBVf1, CER1, CBM2, CBJ2	Tryptophanase-positive strains convert tryptophan to indole
VP	CEF2, CEJ1, MBVf1, CER1, CBM2, CBJ2, CBF5	CBP1, CBF4, CEMm, CBL1, CEL3	Production of acetoin via 2,3-butanediol pathway (neutral end-products)
GEL	CEF2, CEJ1, MBVf1, CER1, CBM2, CBJ2	CBP1, CBF4, CEMm, CBL1, CBF5, CEL3	Proteolytic activity gelatin degradation, possibly for organic matter utilization
GLU	All except CBM2	CBM2	Facultative glucose fermentation (acid production)
MAN	All except CBM2	CBM2	Mannitol fermentation
INO	All except CBJ2	CBJ2	Inositol metabolism via inositol dehydrogenase
SOR	CBP1, CBF4, CEMm, CBL1, CBF5, CEL3	CEF2, CEJ1, MBVf1, CER1, CBM2, CBJ2	Sorbitol fermentation presence of sorbitol dehydrogenase
SAC	CBP1, CBF4, CEMm, CBL1, CBF5, CEL3	CEF2, CEJ1, MBVf1, CER1, CBM2, CBJ2	Sucrose fermentation via invertase
AMY	CBP1, CBF4, CEMm, CBL1, CBF5, CEL3	CEF2, CEJ1, MBVf1, CER1, CBM2, CBJ2	Amygdalin degradation β-glucosidase activity
RHA	CBP1, CBF4, CEMm, CBL1, CBF5, CEL3	CEJ1, CBM2, CBJ2, MBVf1	Rhamnose metabolism via isomerases
ARA	CBP1, CBF4, CEMm, CBL1, CBF5, CEL3	CEJ1, CBM2, CBJ2, MBVf1	Arabinose utilization entry into central metabolism
MEL	All	None	α-galactosidase mediated melibiose fermentation

A total of fifty bacterial strains were successfully isolated and identified from marine macroalgal samples, including *Aeromonas hydrophila, Pantoea spp., Enterobacter spp*., *Pseudomonas luteola*, and *Leclercia adecarboxylata*, with identification probabilities ranging from 75.5% to 100%. Several strains particularly *A. hydrophila* and *P. luteola* demonstrated strong antibacterial activity, especially against *Staphylococcus aureus*, with inhibition zones exceeding 20 mm. Compared to commonly reported macroalgal-associated bacteria such as *Pseudoalteromonas, Zobellia*, or *Flavobacterales*, none of these typical genera were found in our samples. This divergence may reflect the unique environmental conditions of the sampling sites or methodological factors. The dominance of *Aeromonas* and *Pantoea* suggests that macroalgae in this region could host an underexplored reservoir of bacteria with significant biotechnological potential, particularly in the search for novel antimicrobial agents.

General Conclusion

Conclusion

This study has highlighted the promising potential of marine macroalgae and their associated bacteria as natural sources of antibacterial agents. Through a multidisciplinary approach combining ecological observation, microbiological isolation, and bioactivity testing, several key findings have emerged.

Firstly, the diversity of macroalgae collected from the coastal regions of Annaba and El Tarf demonstrated a wide range of morphological characteristics and environmental adaptations. These algae thrived in distinct ecosystems ranging from freshwater to brackish water each characterized by specific salinity levels, pH values, conductivity, and dissolved oxygen content. These environmental parameters were found to influence both the growth of algae and the composition of their microbial communities.

Secondly, the isolation and identification of bacteria associated with the algal samples confirmed the presence of a rich and varied microbiota, some of which belonged to genera known for their bioactive compound production. Several strains showed notable antibacterial activity against a panel of pathogenic indicator bacteria, confirming the hypothesis that epiphytic bacteria from marine macroalgae can act as antimicrobial producers.

In addition, the algae themselves exhibited direct antibacterial properties, particularly when tested as powders or incorporated into culture media. This supports the idea that both algae and their associated microbes contribute to the production of bioactive molecules—either individually or synergistically.

Moreover, environmental stress factors such as salinity and nutrient availability appeared to modulate the expression of these bioactivities. This suggests that algae–bacteria interactions are dynamic and ecologically responsive, possibly influenced by competition, symbiosis, and chemical signaling.

In addressing our initial research question whether marine macroalgae and their associated bacteria can serve as reliable sources of antibacterial agents, and how environmental factors influence these activities our findings provide a clear affirmative. Both the algae and their microbial symbionts demonstrated antimicrobial potential, with observable variation depending on environmental context and microbial identity.

Overall, this work contributes to the expanding field of marine biotechnology by confirming the biological richness and therapeutic potential of marine organisms in underexplored regions like the Algerian coast. It opens the door to further studies aimed at purifying and characterizing specific bioactive compounds, understanding their modes of action, and potentially applying them in medical, agricultural, or environmental applications.

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Annex:

Preparation of Culture Media:

1)BG11 Medium:

- K□HPO□ (Dipotassium phosphate) – 0.04 g
- MgSO□ (Magnesium sulfate) – 0.075 g
- CaCl□ (Calcium chloride) – 0.036 g
- Citric acid – 0.06 g
- EDTA (Ethylenediaminetetraacetic acid) – 0.001 g
- Na□CO□ (Sodium carbonate) – 0.02 g
- 1 mL of trace metal solution, prepared as follows:
  - H□BO□ (Boric acid) – 2.860 g/L
  - ZnSO□ (Zinc sulfate) – 0.22 g/L
  - CuSO□ (Copper sulfate) – 0.097 g/L

1. Dissolve all components in 1 liter of distilled water.

2. Add 1 mL of the prepared trace metal solution.

3. Mix thoroughly until all components are completely dissolved.

4. Sterilize by autoclaving if necessary.

2)Enriched Seawater Medium:

- NaHCO□ (Sodium Bicarbonate) – 11 g
- K□HPO□ (Dipotassium Phosphate) – 0.5 g
- FeSO□ (Iron (II) Sulfate) – 0.01 g
- Natural or artificial seawater – 1 liter

1. Add NaHCO□ to 1 liter of seawater.
2. Incubate the solution at 37°C for 2 hours.
3. After incubation, add: K□HPO□ and FeSO□
4. Mix well until fully dissolved.

3)Nutrient Broth (BN):

- Distilled water 1 liter
- Nutrient broth powder (BN) 8g

1. Dissolve 8 g of BN powder in 1 liter of distilled water.

2. Mix thoroughly until completely dissolved.