People's Democratic Republic of Algeria
Ministry of Higher Education and Scientific Research
University ABOUBEKR BELKAID – TLEMCEN
Faculty of Nature and Life Sciences, and Earth and Universe Sciences
Department of Biology
Natural Products Laboratory (LAPRONA)

Master's thesis

Presented by

BERTAL Rabha and **BENHAMMOU Manar**

In order to obtain the

Master's Degree in Biological Sciences

Specialty: **Microbiology and Quality Control**

Evaluation of the Biological Activities (Antimicrobial and Antioxidant) of Bioactive Compound-Producing Actinomycetes Isolated from the Soil of the Mkhalled Coast (Tlemcen, Western Algeria)

Defended on **Wednesday**, **July 8, 2026,** Tlemcen

Before the following jury composed of :

Presidente:	Mrs. BOUBLENZA Nesrine	MCA	University of Tlemcen
Examiner:	Mrs. ZERHOUNI Khadidja	MCA	University of Tlemcen
Supervisor:	Mr. BELYAGOUBI Larbi	Professor	University of Tlemcen
Co-Supervisor:	Mrs. BELYAGOUBI-BENHAMMOU Nabila	Professor	University of Tlemcen

Academic year: 2025/2026

Dedication

*In the name of **God**, the Most Gracious, the Most Merciful.*

*To Almighty **God** who gave me the health, will, patience and strength necessary to accomplish this work.*

I dedicate this humble thesis,

*To my dear parents, **Nebya** and **Mohammed**, the most precious beings in my eyes. We thank you for your boundless love, silent sacrifices, prayers, trust, and unconditional support. You have always been my light and source of courage. May God grant you health, happiness and a long life.*

*To my sisters **Souad** and **Chahrazed**, and especially my sister **Nassira**, who have always been by my side with their affection, encouragement and presence.*

To all my family members, for their moral support and prayers.

To my dear friends who shared with me the joys and difficulties of this academic journey. Thank you for your loyalty, help and all the wonderful memories.

To all who contributed directly or indirectly to completing this message.

I hope that this work will be a testimony to my deep appreciation and great gratitude to all who supported me during this journey.

*I would like to express my deepest gratitude to my supervisor, Mr. **BELYAGOUBI Larbi**, and his esteemed wife, Mrs. **BELYAGOUBI-BENHAMMOU Nabila**, for their invaluable guidance, continuous support, patience, and encouragement throughout the course of this research. Their expertise, insightful advice, and unwavering commitment have been instrumental in the successful completion of this work.*

Rabha

Dedication

*In the name of **Allah**, the Most Gracious, the Most Merciful.*

*First and foremost, I dedicate this work to **Allah (SWt)**. Every word written on these pages is by His will, guidance, and mercy. I sincerely pray that this work will become an ongoing charity, benefit others and gain acceptance. May Allah accepts my humble work.*

*To my beloved parents, **Farida** and **Ali**, words cannot express my deepest gratitude to you. Your unconditional love, endless sacrifices, unwavering support, and constant prayers have supported every achievement in my life. This achievement belongs to you as much as it belongs to me. Thank you for believing in me, trusting my dreams, and encouraging me to follow my dreams through difficult times.*

*To my dear sisters **Mounia** and **Samia**, thank you for your love, patience, encouragement and for always standing by my side. Your presence has been a source of strength and comfort for me.*

*Without forgetting my dear brother **ilyes** for his care, emotional and financial support and love he gave me by actions all along this months of working , thank you for being there for me*

*To my dear cousins, **Fey** and **Ines**, thank you for your kindness, friendship, joy and motivation that you have brought into my life. Your support meant a lot for me.*

*I would like to express my sincere gratitude to my supervisor, **Mr. BELYAGOUBI Larbi**, and **Mrs. BELYAGOUBI-BENHAMMOU Nabila**, for their valuable guidance, continued support, patience, and encouragement throughout these months of work.*

Finally, I dedicate this work to me for working hard and for believing in my abilities Elhamdoulilah and I appreciate everyone who encouraged me, prayed for me, and believed in me. We hope that this achievement will inspire continuous learning, perseverance, and the pursuit of knowledge for the benefit of humanity and future research, God willing.

Manar

Acknowledgment

In the name of Allah, the Most Gracious, the Most Merciful.

First and foremost, all praise and thanks are due to Allah (SWT), whose infinite mercy, guidance, and blessings gave me the strength, patience, and perseverance and trust to complete this work. I pray that every word written in this thesis is accepted as Sadaqah Jariyah, benefiting others and earning His pleasure.

I would also like to extend my warmest thanks to our supervisor Mr. ***BELYAGOUBI Larbi*** *and Mrs.****BELYAGOUBI-BENHAMMOU Nabila*** *for their kindness, generosity, continuous support, and encouragement. Their help and goodwill have been deeply appreciated and will always be remembered with gratitude*

I would like to express my deepest gratitude to the member of the jury, Mrs. ***BOUBLENZA Nesrine****, for accepting to evaluate this work and for the time and effort devoted to its assessment, for her support and her help in laboratory .*

My sincere appreciation goes to my promoter, Mrs. ***ZERHOUNI Khadidja*** *for her invaluable guidance, constant encouragement, insightful advice, her beautiful smile and unwavering support throughout the realization of this thesis. Her expertise, patience, and constructive comments have been essential to the successful completion of this work.*

My sincere thanks go to all the members of the laboratory LAPRONA for their valuable support, collaboration, and friendly working environment throughout this research. Their assistance has been greatly appreciated.

الملخص

تعد النظم البيئية الساحلية الأرضية والتربة في الجزائر موطنا لمجموعة واسعة من الكائنات الحية الدقيقة التي يمكن أن توفر المواد النشطة بيولوجيا المستخدمة في قطاعات متعددة مثل صناعة الأدوية والزراعة العضوية والطب...

قمنا في دراستنا بتحليل الأنشطة المضادة للميكروبات ومضادات الأكسدة لسلالات الفطريات الشعاعية المعزولة من تربة الغابات والمناطق الجبلية الساحلية في منطقة المخلد.

مستخلصات سلالات الفطريات الشعاعية المكتسبة عن طريق التخمير في اوساط سائلة (وسط Benette وAF وGNG)

تم عزل ما مجموعه 11 سلالة باستخدام ISP2، وسط GNG، وسط TSA، Gauss، إلخ وتم تحديدها باستخدام وسائط مختلفة (الجيلاتين الجيلاتيني، أجار الكازين، TSI، مقايسة السيترات، مقايسة الكاتلاز، صبغة جرام...). ومن خلال تقييم النشاط المضاد للميكروبات باستخدام طريقة أسطوانة الأجار على 5 سلالات نقية، وجد أن E3.2 لديه منطقة تثبيط تبلغ حوالي 11 ملم ضد *باسيلوس سيروس* . و8 ملم ضد *كلابسيلا بنومونيا* أظهرت سلالات مثل E1.9 وE1.11 نشاطًا كبيرًا ضد *بسودوموناس ايروجينوزا* ، مع مناطق تثبيط تبلغ 9.75 و9.5 ملم، اما بالنسبة الى *كانديدا البيكانس* لم يسجل أي نشاط واضح. وفيما يتعلق بطريقة انتشار البئر، لم تظهر جميع السلالات منطقة تثبيط، ربما لأسباب مختلفة : عدم كفاية المستقلبات النشطة، أو المقاومة في الاختبارات البكتيرية، أو المشاكل التقنية مثل عدم كفاية قطر العينة أو عدم كفاية وقت ما قبل الانتشار قبل الحضانة. وفيما يتعلق بالنشاط المضاد للأكسدة الذي تم تحقيقه باستخدام المستخلصات التي تم الحصول عليها بعد التخمير، أظهرت النتائج أن السلالة E1.9 أظهرت أفضل قدرة مضادة للأكسدة إجمالية، بقيمة قصوى تبلغ 8.5 ملغ EAA/جم من المستخلص الجاف على وسط بينيت، بالإضافة إلى أعلى نشاط لاحتجاز جذر DPPH، حيث وصل التثبيط إلى 88٪. أظهرت سلالة E3.2 المزروعة على وسط AF أيضًا نشاطًا مضادًا للأكسدة ملحوظًا، مع قدرة إجمالية مضادة للأكسدة تبلغ 6.7 مجم EAA/جم مستخلص جاف وتثبيط DPPH بحوالي 59% . وهذا يدل على وجود سلالات أقوى مقارنة بالسلالات الأخرى، ويعتمد ذلك على طبيعة البيئات وتكوينها والسلالات المزروعة.

الى جانب ذلك، فإن تنوع التربة الساحلية في الجزائر و اختلاف كائناتها الحية تتمتع بإمكانات بيولوجية كبيرة كمضاد للميكروبات ومضاد للأكسدة ويستحق المزيد من الاستكشاف والقراءة.

الكلمات المفتاحية: التربة الساحلية، سلالات الأكتينوميسيتات، وسائط الزراعة والبكتيريا المختبرة, النشاط المضاد للميكروبات، النشاط المضاد للأكسدة.

Abstract

Algerian coastal terrestrial ecosystems and soil include a large diversity of microorganisms that can provide a remarkable origin of biologically active substances used in multiple sectors such as pharmaceuticals, industry, biological agriculture, and medicine.

In our study, we analyzed the antimicrobial and antioxidant activities of actinomycete strains isolated from the soil of forest and mountainous coastal areas of Mkhalled. Extracts from these actinomycete strains were acquired from fermentation in liquid media (Benett, AF, and GNG medium). A total of 11 strains were isolated using ISP2, GNG medium, TSA medium, Gauss and others and identified using different media (gelatin agar, casein agar, TSI, citrate test, catalase test, gram staining, etc.). Evaluating the antimicrobial activity using the cylinder agar method on 5 purified strains showed that E3.2 exhibits an inhibition zone approximately 11 mm against *Bacillus cereus* and 8 mm against *Klebsiella pneumoniae*, strains such as E1.9 and E1.11 showed remarkable activity against *Pseudomonas aeruginosa*, with inhibition zones of 9.75 and 9.5 mm, and no activity was on *Candida albicans*. Moreover, the well diffusion method indicates that all strains did not show any inhibition zone, possibly due to different reasons: insufficient active metabolites, resistance of the bacteria tests, or technical problems such as unsuitable well diameter or insufficient pre-diffusion time before incubation. Regarding the antioxidant activity achieved with the extracts obtained after fermentation, the results show that strain E1.9 presented the best total antioxidant capacity, with a maximum value of 8.5 mg AAE/g of dry extract on Bennett medium, as well as the highest activity of trapping the DPPH radical, reaching 88% inhibition. The E3.2 strain grown on AF medium also showed remarkable antioxidant activity, with a total antioxidant capacity of 6.7 mg EAA/g dry extract and an inhibition of DPPH of about 59%. This indicates that there are strains that are more potent compared to the others, which depend on nature, the composition of the media, and the cultivated strains.

Besides, the diversity and differentiation of Algerian coastal soils submit a large biological potential as antimicrobial and antioxidant to be more explored and more discovered.

Keywords: actinomycete strains, Mkhalled coastal soil, culture mediums, bacteria tests, antimicrobial activity, antioxidant activity.

Résumé

Les écosystèmes terrestres côtiers et le sol algérien abritent une grande diversité de micro-organismes qui peuvent fournir une origine reconnaissable à des substances actives biologiques utilisées dans de multiples secteurs, tels que l'industrie pharmaceutique, l'agriculture biologique, la médecine...

Dans notre étude, nous avons analysé les activités antimicrobiennes et antioxydantes de souches d'actinomycètes isolées du sol des zones forestières et côtières montagneuses de Mkhalled. Extraits de ces souches d'actinomycètes acquis par fermentation en milieu liquide (Benette, milieu AF et GNG). Un total de 11 souches ont été isolées à l'aide d'ISP2, du milieu GNG, du milieu TSA, de Gauss et autres et identifiées en utilisant différents milieux (gélatine gélatineuse, gélose caséine, TSI, test au citrate, test à la catalase, coloration de Gram...). et en évaluant l'activité antimicrobienne à l'aide de la méthode de cylindre d'agar sur 5 souches purifiées, il a été montré que E3.2 présente une zone d'inhibition d'environ 11 mm contre *Bacillus cereus* et de 8 mm contre *Klebsiella pneumoniae*, les souches telles que E1.9 et E1.11 ont montré une activité remarquable contre *Pseudomonas aeruginosa*, avec des zones d'inhibition de 9.75 et 9.5 mm, et aucune activité était sur *candida albicans*. Concernant la méthode de diffusion en puits, toutes les souches n'ont pas montré de zone d'inhibition, peut-être pour différentes raisons : métabolites actifs insuffisants ou des problèmes techniques tels qu'un diamètre de puits inadapté ou un temps de prédiffusion insuffisant avant l'incubation, concernant l'activité antioxydante obtenue avec les extraits obtenus après fermentation, les résultats montrent que la souche E1.9 présentait la meilleure capacité antioxydante totale, avec une valeur maximale de 8,5 mg EAA/g d'extrait sec sur milieu Bennett, ainsi que la plus forte activité de piégeage du radical DPPH, atteignant 88% d'inhibition. La souche E3.2 cultivée en milieu AF a également montré une activité antioxydante remarquable, avec une capacité antioxydante totale de 6,7 mg d'EAA/g d'extrait sec et une inhibition du DPPH d'environ 59%. Cela indique qu'il y'a des souches plus puissantes par rapport aux autres, dépendant de nature, de la composition des milieux, et les souches cultivées.

En outre, la diversité et la décomposition des sols côtiers algériens présentent un potentiel biologique important en tant qu'antimicrobien et antioxydant qui mérite d'être davantage exploré et découvert.

Mots clés : actinomycètes, souches, sol côtier de Mkhalled, milieux de culture, bactéries tests, activité antimicrobienne, activité antioxydante.

Table of contents

List of figures---------- I
List of tables---------- II
List of abbreviations---------- III
General introduction ---------- 1
Material and methods---------- 10
1. Collection of samples ---------- 11
1.2.Simpling ---------- 12
1.3. Measurement of the physico-chemical parameters of the samples ---------- 12
1.3.1. Measurement of pH ---------- 12
1.3.2. Measurement of electrical conductivity and salinity---------- 12
2.Isolation and purification of actinomycetes ---------- 12
3. Identification ---------- 13
3.1. Morphological characterization ---------- 13
3.1.1. Macroscopic observation ---------- 13
3.1.2. Microscopic observation---------- 13
3.2. Physiological and biochemical analysis---------- 13
3.2.1. Hydrolysis of casein---------- 14
3.2.2. Starch hydrolysis ---------- 14
3.2.3. Gelatin hydrolysis ---------- 14
3.2.4. Action on skimmed milk ---------- 14
3.2.5. Catalase test ---------- 15
3.2.6. Citrate utilization ---------- 15
3.2.7. Use of different carbon substrates on TSI medium agar - ---------- 15
4. Fermentation and extraction of metabolites ---------- 15
4.1. Optimization, fermentation and extraction of active substances in liquid medium ----------16
5. Assessment of biological activities ---------- 16
5.1. The antimicrobial activity of actinomycetes ---------- 16
5.1.1 Tested strains ---------- 16
5.1.2. Agar cylinder technique ---------- 17
5.1.3. Diffusion method for agar wells ---------- 17

5.2. The antioxidant activity of the extract ---------- 18
5.2.1. Total antioxidant capacity (TAC) ---------- 18
5.2.2. DPPH free radical scavenging assay ---------- 18
Results and discussion ---------- 19
1. Physico-chemical analyses of soil samples ---------- 20
2. Isolation of actinomycetes ---------- 20
3. Identification ---------- 23
3.1. Morphologic characterization ---------- 23
3.1.1 Macroscopic observation ---------- 23
3.1.2 Microscopic observation ---------- 23
3.2. Physiological and biochemical analysis ---------- 24
4. Assessment of biological activities ----------26
4.1. Evaluation of the antimicrobial activity of the isolated strains ---------- 26
4.1.1.Agar cylinder method ---------- 26
4.1.2. Well method ---------- 29
4.2. Evaluation of the antioxidant activity of the isolated strains ---------- 29
4.2.1. Total antioxidant capacity (TAC) ---------- 29
4.2.2. DPPH free radical scavenging assay ----------30
Conclusion ---------- 32
References ---------- 34
Annexes---------- 42

List of figures

Figure1. Diversity of actinomycetes habitats. ---------- 3
Figure2. Actinomycetes' biofilm and aggregations' structures. ---------- 4
Figure3. The life cycle of actinomycetes. ---------- 5
Figure4. Distribution of the discovered antibiotics according to their origin. ---------- 7
Figure5. Geographical location of the sampling area----------11
Figure6. Photo of isolation of actinomycetes ----------21
Figure7. Pure culture of isolated actinomycetes.----------22
Figure8. Macroscopic aspect of actinomycete strains ----------23
Figure9. Microscopic observation of actinomycete strains ----------24
Figure10. Coagulation of skimmed milk ----------25
Figure11. Result of catalase production from the 5 strains ----------26
Figure12. Antimicrobial activity of actinomycete strains by the cylinder method of agar. ----------27
Figure 13. The total antioxidant capacity (TAC) of extracts from actinomycete strains --------30
Figure 14. Antioxidant activity of extracts from actinomycete strains evaluated by the DPPH test ----------31

List of tables

Table 1. List of some antibiotics produced by *Streptomyces sp*.----------8
Table 2. Geographical location of the sampling stations ---------- 11
Table 3. Strains used in the various antimicrobial activity tests ----------16
Table 4. Physico-chemical parameters of soil samples ----------20
Table 5. Results of the macroscopic aspect study of isolate ----------22
Table 6. Biochemical characteristics of actinomycete strains ----------24

List of abbreviations

TSA: Tryptic Soy Agar

ISP: International Streptomyces Project

GNG: Nutritious gelatin.

TSI : Triple Sugar Iron Agar.

AF: Actinomycete fermentation

rmp: Revolution Per Minute

UFC: Colony Forming Unit

ATCC : American Type Culture Collection.

% : Percent

°C: Degree Celsius

C+G: Cytosine+Guanine

OD: Optical Density

pH: Potential of Hydrogen

Generalities

Generalities

With an area of roughly 2.38 million km, Algeria is the largest country in Africa **(Saidi *et al*., 2023)**, going from the Mediterranean coastline (north) to the Sahara Desert (south), passing through the Tell Atlas and Saharan mountains, as well as several semiarid plateaus. A great climatic diversity coexists with this wide range of relief: semi-arid climates in the steppe, arid to hyperarid climates in the Saharan regions, and humid Mediterranean climates in the north. Different regions have different biological circumstances due to significant variations in precipitation, temperatures, winds, and evaporation. Algerian ecosystems present a great diversity of ecological conditions, ranging from the Mediterranean coastal areas to mountainous, steppe, and Saharan regions. This diversity is reflected in the characteristics of soils, which differ by their texture, pH, organic matter content, salinity, and nutrient availability. These properties strongly influence the composition, diversity, and activity of microbial communities.

The soil is the outermost layer of the Earth's crust formed by the disintegration or fragmentation of the underlying parent rock under the influence of physicochemical and biological factors. It consists of layers (horizons) of worn mineral materials, organic matter, air, and water. It is a surface formation that is an essential component of biotopes unique to continental ecosystems. Their ensemble, called the pedosphere, results from the interaction of two biospheric compartments: the atmosphere and the surface layers of the lithosphere **(Manneville *et al*., 1999)**.

As we know, the physicochemical characteristics of soil play an essential role in shaping microbial communities, as various environmental and biological factors directly influence the diversity, activity, and distribution of soil microorganisms.

Soil microbial communities are among the most widespread and diverse biological groups in the natural world, and they are very small organisms, including bacteria, fungi, protozoa, nematodes, and actinomycetes. All of them play an essential role in the natural cycle and help to create a productive soil, well aerated and rich in nutrients easily assimilated by plants.

Bacteria are the most abundant soil microbes, typically outnumbering all other species combined. They may number between 100 and 200 million per gram of fertile soil. They are so small (less than a millionth of an inch) and reproduce quickly. Depending on the organism and the surroundings, a population can double in 20 minutes; it depends on the species. Soil bacteria are incredibly diverse, with over 200 primary kinds present in different soils.

Generalities

Bacteria have a significant role in breaking down cellulose and other structural components, hence reducing thatch. Nitrobacteria (*Nitrosomonas* and *Nitrobacter*) play a capital role in the process of nitrification, which converts ammonium to nitrate for plant use. This mechanism releases nitrogen from soil organic matter, as well as various slow-release and quick-release fertilizers. Besides, cellulose and lignin in the soil are broken down by decomposers, also known as saprophytic fungi. Simple sugars are broken down by sugar fungus known as *zygomycetes*, but most fungi break down more difficult-to-decompose organic wastes that are rich in cellulose, hemicellulose, lignin, or cell walls. Plant roots are shielded from pathogenic microbes by the fungus *Trichoderma*.

Protozoa are unicellular, eukaryotic, heterotrophic microorganisms (they feed on organic matter) that can move. Although it is variable in size, a typical protozoan can be ten times larger than a bacterium but still remain microscopic. Protozoa are distinguished by the way they move: amoebas move their cytoskeletons and advance thanks to their pseudopods; ciliates such as Paramecium move by undulating movement of their cilia; flagellates use one or more of their long whip-shaped flagella to move. Although much remains to be learned about the diversity of protozoa and their roles in soil, it is known that many feed on bacteria, sometimes fungi. This predatory role involves them in the structuring of microbe's communities, carbon mineralization, and the mineralization of nutrients such as nitrogen and phosphorus from microorganisms they have grazed or from small particles of soil organic matter **(Basiliko *et al*., 2021)**. Beyond the microscopic *protozoa*, soil can contain *nematodes*, which are microscopic worms belonging to the phylum *Nematoda*. They live in the pores of the soil where they move thanks to the water present between the particles. Based on their dietary habits, they can be divided into four functional categories. In agricultural soils, fungivores and bacterivores are the most prevalent groups, consuming fungi and bacteria, respectively. This consumption helps control microbial populations and contributes to nutrient cycling and nitrogen mineralization **(Van den Hoogen *et al*., 2019)**.

Among these bacteria there are actinomycetes, the term "actinomycetes" was derived from the Greek words "aktis" (a ray) and "mykes" (fungus), reflecting their filamentous morphology **(Chaudhary *et al*., 2013; Siro and Pipite, 2024).** Actinomycetes are Gram-positive filamentous bacteria, belonging to the order *Actinomycetales*, within the phylum *Actinobacteria*, and their genome contains high GC (guanine and cytosine) content (60–78%); most are strict saprophytes, but some form parasitic or mutualistic associations with plants and animals **(Goodfellow and Williams, 1983; Tripathis *et al*., 2015).**

Generalities

Numerous environments, including terrestrial (soil, rhizosphere), aquatic (marine and freshwater), and extreme (deserts, drylands, hot springs, and polar regions), have been shown to harbor Actinobacteria. The majority of studies in the current literature, however, disproportionately focus on Actinobacteria from agricultural and forest soils in tropical and subtropical regions, particularly in Asia and Latin America, indicating a significant geographical and ecological bias **(Genilloud, 2017)**. This overrepresentation may neglect unique and metabolically diverse strains that can occupy underexplored niches such as deep-sea sediments **(Subramani and Aalbersberg, 2012)**, polar soils **(Zhang *et al.*, 2019),** or saline and alkaline ecosystems.

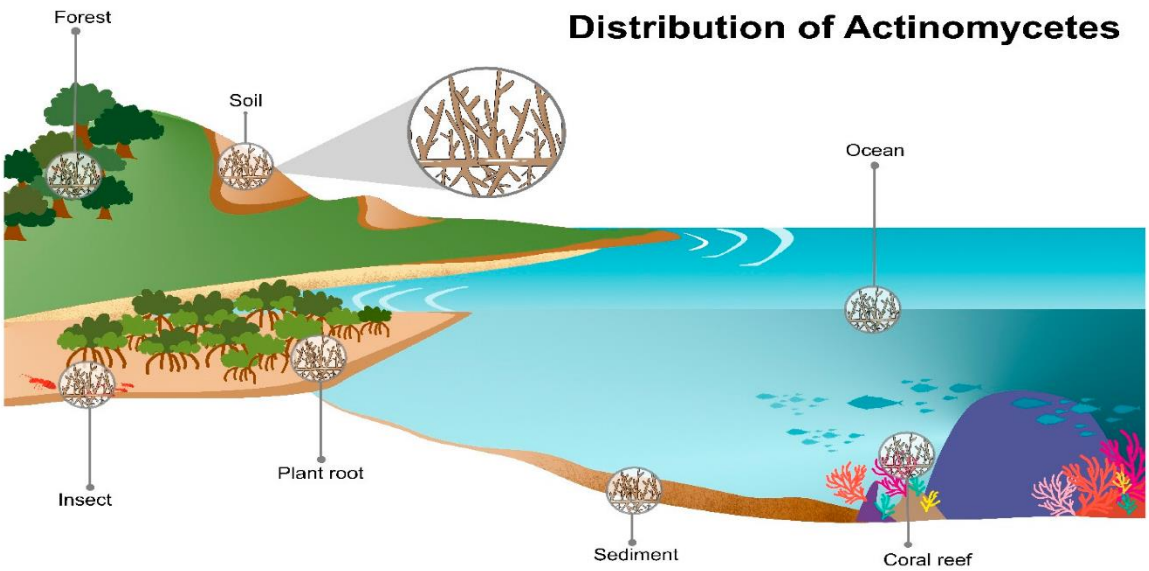


Figure 1. Diversity of actinomycetes habitats **(Van *et al.*, 2017)**.

Actinomycetes of soil develop as hyphal fungi, responsible for the characteristic "earthy" smell of freshly turned and healthy soil. Because actinomycetes need oxygen, their population is most significant in the soil's surface layer and progressively declines with depth **(Bhatti *et al.*, 2017).** They are sensitive to low acidity/pH (the optimal pH range is 6.5 to 8.0) and water-saturated soil conditions. These microorganisms are mainly mesophilic, living at temperatures between 25°C and 30°C.

Generalities

Actinomycetes can be heterotrophic or chemoautotrophic, but most are chemoheterotrophic **(Ait Barka *et al*., 2016);** their average generation period (2 to 3 hours) is longer than that of other bacteria **(Kitouni, 2007)**, and they grow slowly as branched filaments **(Nanjwad *et al*., 2010)**. The mycelial structures can be aerial or adhered to the substrate **(Wu *et al*., 2020; Cimmino *et al*., 2016).** This characteristic, together with the fact that some individuals produce spores as a form of asexual reproduction, has already led actinomycetes to be considered transitional microorganisms between bacteria and fungi.

The distinction between substrate and aerial mycelium is based on their functional and morphological differences. The substrate mycelium, called vegetative or primary mycelium, plays a major role in absorbing nutrients from the medium, thus allowing the growth of actinomycetes. It also has a wide variety of colors. Conversely, the aerial mycelium, also called secondary mycelium, consists of the hyphae produced by the vegetative mycelium under stress conditions. Its main function is reproduction, during which spores develop on aerial hyphae. These hyphae appear bright in phase contrast, refractive and coarse-shaped **(Chaudhary *et al*., 2013).**

Figure 2 shows the diverse growth characteristics shown by some actinomycetes and their ability to form biofilm structures and aggregations.

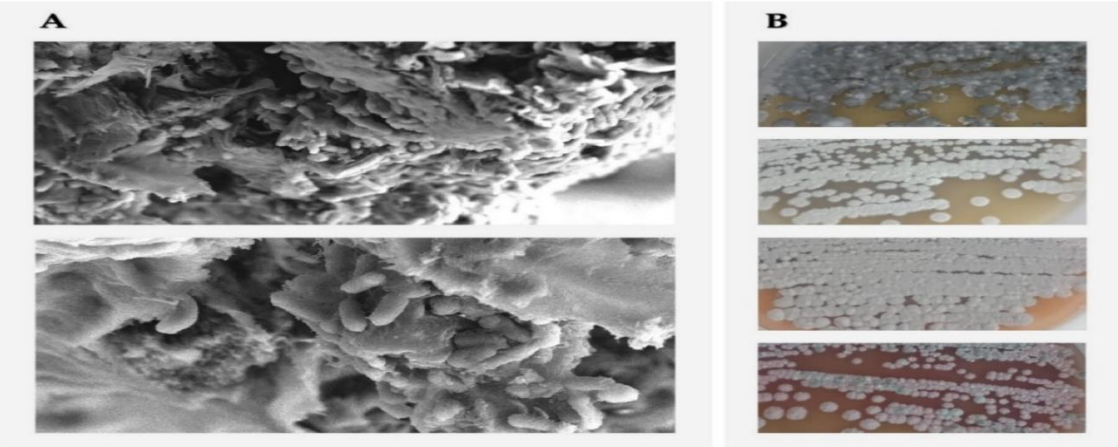


Figure 2. Actinomycetes' biofilm and aggregations' structures formed on surfaces visualized by scanning electron microscopy **(A)** and growth shapes and colors of spores formed on agar plates of the International *Streptomyces* Project n° 2 **(B). (Farda *et al*., 2022).**

Generalities

Most actinomycetes are aerobic microorganisms with complex life cycles **(Figure 3)**, except for *Corynebacterium* unicellular and *Mycobacterium* (**Prudence *et al*., 2020).** Throughout their life cycle, their well-developed radial mycelium divides into substrate and aerial mycelium. The aerial mycelium develops after the substrate mycelium has grown in the medium to absorb nutrients. On the other hand, the hyphae curl and form a septum when actinomycetes grow in a deficient environment. After the development of the septum, conidiospores are formed inside the hyphae. Other genera exhibit unique traits, such as *Micromonospora* (single non-mobile spore), *Microbispora* (two spores in a chain), and *Streptosporagium* (carrying sporangium as a spore vesicle), with the exception of Streptomyces, which has long-chain spores. When favorable conditions are met, spores will be discharged into the environment and germinate. The spore will protrude germ tubes during the germination stage. Once the germ tubes reach the vegetative growth stage, the cycle is repeated.

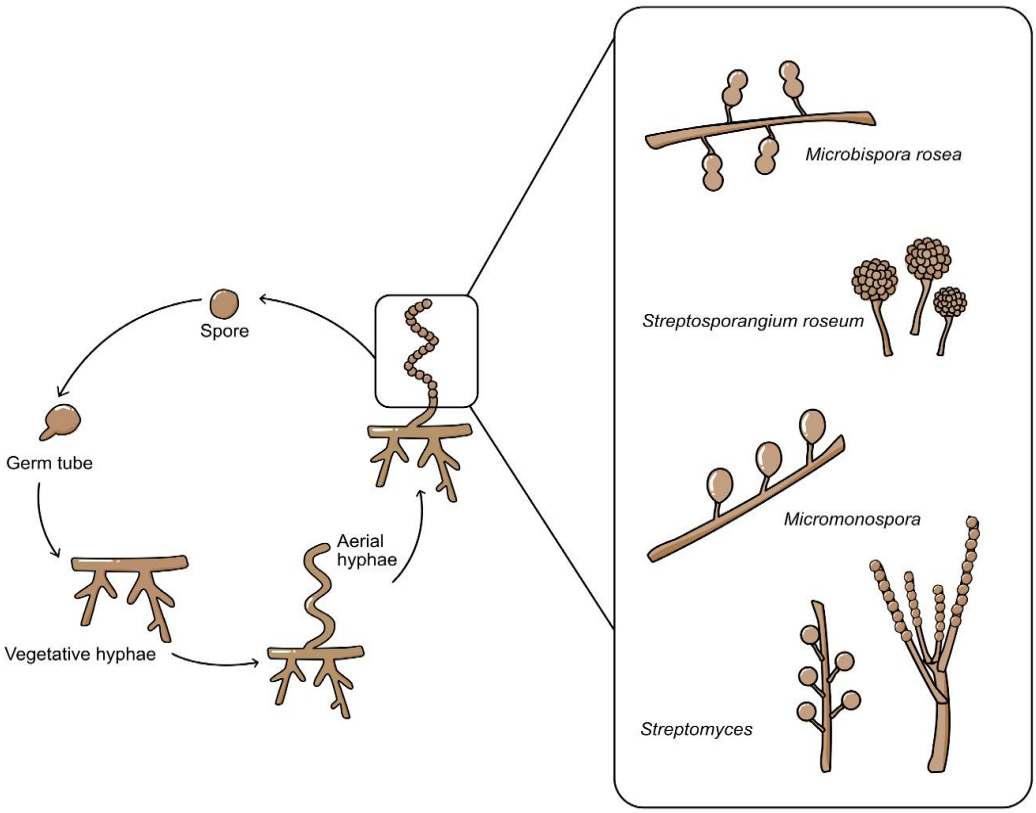


Figure 3. The life cycle of actinomycetes, starting from conidiospores until sporulation. The various types of conidiospores are shown in the expanded box.

Generalities

Recent studies have shown that actinomycetes are members of the phylum *Actinobacteria*, and *Actinomycetales*, *Streptomycetales*, *Micromonosporales*, *Pseudonocardiales*, and other ecologically and industrially significant orders are included in the class Actinobacteria within this phylum. These orders include a wide range of genera that are recognized for their environmental significance and metabolic adaptability. The genus *Streptomyces* has been investigated the most among them, especially because of its exceptional ability to produce antibiotics and their role in the breakdown of organic materials. Furthermore, other genera, including *Rhodococcus*, *Nocardia*, *Micromonospora*, and *Nocardiopsis*, have shown remarkable skills in bioremediation, particularly in breaking down complex organic contaminants and withstanding heavy metals **(Hashim *et al*., 2026).**

Actinomycetes play key ecological roles in soils, contributing to the decomposition of organic matter and nutrient dynamics, these functions support soil structure and long-term fertility. They degrade complex substrates such as lignin, cellulose and chitin, thus contributing to the mineralization of soil organic matter **(Grigoryan and Bataeva, 2023)**. Several actinomycetes interact with plants as rhizobionts or endophytes and promote their development through multiple mechanisms. These interactions can reduce the use of chemical inputs and support sustainable agricultural practices. They mobilize nutrients (e.g., phosphate solubilization) and produce siderophores and growth hormones that stimulate the plant **(Negi *et al*., 2024)**. They produce enzymes that are crucial to the food, fermentation, and textile industries, including cellulase, lipase, amylase, and protease **(Sharma *et al*., 2014).** In addition to their contribution to soil fertility and microbial interactions, actinomycetes are recognized as prolific producers of secondary metabolites, which are generally synthesized during the stationary phase of growth, exhibiting a remarkable diversity of biological activities that extend beyond their well-documented antimicrobial properties.

The potential use of actinomycetes as a source of bioproducts began in the 1920s, with the discovery of antibiosis in a species from this genus. Later, in the 1940s, the first antibiotic compounds produced by actinomycetes were identified, including streptomycin, which became the first active treatment for tuberculosis **(Waksman, 2010)**. Members of the order *Actinomycetales* are particularly renowned for their prolific production of bioactive compounds, with an extensive record of over 10,000 antimicrobial agents currently utilized in pharmaceutical applications (**Sharma, 2014**). Among these bioactive compounds, antibiotics represent the most significant group, with approximately 75% of clinically important antibiotics derived from actinomycetes **(Figure 4) (Hong *et al*., 2017)**.

A vast array of these antibacterials exhibits a broad spectrum of activity and employs diverse mechanisms of action. They are highlly effective against a wide range of both Gram-positive and Gram-negative bacteria **(Hassani, 2014).**

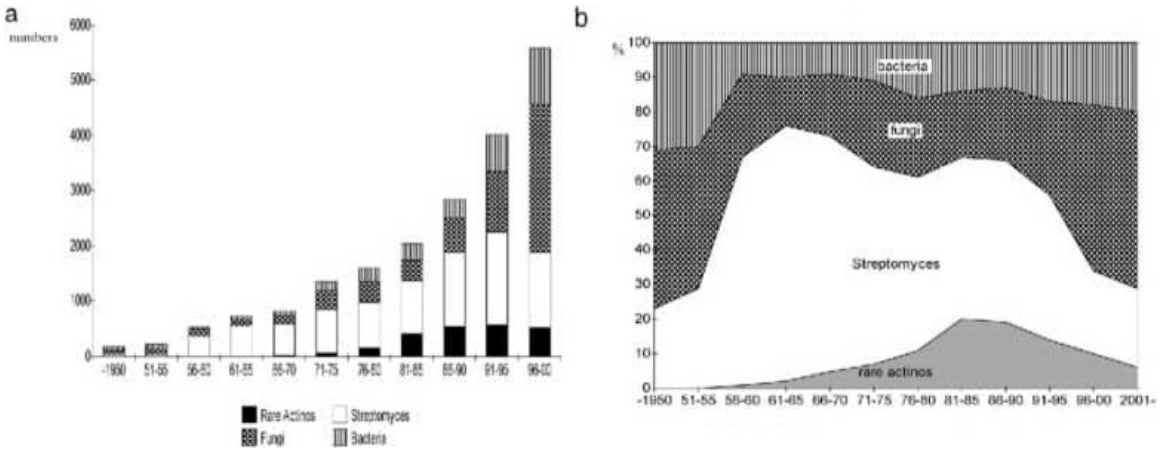


Figure 4. Distribution of the discovered antibiotics according to their origin **(a)** number and **b** (percentage)**. (Hong et al., 2017).**

While several actinobacterial genera contribute to the production of antibacterial compounds, *Streptomyces* is considered the most prolific producer of these metabolites. This bacterium is a genus of Gram-positive bacteria that resembles filamentous fungi in shape and flourishes in a variety of settings. Streptomyces undergoes morphological differentiation by forming a layer of hyphae that can develop into a chain of spores. The capacity of Streptomyces to generate bioactive secondary metabolites, including antifungals, antivirals, antitumorals, antihypertensives, immunosuppressants, and particularly antibiotics **(Table 1)**, is its most intriguing characteristic. The majority of antibiotics are species-specific, and Streptomyces species depend on these secondary metabolites to compete with other microbes, even those in the same genus.

Table1. List of some antibiotics produced by *Streptomyces sp*. **(Kiesier *et al*., 2000).**

Streptomyces sp.	Antibiotics	*Streptomyces .sp*	Antibiotics
S. orchidaccus	Cycloserin	*S.erythraeus*	Erythromycin
S.oriantalis	Vancomycin	*S.vansuella*	Chloramphenicol
S. fradiae	Neomycin Actinomycin Fosfomycin Dekamycin	*S.aureofaciens*	Chlortetracycline, D Imethylchlor Tetracycline
S. nodosus	Amphotericin B	*S.ambofaciens*	Spiramycin
S.noursei	Nystatin	*S.avermitilis*	Avermicin
S.mediterranei	Rifampicin	*S.alboniger*	Puromycin
S.griseus	Streptomycin	*S.niveus*	Novobicin
S.knanamyceticus	Kanamycin	*S.platensis*	Platenmycin
S.tenebrarius	Tobramycin	*S.rososporus*	Daptomycin
S.spectabilis	Spectinomycin	*S. .ribosidificus*	Ribostamycin
S.viridifaciens	Teracyclin	*S. garyphalus*	Cycloserine
S.lincolensis	Lincomycin Clindamycin	*S.vinaceus*	Viomycin
S. .rimosus	Oxytetracyclin	*S.clavuligerus*	Cephalosporin

The ability of actinobacteria to produce antibiotics reflects their exceptional capacity for secondary metabolite biosynthesis. This biosynthetic diversity is not limited to compounds with antibacterial properties only. *Streptomyces avermitilis* is a bacterium isolated from the soil, and during their fermentation, they synthesize the molecules avermectins, which are 16-membered, naturally occurring or semi-synthetic macrocyclic lactone derivatives; however, this bacterium has both insecticidal and anthelmintic activities. It is utilized to control mite pests and insects in vegetable, fruit, agronomic, and ornamental crops **(Zhang *et al*., 2020)**. Besides this, *Streptomyces* bacteria are rich in potentially antiviral compounds, including a variety of secondary metabolites that show promise against viral infections. These compounds, generated from several Streptomyces species, use a variety of ways to target different stages of viral replication or the host cell. Streptomyces-derived chemicals can destroy many viruses, including herpesviruses, HIV-1, and influenza A **(Acquah *et al*., 2024**).

General introduction

Actinomycetes are recognized as one of the most important microbial sources of natural antioxidants due to their ability to produce a wide variety of bioactive secondary metabolites with free radical-scavenging properties. Oxidative stress arises from a raised intracellular quantity of free oxygen radicals that induce lipid peroxidation, protein denaturation, DNA hydroxylation, and apoptosis, ultimately decreasing cell viability. Antioxidants further aid in preventing cellular damage by scavenging free radicals and lowering oxidative stress. Since ancient times, the main sources of antioxidants have been medicinal plants, fruits, and spices. Unlike plants, microbes have the advantage of rapid growth under regulated settings, making them a useful source of antioxidants. Furthermore, in contrast to synthetic antioxidants, microbe-based antioxidants are biodegradable, harmless, and non-carcinogenic **(Rani *et al*., 2021).**

In a context where antimicrobial resistance is a growing global threat and the discovery of new antibiotics is slowing down considerably, Algerian soils, particularly those on the coastal soil of Mkhalled, Do they represent an untapped reservoir of actinomycetes that produce secondary metabolites with potentially innovative biological activities (antibacterial, antifungal, and antioxidant) ?

The present work aims to isolate and identify the actinomycete strains present in the Mkhalled beach samples, then to cultivate them on different culture media (ISP2, Bennett, TSA, GNG), then identify them, afterwards the evaluation of antimicrobial activity is tested by two methods : cylinder agar and wells method. following this , the fermentation of strains in different mediums (Bennett, GNG, AF) for antioxidant activity assessment containing the DPPH and TAC.

Material and methods

1. Collection of samples

Our work was carried out at the laboratory level, the research of Natural Products (LAPRONA).

The soil samples analyzed were taken from the rhizophric coastal soil of Mkhalled, located in the wilaya of Tlemcen (Algeria**).** It is situated east of the port of Honaïne in the Wilaya of Tlemcen, located about 60 km northwest of the city of Tlemcen and 120 km west of Sidi Bel Abbès, in the commune of Béni Khelad, under the daïra of Honaïne **(Directorate of Agricultural Services of Tlemcen, 2020)**.

Table 2. Geographical location of the sampling stations**.**

Sample	**Site of sampling**	**Localization**	**Code of the isolated actinomycetes**	**Type of soil**
S1	Coastal soil of Mkhalled area	35°12'43.9"N 1°36'52.6"W	E1	Forest
S2	Coastal soil of Mkhalled area	35°12'43.9"N 1°36'52.6"W	E2	Forest
S3	Coastal soil of Mkhalled area	35°13'03.4"N 1°36'57.0"W	E3	mountain



Figure 5. Geographical location of the sampling area of E1, E2 (Forest), and E3 (Mountain) **(Google maps, 2026).**

1.2. Sampling

The 3 soil samples was taken from Mkhalled's coastal soil under aseptic conditions using a sterile spatula. The soil was collected in carefully sealed sterile plastic bags and stored in the laboratory at 4°C before analysis.

1.3. Measurement of the physico-chemical parameters of the samples

In order to see the relationship between microorganisms and the characteristics of their ecosystems, parameters such as pH, conductivity, and salinity are studied.

1.3.1. Measurement of pH

To determine the pH, 10 g of each soil sample is mixed with 50 mL of distilled water. After a good agitation for 15min, the suspension is left to rest for 2 hours **(El Karkouri *et al.*, 2019)**. A pH meter (Adwa AD 1030) is used where the electrode was inserted directly into each sample; two repetitions were carried out.

1.3.2. Measurement of electrical conductivity and salinity

5g of soil are mixed with 25mL of distilled water. After stirring for 30 minutes, the suspension is left to rest for 15 min and then centrifuged (3000 rpm/10 min), and the conductivity and salinity are measured using the conductivity meter (WTW Multi 340i) **(El Karkouri *et al.*, 2019).**

2.Isolation and purification of actinomycetes

The isolation technique used is the serial dilution method. 10 g of each soil sample are mixed in 90 mL of sterile physiological water (0.9% NaCl), which represents the 10^{-1} dilution (figure 10). After homogenization using a vortex, decimal dilutions are carried out in sterile physiological water up to 10^{-7} **(Kitouni, 2007).** Then 100 µL of each dilution is spread on the surface of the culture media: middle of GNG, ISP2, TSA, Gausse, and Benett. All media used in this study contain an antifungal, amphotericin B de marque mytricine (used to prevent the growth of yeast) at a concentration of 50 µg/mL, and an antibiotic, nalidixic acid (which helps to reduce bacterial growth) (10 µg/mL) **(Belyagoubi, 2014 and 2018)**. After seeding, the petri dishs are incubated at 28±1°C for 2 to 3 weeks and regularly examined, colonies that appeared dry and chalky were selected. After being divided in a Petri dish, each of the distinct

actinomycete's colonies was sub-cultured in a test tube and Petri dish containing GNG culture medium, incubated at 28 °C for 7 days, and then stored as the stock in the refrigerator at 4 °C Every two to three weeks, the isolates were subcultured again to keep the cultures from dying out (**Budhathoki and Shrestha, 2020).**

Cryopreservation in glycerol is a widely used and reliable method for the long-term preservation of actinomycetes. Glycerol concentrations typically range from 10%, which is effective for preserving Streptomyces spores **(Sineva et al., 2019),** to 30%, which is suitable for maintaining a broader diversity of actinomycete isolates **(Ortiz et al., 2016).**

3. Identification

3.1. Morphological characterization

3.1.1. Macroscopic observation

Actinomycete isolates were inoculated in ISP culture media (ISP2, ISP6, and ISP7) to examine their morphological characteristics. Pure isolates were aseptically streaked and incubated for 14 days at 28 °C. Characterization of actinomycetes refers to the study of the formation, shape, and structure of aerial mycelia and substrate **(Shirling, 1966).**

3.1.2. Microscopic observation

Fresh cultures were examined under a microscope at 100× magnification to identify cell morphology (shape, clustering mode, and density), which is a crucial stage in taxonomic orientation (**Williams *et al*., 1998**) using the method of gram staining.

On a glass slide, a loopful of the isolate was applied, allowed to air dry, and then heat-fixed. After applying crystal violet on the smear for 30 seconds, distilled water was used to remove it. After adding Gram's iodine solution and letting it rest for a minute, distilled water was used to rinse it off. After 15 to 20 seconds of dropwise decolorization with 95% ethanol, the smear was cleaned with distilled water. Safranin was applied to the smear for one minute followed by a distilled water wash and drying. A bright field microscope with a magnification of 1000x was used to analyze the smear **(Smith *et al*., 2021).**

3.2. Physiological and biochemical analysis

Gram-positive isolates were selected for biochemical characterization. The biochemical tests performed were:

3.2.1. Hydrolysis of casein

The test for skimmed milk is based on the hydrolysis of casein, the main milk protein, by extracellular proteolytic enzymes produced by certain microorganisms. When these proteases are secreted in casein-containing medium, they break down this protein into peptides and soluble amino acids. (**Yokota *et al.*, 1988**). As a result, a clear halo around actinomycetes' colonies will appear **(Goodfellow, 1971**).

3.2.2. Starch hydrolysis

The starch agar is a differential medium that tests the ability of an organism to produce the extracellular enzymes (exoenzymes) α-amylase and oligo-1,6-glucosidase that are secreted out of the bacteria and diffuse into the starch agar. These enzymes hydrolyze starch by breaking the glycosidic linkages between glucose subunits and allow the products of starch hydrolysis to enter the cell. Starch agar is also used in differentiating members of various genera that have both amylase-positive and amylase-negative species. When bacteria capable of producing α-amylase and oligo-1,6-glucosidase are grown on starch agar, they secrete these enzymes into the surrounding areas and hydrolyze the starch. To detect the hydrolysis of starch, Gram's iodine (Lugol's iodine) is used. Gram's iodine reacts with starch to form a dark blue, purple, or black complex depending upon the concentration of iodine **(Leboffe, 2010).**

3.2.3. Gelatin hydrolysis

Inoculate nutrient gelatin (4%) in Petri dishes and inoculate the actinomycete species on it in a streak form, then incubate it at 28°C for 14 days . After adding a mercuric chloride solution to it, a clear zone appears around gelatinase-positive colonies **(Aryal, 2022).**

3.2.4. Action on skimmed milk

Samples containing a 10% solution of skimmed milk powder dissolved in distilled water are inoculated with strains of actinomycetes and maintained at a temperature of 28°C. Systematic observations over a 14-day period make it possible to identify coagulation. or the peptonization of milk induced by the strains (**Williams and Cross.,1971).**

3.2.5. Catalase test

Catalase is an enzyme present in most strict aerobic bacteria and facultative anaerobes. This enzyme allows the degradation of H_2O_2, which results from the oxidation of air by oxygen:

$$H_2O_2 \xrightarrow{\text{Catalase}} H_2O + 1/2O_2$$

This reaction is demonstrated simply by contact of the culture with a solution of fresh H_2O_2 at 10 volumes: a drop of hydrogen peroxide is placed on a slide in the presence of a culture sample. An abundant gas evolution in the form of bubble mousse indicates the decomposition of oxygen: the catalase test is positive, and if there are no bubbles, the catalase test is negative **(Delarras, 2007).**

3.2.6. Citrate utilization

It is used to test the ability of an organism to survive by using citrate as the sole carbon and energy source and ammonium salts as the sole nitrogen source. It contains a pH indicator called bromothymol blue, which is forest green in neutral pH. If the bacterial species have the required enzyme, such as citrate permease, then citrate and ammonium salts are utilized for growth, and alkaline byproducts are produced **(MacWilliams, 2009).**

3.2.7. Use of different carbon substrates on TSI medium agar

This medium consists of a base and a slope (containing glucose, lactose, and sucrose and permitting the detection of HS and gase). Tubes containing the TSI medium are seeded in the central puncture and on the inclined surface, then incubated for 7 days at 28°C.

The results are as follows:

Lactose-sucrose positive: yellow coloration of the slope.

Positive glucose: yellow coloration of the base.

H_2S positive: blackening of the medium at the junction between the slope and the base.

The production of gases (hydrogen and carbon dioxide) resulting from fermentations is either the appearance of bubbles or the fragmentation of the agar (**Belyagoubi, 2014**).

4. Fermentation and extraction of metabolites

4.1. Optimization, fermentation and extraction of active substances in liquid medium

To optimize the production of metabolites, three culture media were used: GNG, Bennett medium and AF medium. The strain was inoculated into vials each containing 100 mL of these media, with a pH adjusted to 7 ± 0.2. The cultures were incubated for two weeks at a temperature of 28 ± 2 °C. After incubation, the filtrates were centrifugated ,recuperated and tested to assess their antimicrobial activity.

The extraction of antimicrobial compounds was carried out using ethyl acetate or chloroform (V/V). The organic phase was separated using a separation funnel, then concentrated at 45 °C using a rotary evaporator to obtain a crude
extract. This was recovered using 5% DMSO, then stored at 4 °C for the antioxidant activity test **(Djebbah, 2022**).

5. Assessment of biological activities

5.1. The antimicrobial activity of actinomycetes

5.1.1 Tested strains

In order to evaluate the antimicrobial activity of actinomycete strains isolated from it, reference strains were used. They are presented in the following table (Table 2).

Table 3. Strains used in the various antimicrobial activity tests.

	Microorganisms	Gram	Code
Bacteria	*Staphylococcus aureus*	Positive	ATCC 25923
	Bacillus cereus		ATCC 11778
	Micrococcus luteus		ATCC 15307
	Pseudomonas aeruginosa	Negative	ATCC 27853
	Escherichia coli		ATCC 25922
	Klebsiella pneumoniae		ATCC 70603
Yeast	*Candida albicans*		ATCC 10231

ATCC : American Type Culture Collection.

5.1.2. Agar cylinder technique

In order to evaluate the production of antimicrobial molecules against pathogenic microorganisms using the agar cylinder method, the isolated strains were inoculated on Bennett medium and then incubated at 28°C for 14 days. From the incubation, 6 mm diameter disks were taken using a Pasteur pipette and then deposited on the surface of the Sabouraud agar, previously seeded with the yeast *Candida albicans* at a final load of $\approx 10^6$ CFU/mL. In parallel, discs were also placed on the Mueller-Hinton medium, seeded with reference bacterial strains at a final load of $\approx 10^8$ UFC/mL **(CLSI, 2017)**.

The assembly is kept refrigerated at 4 °C for two hours to promote the diffusion of active substances in agar. Subsequently, the cans are incubated at 37 ± 1°C for 24 hours at 48 hours.

The diameters of the inhibition zones formed around the agar cylinders are then measured.

Amoxicillin disks (25 µg) were used as positive controls for bacteria, and disks of cycloheximide (Sigma-Aldrich, 100 µg) and fluconazole (25 µg) for the yeast *Candida*, then incubated at 37°C for 24 hours for bacteria and 25°C for yeast for 48 hours **(Gungi *et al*., 1983).** The diameters of the inhibition zones formed around the agar cylinders are then measured **(Boudjelal *et al*., 2011).**

5.1.3. Diffusion method for agar wells

Actinomycete strains are first cultured in 5 mL of Bennett broth and incubated at 28 ± 1°C for 14 days, with shaking every two hours. This culture phase allows the production of secondary metabolites. At the end of incubation, the culture is centrifuged at 5000–10000 RPM for 10–15 minutes to separate the cells from the culture fluid. The recovered supernatant, which contains diffusible substances produced by actinomycetes, filtered to remove residual particles and obtain a sterile extract.

The well agar diffusion method is widely used to evaluate the antimicrobial activity of microbial extracts; it starts with homogeneous seeding of the agar surface with a microbial suspension. Then, a sterile well of about 6 to 8 mm in diameter is dug into the agar using a sterile pipette. A precise volume, generally between 50 and 100 µL, of the extract or solution containing the antimicrobial agent at the desired concentration is deposited in each well. The boxes are placed in the oven at a temperature appropriate for the target microorganisms,

generally 37°C for 24 hours for bacteria and 28°C for 48 hours for yeasts. During this period, the antimicrobial agent diffuses through the agar medium and forms a visible zone of inhibition around the well**. (Balouiri *et al*., 2016).**

5.2. The antioxidant activity of the extracts

5.2.1. Total antioxidant capacity

The total antioxidant capacity (TAC) of different filtrates was determined using the phosphomolybdenum method **(Prieto *et al*., 1999)**. A volume of 300 µL of diluted extract was mixed with 3 mL of reagent solution containing sulfuric acid, sodium phosphate, and ammonium molybdate. The mixtures were incubated at 95 °C for 90 minutes, cooled, and their absorbance read at 695 nm against a reagent blank. A calibration curve prepared with ascorbic acid enabled TAC values to be expressed as milligrams of ascorbic acid equivalents per gram of dry extract (mg AAE/g DE). All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation.

5.2.2. DPPH free radical scavenging assay

A single concentration of the filtrate (10 mg/mL) was evaluated for antioxidant activity. Briefly, 50 µL of the filtrate was mixed with 1950 µL of a freshly prepared methanolic DPPH solution (0.025 g/L). The reaction mixture was incubated in the dark at room temperature for 30 min, after which the absorbance was measured at 515 nm.

A negative control consisting of methanol instead of the sample was used, while ascorbic acid served as the positive reference standard according to the method described by **(Brand-Williams *et al*.,1995).** The DPPH radical-scavenging activity was expressed as the percentage of inhibition using the following equation:

$$\text{Inhibition (\%)} = [(A_C - A_T) / AC] \times 100$$

Where: A_C : represents the absorbance of the negative control; A_T : represents the absorbance of that of the tested sample. All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation.

Results and discussion

Results and discussion

1. Physico-chemical analyses of soil samples

The table below (**Table 4**) summarizes all of pH electrical conductivity, redox and salinity results from soil sampling.

Table 4. Physico-chemical parameters of soil samples.

Samples	pH	Electrical conductivity (Us/cm)	Redox	Salinity (g/L)
E1	8.45	1031	-72.1	0.3
E2	8.11	1092	-55	0.3
E3	8.94	1598	-84.8	0.6

All soils are alkaline, according to the pH values (8.11–8.94). Therefore, according to the mentioned pH values, this indicates that all these environments are favorable for the growth of actinomycetes, particularly Streptomyces, which grows in alkaline conditions (approximately pH = 7–9) **(Kontro and Lignell, 2005)**. Besides, the EC indicates the concentration of ions in soils; therefore, E3 shows the highest value of EC, which signifies a better concentration of soluble nutrients and mineral salts in forests than in mountain areas. As for the redox potential, all samples have a negative value of redox potential, indicating decreased conditions that indicate a lack of oxygen and the presence of reducing agents in the forest area more than in the mountains **(Oktyabrskii and Smirnova, 2012)**. In addition, the measured salinity values indicated that the three soil's samples are non-saline soils. Although E3 has twice the salinity of E1 and E2, E3's concentration is still negligible and is not expected to inhibit microbial activity.

2. Isolation of actinomycetes

Figure 6 shows the number of strains that were isolated from our soil sample and cultivated on various growing media, which are the TSA, GNG, and Bennett and Goss media. We used these to isolate those strains; we obtained 5 successful strains, shown in the figures below.

Results and discussion



Figure 6. Photo of isolation of actinomycetes **(Personal photo, 2026).**

The results of isolation on TSA, GNG, ISP2, and Bennett media revealed abundant and diversified microbial growth. The colonies observed exhibited different morphologies in terms of size, shape, texture, and surface appearance, indicating significant microbial biodiversity in the soil sample studied **(Figure 6)**. It was shown that ISP2 and GNG media allowed the isolation of a larger number of actinomycete colonies than other media tested. This abundance can be explained by the nutritional composition of these environments, which is particularly adapted to the metabolic requirements of actinomycetes. The ISP2 medium contains glucose, barley extract, and yeast extract, which provide sources of carbon, nitrogen, vitamins, and growth factors that promote fungal growth and spore formation. Likewise, the GNG medium, composed of gelatin and nutrient agar, provides an easy-to-absorb energy source as well as complex nitrogenous compounds that promote the growth of actinomycetes. The nutritional richness of these two environments likely stimulated spore germination and colony development, resulting in greater morphological diversity and a greater number of isolates. The colonies observed had characteristics typical of actinomycetes, including a dry, powdery, or chalky appearance, as well as filamentous growth reminiscent of fungi and adhesion to agar.

These results are consistent with those of (**Hayakawa and Nonomura,1987),** who showed that carbon- and nitrogen-rich environments promote the isolation of actinomycetes from soil and increase the recovery of Streptomyces species. The actinomycetes were well isolated in glycerol. At the end of this time and after isolation and observation, colonies having macroscopic characteristics similar to actinomycetes were transplanted by streaking on fresh GNG and TSA, Bennett medium, and even ISP2 medium. In order to create colonies that are more or less uniform (pure cultures) **(KPODA and TAPSOBA, 2024)**.

Results and discussion

From 11 isolated and purified actinomycete strains, 5 strains were selected in our study. The results observed after 14 days of incubation are mentioned in **Figure 7**.

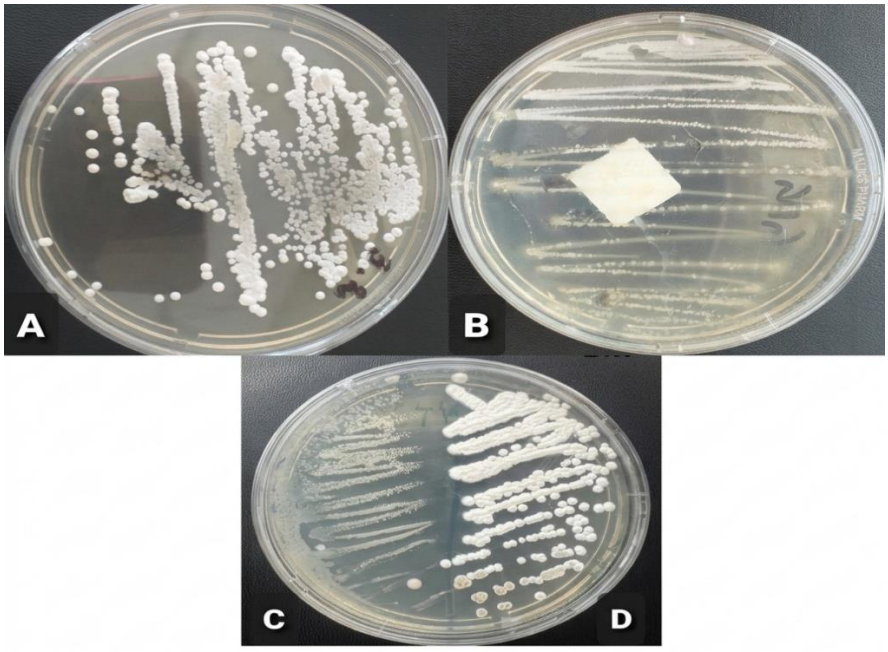


Figure 7. Pure culture of isolated actinomycetes A: E3.2 on GNG, B: E 2.3 on GNG, C: E1.9 on Bennett and D: E1.11 on Bennett

Table 5. Results of the macroscopic aspect study of isolates

Strain code	**Source of isolation (medium)**	**Macroscopic appearance**
E1.1	Gauss 10^{-3}	Medium white colony in the middle of agar
E1.9	ISP2 10^{-3}	White medium colony
E1.11	ISP2 10^{-3}	Medium white colony
E2.3	Isp2 10^{-3}	Small gray/white colony
E3.2	GNG 10^{-5}	White medium colony

3. Identification

3.1. Morphologic characterization

3.1.1 Macroscopic observation

Colonies of actinomycetes strains appear after incubation for 2 to 4 weeks at 28 °C, following seeding on the ISP media and showing abundant growth on the culture medium **(Figure 8).** They are circular, white to grey with an opaque appearance at the center, dry and powdery, and compatible with the formation of an aerial mycelium.



Figure 8. Macroscopic aspect of actinomycete strains **(Personal photo, 2026)**.

3.1.2 Microscopic observation

Gram staining showed that all isolates retained crystal violet and appeared in purple under the microscope, indicating their positive Gram character. The cells exhibited a branched filamentous morphology, forming a mycelium, characteristic of actinomycetes **(Figure 9)**.

The Gram-positive trait observed is explained by the presence of a thick layer of peptidoglycan in the cell wall of actinomycetes. The filamentous morphology and the formation of a mycelial network are important criteria for their preliminary identification.

These results are consistent with those reported by (**Goodfellow and Williams,1983)**, who describe actinomycetes as Gram-positive bacteria with filamentous growth forming a vegetative and aerial mycelium. Gram staining thus allowed an initial taxonomic orientation of the isolates before their further morphological and biochemical characterization.

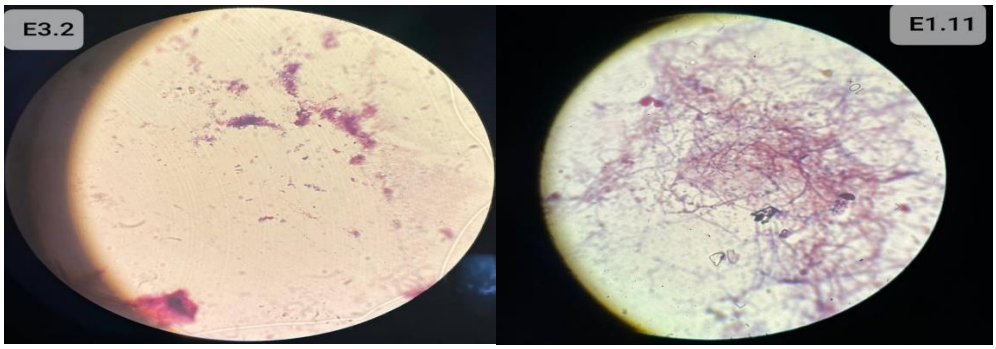


Figure 9. Microscopic observation of actinomycete strains (G × 100) after Gram staining **(Personal photo, 2026).**

3.2. Physiological and biochemical analysis

The results obtained are presented in the following table.

Table 6. Biochemical characteristics of actinomycete strains**.**

Characteristics		**Strains**					
		T	**E1.1**	**E1.9**	**E1.11**	**E2.3**	**E3.2**
Hydrolysis of casein		-	-	-	-	-	+
hydrolysis of starch		-	-	-	+	+	+
Hydrolysis of gelatin		-	-	-	-	+	-
Coagulation of skimmed milk		-	+	+	+	+	+
Catalase Search		-	+	+	+	+	+
Use of citrate as the only carbon source		-	-	-	-	-	-
TSI	**Glucose/Lactose/ Sucrose**	-	---	+++	+++	+++	+++
	Production of H2S	-	-	+	-	-	-
	Production of gas	-	-	-	-	-	-
Production of pigments Melanoid in ISP6		-	-	-	-	+	+
Production of pigments Melanoid in ISP7		-	-	-	+	-	+

(-): Negative test.; **(+)** : Positive test.; **(T)**: Control

For the casein hydrolysis test alone, the strain E3.2 is positive, indicating its ability to produce extracellular proteases capable of degrading casein. The results obtained in the starch hydrolysi test show that strains E1.11, E2.3, and E3.2 are positive after

adding the Lugol reagent, so they produce amylases capable of degrading complex polysaccharides into simple sugars **(Stamford *et al*., 2001).**

In this study, only one strain (E2.3) showed a positive result for gelatin hydrolysis, while strains E1.1, E1.9, E1.11, and E3.2 were negative. This result indicates that only the E2.3 strain is capable of producing gelatinases, extracellular enzymes that hydrolyze gelatin into peptides and assimilable amino acids and which is an important physiological feature in certain actinomycetes, including species of the genus *Streptomyces*.

Similar results have been reported by **(Shirling and Gottlieb .,1966)**, who showed that the hydrolysis of gelatin varies among species of Streptomyces and is a useful criterion for their phenotypic characterization.

The strains studied showed a positive reaction to the coagulation test of skimmed milk, characterized by the formation of a compact white clot at the bottom of the tubes. This observation reflects the ability of isolates to produce proteolytic enzymes acting on casein, the major protein in milk **(Figure 10)**.

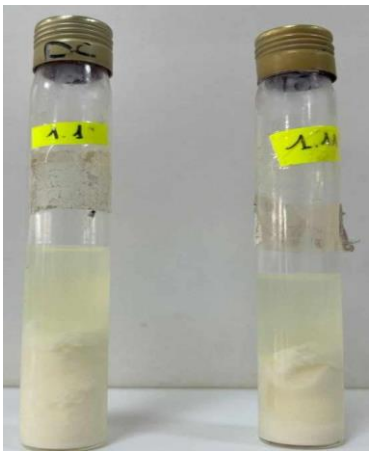


Figure 10. Coagulation of skimmed milk **(Personal photo, 2026).**

According to the results obtained, all isolates are catalase-positive (abundant release of gas in the form of foam), which reflects their ability to degrade hydrogen peroxide ($H_2 O_2$) into water and oxygen, reflecting a strict aerobic metabolism **(Figure 11).**

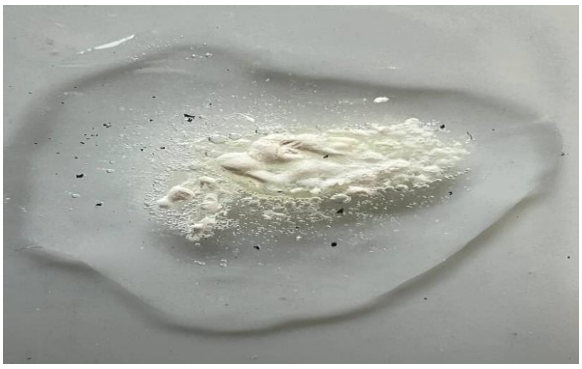


Figure 11. Result of catalase production from the 5 strains **(Personal photo, 2026).**

Regarding the use of citrate as the only carbon source, all strains are negative. This indicates that these isolas prefer more complex carbon substrates and proves the absence or lack of expression of this enzyme, forcing the strain to grow on other nutrients. The same results are obtained by (**Abbes and Boutraa ,2017).**

The results of the TSI test reveal that strains E1.9, E1.11, E2.3, and E3.2 ferment the three sugars in the medium (glucose, lactose, and/or sucrose), as indicated by the positive reaction. On the other hand, strain E1.1 shows no fermentation. The production of H_2 S was only observed in strain E1.9, suggesting its ability to reduce sulfur compounds, and the absence of gas production for all strains confirms their strict aerobic profile.

4. Assessment of biological activities

4.1. Evaluation of the antimicrobial activity of the isolated strains

4.1.1. Agar cylinder method

Figure 12 presents the results obtained during the evaluation of antimicrobial activity by the agar cylinder method.

The antimicrobial screening revealed that the actinomycete isolates exhibited different inhibition profiles against the tested microorganisms, indicating variability in their capacity to produce bioactive secondary metabolites. None of the isolates displayed broad-spectrum activity against all test organisms; instead, each strain exhibited a specific antimicrobial spectrum.

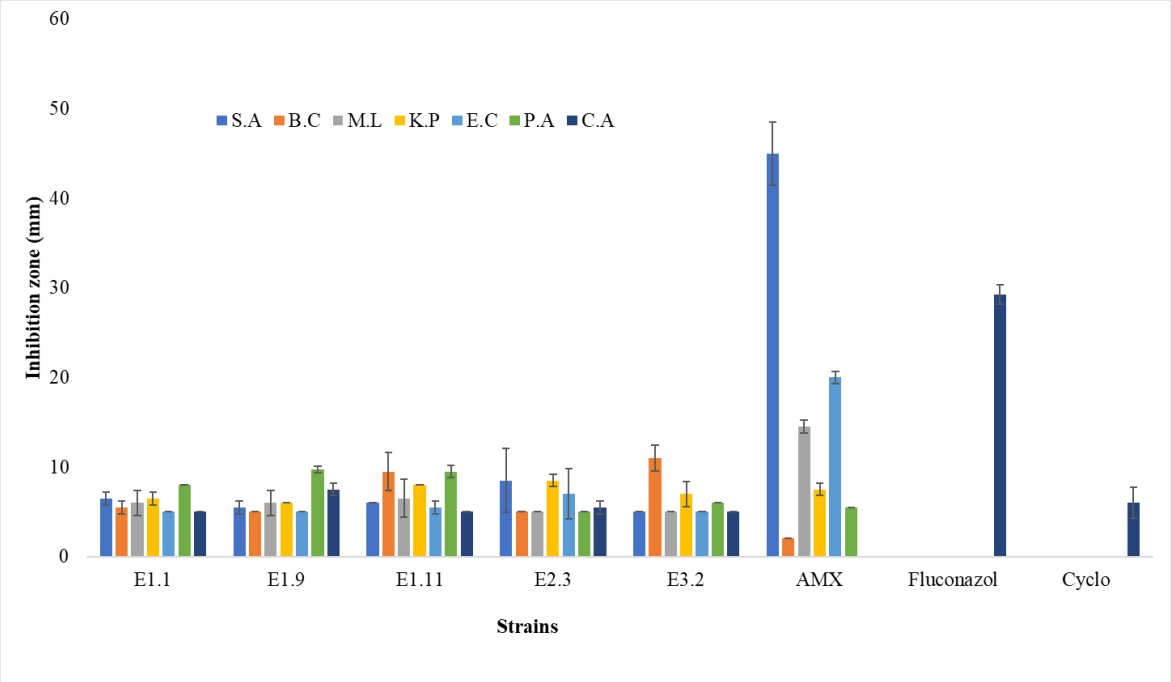


Figure 12. Antimicrobial activity of actinomycete strains by the cylinder method of agar. **AMX:** Amoxicillin; **Cyclo:** Cycloheximide.

Among the Gram-positive bacteria, *Bacillus cereus* was inhibited by all actinomycetes isolates, although the inhibition zones varied between strains. The highest activity was observed for the strain E3.2, which produced an inhibition zone of approximately 11 mm, followed by E1.11 (9.5 mm). Although these inhibition zones were considerably smaller than those produced by the positive control (amoxicillin, approximately 46 mm), they demonstrate that these isolates synthesize compounds capable of inhibiting B. cereus growth. Identically, *Staphylococcus aureus* was susceptible to all tested isolates. The strongest activity was recorded for strain E2.3 (approximately 8.5 mm), whereas the remaining strains produced inhibition zones ranging from 5 to 6.5 mm. These findings confirm that several actinomycetes isolated produce metabolites active against Gram-positive bacteria, although their activity remains lower than that of amoxicillin. About *Micrococcus luteus*, all strains showed a low zone estimated by 6 to 7mm except E1.11 that showed a little high zone than other strains with a positive control reaches 15 mm. Concerning *Klebsiella pneumoniae*, all isolates exhibited measurable antibacterial activity. The greatest inhibition was also produced by E2.3, with a zone of approximately 9 mm, suggesting that this strain secretes metabolites effective against

this opportunistic pathogen. Although the inhibition was comparable to that of the other isolates, it remained much lower than that of conventional antibiotics. For *Pseudomonas aeruginosa,* only moderate inhibition was observed. The highest activities were produced by E1.9 and E1.11, with inhibition zones of approximately 9.75 mm and 9.5 mm comparable of E2.3 and E3.2 (approximately 6-7mm), respectively These results suggest that these isolates produce antimicrobial compounds capable of partially overcoming the resistance mechanisms of P. aeruginosa. However, the inhibition remained limited compared with standard antimicrobial agents.

The tested isolates exhibited no activity against *Escherichia coli*. Only E2.3 produced a measurable inhibition zone (approximately 7 mm) and E1.11, while the remaining isolates showed weak inhibition (approximately 5–6 mm). The relatively low susceptibility of *E. coli* may be attributed to their highest resistance. In contrast, none of the actinomycetes isolated exhibited antifungal activity against *Candida albicans*. The absence of inhibition zones indicates that the metabolites produced by the isolates were ineffective against this yeast. Cycloheximide, used as the positive control, produced a clear inhibition zone (approximately 6 mm), whereas fluconazole produced a much larger inhibition zone (approximately 29-30 mm), confirming the susceptibility of the test organism and validating the assay conditions.
All in all, E2.3 demonstrated the broadest antimicrobial spectrum, showing activity against all tested bacterial species, including *E. coli,* whereas E3.2 displayed the highest activity against Bacillus cereus and remarkable activity against *Klebsiella pneumoniae,* conversely E1.1 showed the lowest activity against the microorganisms tests. In final, the observed antimicrobial activities indicate that these isolates possess the ability to produce antibacterial metabolites with selective activity spectrum. Further investigations should focus on optimizing culture conditions, characterizing the active compounds, and evaluation their efficacy against multidrug-resistant pathogens to assess their potential as novel antimicrobial agents.

4.1.2. Well method

No inhibition zones were observed around the wells after the agar well diffusion assay, indicating that the fermentation filtrates did not exhibit detectable extracellular antimicrobial activity under the experimental conditions, despite the normal diffusion of the liquid through the agar. This lack of activity may be attributed to the absence or low concentration of

secondary metabolites, poor diffusion of bioactive compounds, or unsuitable fermentation conditions This discussion is supported by **(Daquioag and Penuliar, 2021).**

4.2. Evaluation of the antioxidant activity of the isolated strains

4.2.1. Total antioxidant capacity

The total antioxidant capacity (TAC) of extracts from different strains of actinomycetes was evaluated to determine their ability to produce compounds with antioxidant activity. The values obtained, expressed in mg of ascorbic acid equivalent per gram of dry extract (mg AAE/g DE), are presented in the following figure **(Figure 13)**.

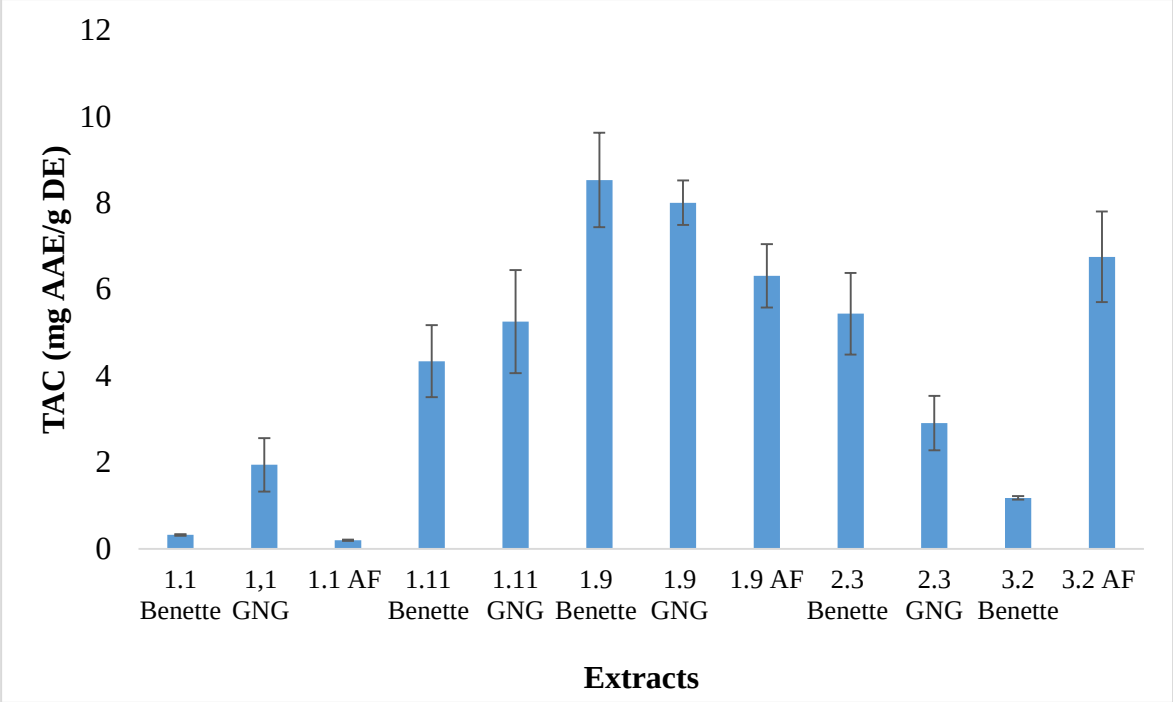


Figure 13. The total antioxidant capacity of extracts from actinomycete strains.

The evaluation of TAC of different actinomycetes extracts revealed significant variability depending on the actinomycete strain. The recorded values range from 0.2 to 8.5 mg AAE/g DE, reflecting a marked heterogeneity in the production of secondary metabolites with antioxidant activity between the different strains. Strain E1.9 shows the highest antioxidant capacity, regardless of the culture media used (Benett: 8.5; GNG: 8.0; AF: 6.3 mg AAE/g DE), suggesting that this strain has a particularly rich secondary metabolism of bioactive compounds, such as pigments, polyphenols or other antioxidant molecules typically synthesized by actinomycetes. In contrast, strain E1.1 shows the lowest values, especially

with Benett medium (0.3 mg AAE/g DE) and AF (0.2 mg AAE/g DE), which could indicate a low production of antioxidant metabolites by this strain or unfavorable culture conditions for their recovery.

Whereas the GNG medium was significantly more efficient for strain E1.1 (2.0 mg AAE/g DE), while the AF medium provided the best yields of antioxidant compounds for strain E3.2 (6.7 versus 1.2 mg AAE/g DE with Bennette medium). For strain E1.11, GNG medium yielded a higher value (≈ 5.2 mg AAE/g ED) than Bennette medium (≈ 4.3 mg AAE/g ED), indicating that the nutritional composition of GNG medium promotes biosynthesis of antioxidant metabolites in this strain. However, strain E2.3 showed the opposite behavior, with a higher TAC on Bennette medium (≈ 5.4 mg AAE/g ED) than on GNG medium (≈ 2.9 mg AAE/g ED).

These results are consistent with the work of **Barka *et al*. (2016)**, which showed that the metabolic potential of actinomycetes is highly dependent on culture conditions, as well as with **(Subramani and Aalbersberg, 2012)**, who highlighted that the expression of biosynthetic pathways for bioactive metabolites varies depending on the composition of the culture medium.

4.2.2. DPPH free radical scavenging assay (2,2-diphenyl-1-picrylhydrazyl)

The results of the antioxidant activity of different actinomycete extracts, determined by the DPPH test, are presented in Figure 14. The extracts were evaluated at concentrations of 4 for strain E2.3 on bennette medium and 10 mg/mL for the others, and their ability to inhibit the DPPH radical is expressed as a percentage of inhibition.

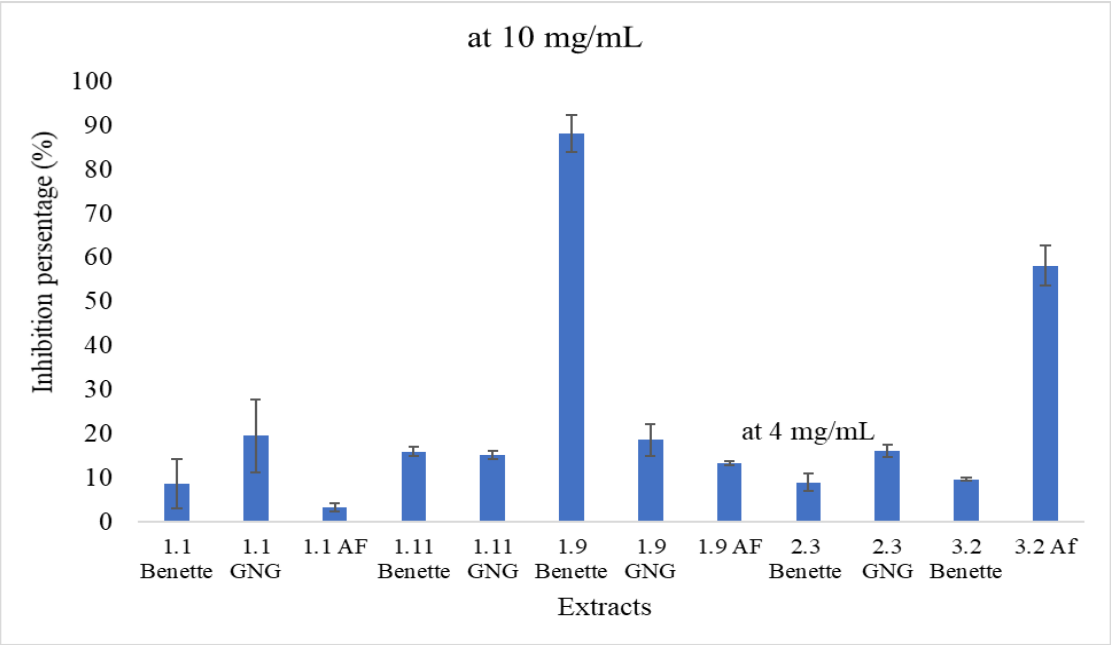


Figure 14. Antioxidant activity of extracts from actinomycetes strains evaluated by the DPPH test at concentrations of 4 (E2.3 Bennett) and 10 mg/mL.

Antioxidant activity varies practically across the medium of the 12 strains tested, with a clear advantage:E1.9 grown on Bennett's medium showing excellent activity, with inhibition reaching about 88%, far ahead of every other sample and indicating a strong ability to eliminate free radicals. The same applies to strain E3.2 on AF medium, with approximately 59% inhibition, which represents good and significant stimulation. After this sharp decline, a range of extracts show only moderate to low activity in the range of 13–20%, including E1.1 GNG (20%), E1.9 GNG (19%), E2.3 GNG (16%), and E1.11 Bennett (16%), 1.11 GNG (15%), and E1.9 AF (13%).Notably, the GNG medium appears frequently here which suggests that it produces consistent but unnoticed antioxidant potential regardless of breed. At the lower point, activity becomes inferior, with E3.2, E1.1 Bennett and E2.3 Bennett performing around 9-10% and the latter E1.1 AF being the weakest performer ever at 3%.
Compared to the medium, Bennett produces the best result (E1.9) and many of the moderate results (E1.1, E2.3, E3.2), AF does the same in reverse (ideal for E3.2 and limited activity for E1.1), while GNG is the most stable medium. This pattern indicates a strong strain-medium interaction effect: the antioxidant potential of these extracts is not an integral constant property of the strain or culture medium alone, but arises from specific combinations of the

two, with E1.9 on Bennett and E3.2 on AF medium being the two groups worth pursuing for their strong free radical scavenging activity.

The results of our study confirm that the antioxidant capacity is directly related to the culture media and its composition, and the same results were observed by **(Krupodorova *et al.*,2024**) where they found that the antioxidant activity of fungal extracts is strongly dependent on the medium.

Finally, these results highlight the interesting potential of strains E1.9 and E3.2 as promising candidates for further exploration for their antimicrobial and antioxidant properties, for biotechnology and pharmaceutical applications.

Conclusion

Conclusion

Our study demonstrated that the coastal soils of Mkhalled (forest and mountain) constitute a valuable wide different isolation of actinomycetes with different biological activities(antimicrobial and antioxidant). after isolation, purification, and characterization, several actinomycete strains were successfully obtained and evaluated for their antimicrobial and antioxidant activities.

The antimicrobial screening revealed that the isolates exhibited variable inhibitory effects against the tested pathogenic bacteria. While some strains showed no activity or less than other strains, others produced clear inhibition zones against both Gram + and Gram bacteria, indicating their capacity to produce bioactive secondary metabolites capable to inhibit their growth. Notably, certain isolates inhibited microorganisms that were resistant to the reference antibiotic (AMX and antifungals), highlighting the potential of these actinomycetes as sources of novel antimicrobial compounds. In parallel The antioxidant evaluation also demonstrated differences among the isolates including the DPPH radical scavenging assay validated that several strains possess antioxidant properties, same about TAC varied according to the isolate. These findings suggest that actinomycetes from coastal soils are capable of producing large secondary metabolites that contribute to protection against oxidative stress

In conclusion, the actinomycetes isolated from Mkhaled's coastle soil represent a promising reservoir of potent actinomycetes. These findings contribute to the growing body of evidence supporting the exploitation of environmental actinomycetes as valuable resources in the search for new natural bioactive compounds capable of addressing the increasing challenges of antimicrobial resistance and oxidative stress-related disorders.

As perspective , our research provides a large spectre in future , especially coastle soil that contain several microorganisms and powerful actinomycetes , we suggest that reaserches should discover more about this areas shouland focus on the molecular identification of the most promising isolates through 16S rRNA gene sequencing to accurately determine their taxonomic positions, Furthermore, to investigate their mechanisms of action, evaluating the safety and cytotoxicity of the active metabolitesproduced by this actinomycets, and finally lead to other underexplored ecosystems searching fornovel actinomycete species and previously unidentified bioactive compounds with high potential applications in different sectors of biology.

.

References

References

- Abbes S., and Bouteraa I., (2017). Phenotypic identification of some actinobacteria isolates showing antimicrobial activity against pathogenic microorganisms. Thesis of MSILA.
- Bergeys manual of systematic bacteriology (2012). : volume 5: the Actinobacteria. Springer Science & Business Media.
- Acquah, K. S., Gammon, D. W., & Beukes, D. R. (2024). South African actinobacteria: A treasure trove of novel bioactive metabolites for drug discovery. *South African Journal of Science*, *120*(1-2), 1-11.
- Aryal, S. (2022, August 10). Gelatin hydrolysis test: Principle, procedure, uses and interpretation. Microbiology Info. https://microbiologyinfo.com/gelatin-hydrolysis-test/
- Balouiri, M., Sadiki, M., & Ibnsouda, S. K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, *6*(2), 71-79.
- Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H. P., ... & van Wezel, G. P. (2016). Taxonomy, physiology, and natural products of Actinobacteria. Microbiology and molecular biology reviews, 80(1), 1-43.
- Basiliko, N., Dunfield, K., & Tenuta, M. (2021). La biodiversité et l'écologie des sols. *Introduction à la science du sol: de la théorie à la pratique en sols canadiens*.
- BELYAGOUBI Larbi. (2014). Antibiotiques produits par des bactéries (actinomycètes et bactéries lactiques) issus de différents écosystèmes naturels Algériens. Thèse de doctorat. Université Aboubakr Belkaïd-Tlemcen, P 143
- Beroigui, O., & Errachidi, F. (2023). Streptomyces at the Heart of Several Sectors to Support Practical and Sustainable Applications: A Review. https://doi.org/10.36877/pmmb.a0000345
- Bhatti, A. A., Haq, S., & Bhat, R. A. (2017). Actinomycetes benefaction role in soil and plant health. *Microbial pathogenesis*, *111*, 458-467.
- Boudjelal, F., Zitouni, A., Mathieu, F., Lebrihi, A., & Sabaou, N. (2011). Taxonomic study and partial characterization of antimicrobial compounds from a moderatel halophilic strain of the genus Actinoalloteichus. *Brazilian Journal of Microbiology*, *42*(3), 835-845.
- Brady, N. C., Weil, R. R., & Weil, R. R. (2008). *The nature and properties of soils* (Vol. 13, pp. 662-710). Upper Saddle River, NJ: Prentice Hall.

References

- Budhathoki, S., & Shrestha, A. (2020). Screening of actinomycetes from soil for antibacterial activity. *Nepal Journal of Biotechnology*, *8*(3), 102-110.
- Campos-Herrera, R., Gomez-Ros, J. M., Escuer, M., Cuadra, L., Barrios, L., & Gutiérrez, C. (2008). Diversity, occurrence, and life characteristics of natural entomopathogenic nematode populations from La Rioja (Northern Spain) under different agricultural management and their relationships with soil factors. *Soil Biology and Biochemistry*, *40*(6), 1474-1484.
- Carip, C. (2008). Microbiologie. Hygiene Medicales Internationales. Mic/015. p. 9.10.19
- Chaudhary, H. S., Soni, B., Shrivastava, A. R., & Shrivastava, S. (2013). Diversity and versatility of actinomycetes and its role in antibiotic production. *Journal of Applied Pharmaceutical Science*, *3*(8,), S83-S94.
- Cimmino, T., Metidji, S., Labas, N., Le Page, S., Musso, D., Raoult, D., & Rolain, J. M. (2016). Genome sequence and description of Actinomyces polynesiensis str. MS2 sp. nov. isolated from the human gut. *New Microbes and New Infections*, *12*, 1-5.
- Daquioag, J. E. L., & Penuliar, G. M. (2021). Isolation of actinomycetes with cellulolytic and antimicrobial activities from soils collected from an urban green space in the Philippines. *International journal of microbiology*, *2021*(1), 6699430.https://doi.org/10.1155/2021/6699430
- Delarras, C. A. M. I. L. L. E. (2007). Microbiologie pratique pour le laboratoire d'analyses ou de contrôle sanitaire: Aliments, produits cosmétiques, eaux, produits pharmaceutiques. Éditions Médicales Internationales, Lavoisier.
- Directorate of Agricultural Services of Tlemcen. (2020). Monograph of the wilaya of Tlemcen. Tlemcen: DSA Tlemcen.
- El Karkouri, A., Ait Assou, S., & El Hassouni, M. (2019). Isolation and screening of actinomycetes producing antimicrobial substances from an extreme Moroccan biotope. *The Pan African Medical Journal*, *33*, 329.
- Farda, B., Djebaili, R., Vaccarelli, I., Del Gallo, M., & Pellegrini, M. (2022). Actinomycetes from caves: an overview of their diversity, biotechnological properties, and insights for their use in soil environments. *Microorganisms*, *10*(2), 453.
- Genilloud, O. (2017). Actinomycetes: still a source of novel antibiotics. *Natural product reports*, *34*(10), 1203-1232.

References

- GonSaidi, F. A., Phinzi, K., & Molnár, E. (2023). Urbanization in Algeria: Toward a more balanced and sustainable urban network?. *Social Sciences*, *12*(3), 174.
- Goodfellow M. (1971). Numerical taxonomy of some nocardioform bacteria. Journal of general microbiology, 69(1), 33–80. https://doi.org/10.1099/00221287-69-1-33
- Goodfellow, M., and Williams, S. T. (1983). Ecology of actinomycetes. Annual review of microbiology., 37(1), 189-216.
- Grigoryan, L. N., and Bataeva, Y. V. (2023). Ecological features and biotechnological possibilities of soil actinobacteria. Theoretical and Applied Ecology, 2, 6-19.
- GUNGI S., ARIMA K., BEPPY T. (1983). Screening of antifungal antibiotics according to activities inducing morphological abnormalities. Agricultural and Biological Chemistry. 47: 2061-206
- Gunji, S., Arima, K., & Beppu, T. (1983). Screening of antifungal antibiotics according to activities inducing morphological abnormalities. *Agricultural and Biological Chemistry*, *47*(9), 2061-2069.
- Hasani, A., Kariminik, A., & Issazadeh, K. (2014). Streptomycetes: characteristics and their antimicrobial activities ,63-75.
- Hashim, H. S., Zayan, M. M., Mohamed, A. A., & El rahman Abulila, H. I. A. (2026). Actinomycetes in the spotlight: biodiversity and their role in bioremediation. World Journal of Microbiology and Biotechnology, 42(2), 44.
- Hayakawa, M., & Nonomura, H. (1987). Humic acid-vitamin agar, a new medium for the selective isolation of soil actinomycetes. Journal of Fermentation Technology, 65(5), 501–509.
- Hinsinger, P., Bengough, A. G., Vetterlein, D., & Young, I. M. (2009). Rhizosphere: biophysics, biogeochemistry and ecological relevance ,117-152.
- Hong, H., Samborskyy, M., Usachova, K., Schnatz, K., & Leadlay, P. F. (2017). Sulfation and amidinohydrolysis in the biosynthesis of giant linear polyenes. *Beilstein Journal of Organic Chemistry*, *13*(1), 2408-2415.
- Hutchings, M. I., Truman, A. W., & Wilkinson, B. (2019). Antibiotics: past, present and future. *Current opinion in microbiology*, *51*, 72-80.
- Institute, C. L. S. (2017). Performance standards for antimicrobial susceptibilit testing.

References

- Jones, J. T., Haegeman, A., Danchin, E. G., Gaur, H. S., Helder, J., Jones, M. G., ... & Perry, R. N. (2013). Top 10 plant- parasitic nematodes in molecular plant pathology. *Molecular plant pathology*, *14*(9), 946-961.
- Kekuda, P. T. R., Shobha, K. S., & Onkarappa, R. (2010). Studies on antioxidant and anthelmintic activity of two Streptomyces species isolated from Western Ghat soils of Agumbe, Karnataka. *Journal of Pharmacy Research*, *3*(1), 26-29.
- Kelly, K. L., and Judd, D. B. (1976). *Color: universal language and dictionary of names* (Vol. 440). US Department of Commerce, National Bureau of Standards.
- Kieser, T., Bibb, M. J., Buttner, M. J., Chater, K. F., & Hopwood, D. A. (2000). Practical Streptomyces genetics: John innes foundation. *Norwich Research Park, Colney*, *2000*, 44-61.
- Kitouni M. (2007). Isolement de bactéries actinomycétales productrices d'antibiotiques à partir d'écosystèmes extrêmes. Identification moléculaire des souches actives et caractérisation préliminaire des substances élaborées. Thèse de doctorat en microbiologie appliquée. Université Mentouri, Constantine, P170.
- Kontro, M., Lignell, U., Hirvonen, M. R., & Nevalainen, A. (2005). pH effects on 10 Streptomyces spp. growth and sporulation depend on nutrients. Letters in applied microbiology, 41(1), 32-38.
- Kropodurova, T., Barshtin, F., Djajan, F., Plojnik, A., Zaichenko, T., and Bloom, Y. (2024). Enhance the antioxidant activity and total phenolic content of Fomitopsis pinicola extract. Mycobiology and Biotechnology, 11(1), 18.
- Leboffe, M. J., & Pierce, B. E. (2015). Microbiology: laboratory theory and application. Morton Publishing Company.
- MacWilliams, M. P. (2009). Citrate test protocol. American Society for Microbiology.
- Manneville, O. (1999). Tourbières et marais. *Delachaux et Niestlé*.
- Martínez-Suárez, J. V., Ortiz, S., & López-Alonso, V. (2016). Potential impact of the resistance to quaternary ammonium disinfectants on the persistence of Listeria monocytogenes in food processing environments. *Frontiers in microbiology*, *7*, 638.
- Nanjwade B. K., Chandeashekhara S., Prakash S., Goudanavar M. S., Fakirappa V. M. (2010). Production of antibiotics from soil isolated actinomycetes and evaluation of their antimicrobial activities. Tropical Journal of Pharmaceutical Research, 9:332 – 340.

References

- Nazari, M. T., Machado, B. S., Marchezi, G., Crestani, L., Ferrari, V., Colla, L. M., & Piccin, J. S. (2022). Use of soil actinomycetes for pharmaceutical, food, agricultural, and environmental purposes. *3 Biotech*, *12*(9), 232.
- Negi, V., Sharma, P., Amreen, A., & Rawat, G. (2024). A comprehensive review on actinomycetes distribution, novel bioactive compounds and their implementation in the field of agronomy and medical. Journal of Mountain Research, 19(2). https://doi.org/10.51220/jmr.v19-i2.10
- Ngamcharungchit, C., Chaimusik, N., Panbangred, W., Euanorasetr, J., & Intra, B. (2023). Bioactive metabolites from terrestrial and marine actinomycetes. *Molecules*, *28*(15), 5915.
- Oktyabrskii, O. N., & Smirnova, G. V. (2012). Redox potential changes in bacterial cultures under stress conditions. Microbiology, 81(2), 131-142.
- Orgiazzi, A., Bardgett, R. D., & Barrios, E. (2016). *Global soil biodiversity atlas* (pp. 176-pp).
- Parte, A., Whitman, W. B., Goodfellow, M., Kämpfer, P., Busse, H. J., Trujillo, M. E., ... & Suzuki, K. I. (Eds.).
- Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Analytical biochemistry, 269(2), 337-341.
- Prudence, S. M., Addington, E., Castaño-Espriu, L., Mark, D. R., Pintor-Escobar, L., Russell, A. H., & McLean, T. C. (2020). Advances in actinomycete research: an ActinoBase review of 2019. Microbiology, 166(8), 683-694.
- Rani, A., Saini, K. C., Bast, F., Mehariya, S., Bhatia, S. K., Lavecchia, R., & Zuorro, A. (2021). Microorganisms: a potential source of bioactive molecules for antioxidant applications. *Molecules*, *26*(4), 1142.
- Rietz, D. N., & Haynes, R. J. (2003). Effects of irrigation-induced salinity and sodicity on soil microbial activity. *Soil Biology and Biochemistry*, *35*(6), 845-854.
- Rousk, J., Brookes, P. C., & Bååth, E. (2009). Contrasting soil pH effects on fungal and bacterial growth suggest functional redundancy in carbon mineralization. *Applied*

and environmental microbiology, *75*(6), 1589–1596. https://doi.org/10.1128/AEM.02775-08.

- Sharma, M., Dangi, P., & Choudhary, M. (2014). Actinomycetes: source, identification, and their applications. *International Journal of Current Microbiology and Applied Sciences*, *3*(2), 801-832.
- Shirling, E. T., & Gottlieb, D. (1966). Methods for characterization of Streptomyces species. International journal of systematic bacteriology, 16(3), 313-340.
- Sineva, O. N., Ivankova, T. D., and Terekhova, L. P. (2019). Low Temperature Storage of Actinomycetes-Members of the Genus Streptomyces. *Antibiot Khimioter= Antibiotics and Chemotherapy*, *64*(3-4), 3-9.
- Siro, G., and Pipite, A. (2024). Mini-review on the antimicrobial potential of actinobacteria associated with seagrasses. *Exploration of Drug Science*, *2*(1), 117-125.
- Smith, H., & Brown, A. E. (2021). *Benson's microbiological applications: laboratory manual*. McGraw-Hill Higher Education.
- Stamford, T. L. M., Stamford, N. P., Coelho, L. C. B. B., & Araujo, J. M. (2001). Production and characterization of a thermostable α-amylase from Nocardiopsis sp. endophyte of yam bean. *Bioresource Technology*, *76*(2), 137-141.
- Subramani, R., & Aalbersberg, W. (2012). Marine actinomycetes: an ongoing source of novel bioactive metabolites. *Microbiological research*, *167*(10), 571-580.
- Van Den Hoogen, J., Geisen, S., Routh, D., Ferris, H., Traunspurger, W., Wardle, D. A., ... & Crowther, T. W. (2019). Soil nematode abundance and functional group composition at a global scale. *Nature*, *572*(7768), 194-198.
- Van der Meij, A., Worsley, S. F., Hutchings, M. I., & van Wezel, G. P. (2017). Chemical ecology of antibiotic production by actinomycetes. *FEMS microbiology reviews*, *41*(3), 392-416.
- Vitali, L. A., Beghelli, D., Nya, P. C. B., Bistoni, O., Cappellacci, L., Damiano, S., ... & Bramucci, M. (2016). Diverse biological effects of the essential oil from Iranian Trachyspermum ammi. *Arabian Journal of Chemistry*, *9*(6), 775-786.
- Waksman, S. A. (1950). The actinomycetes-their nature, occurrence, activities, and importance.

References

- Waksman, S. A., Schatz, A., & Reynolds, D. M. (2010). Production of antibiotic substances by actinomycetes. Annals of the New York Academy of Sciences, 1213(1), 112.

- Williams, P., Ketley, J., & Salmond, G. (Eds.). (1998). *Methods in microbiology: Volume 27. Bacterial pathogenesis* (pp. xix+-620).
- Williams, S. T., & Cross, T. (1971). Chapter XI actinomycetes. In *Methods in microbiology* (Vol. 4, pp. 295-334). Academic Press.
- Wu, Y., Kang, Q., Zhang, L. L., & Bai, L. (2020). Subtilisin-involved morphology engineering for improved antibiotic production in actinomycetes. Biomolecules, 10(6), 851.
- Yokota, K., Furusawa, N., Abe, T., & Takenaka, S. (1988). Application of casein agar plate method for the determination of protease activity. Eisei Kagaku, 34(3), 241–247.
- Zhang, B., Wu, X., Tai, X., Sun, L., Wu, M., Zhang, W., ... & Dyson, P. (2019). Variation in actinobacterial community composition and potential function in different soil ecosystems belonging to the arid Heihe River Basin of Northwest China. *Frontiers in microbiology*, *10*, 2209.
- https://asm.org/protocols/citrate-test-protocol

Annexes

Annexes 01

Culture Media Composition

GNG (Nutrient Gelatin Agar)

- Nutrient agar: 24 g
- Gelatin: 4 g
- Distilled water: 1000 mL
- pH: 7.2

TSA (Tryptic Soy Agar)

- Trypticase: 17 g
- Soytone: 3 g
- Sodium chloride: 5 g
- Agar: 15 g
- Distilled water: 1000 mL
- pH: 7.2

Bennett Agar

- Yeast extract: 1 g
- Beef extract: 1 g
- Peptone: 2 g
- Glucose: 10 g
- Agar: 15 g
- Distilled water: 1000 mL
- pH: 7.0 ± 0.2

ISP2

- Yeast extract: 4 g
- Malt extract: 10 g
- Glucose: 4 g
- Agar: 20 g
- Distilled water: 1000 mL
- pH: 7.0 ± 0.2

ISP6

- Peptone: 15 g
- Proteose peptone: 5 g
- Ferric ammonium citrate: 0.5 g
- Sodium thiosulfate: 0.08 g
- Yeast extract: 1 g
- Dipotassium hydrogen phosphate ($K_2\ HPO_4$): 1 g
- Agar: 20 g
- Distilled water: 1000 mL
- pH: 7.0 ± 0.2

ISP7

- Glycerol: 15 g
- L-asparagine: 1 g
- Dipotassium hydrogen phosphate ($K_2\ HPO_4$): 0.5 g
- Sodium chloride (NaCl): 0.5 g
- Ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2\ O$): 0.01 g
- Agar: 20 g
- Distilled water: 1000 mL
- pH: 7.0 ± 0.2

Gause Medium

- Glucose: 10 g
- Peptone: 1 g
- Sodium chloride (NaCl): 1 g
- Agar: 15 g
- Distilled water: 1000 mL

Mueller–Hinton Agar

- Beef infusion: 2 g
- Starch: 1.5 g
- Casein hydrolysate: 17.5 g
- Agar: 17 g
- Distilled water: 1000 mL
- pH: 7.

Sabouraud Agar

- Peptone: 10 g
- Glucose: 20 g
- Agar: 17 g
- Distilled water: 1000 mL
- pH: 7.2

AF Broth

- Yeast extract: 4 g
- Malt extract: 10 g
- Glucose: 2 g
- Sodium chloride (NaCl): 2.5 g
- Calcium carbonate ($CaCO_3$): 1 g
- Distilled water: 1000 mL
- pH: 7.0 ± 0.2

Mueller–Hinton Broth

- Beef extract: 2 g
- Acid hydrolysate of casein: 17.5 g
- Starch: 1.5 g
- Distilled water: 1000 mL
- pH: 7.2

Triple Sugar Iron (TSI) Agar

- Meat peptone: 15 g
- Proteose peptone: 5 g
- Beef extract: 3 g
- Yeast extract: 3 g
- Glucose: 1 g
- Sucrose: 10 g
- Lactose: 10 g
- Ferric ammonium citrate: 0.3 g
- Sodium chloride: 5 g
- Sodium thiosulfate: 0.3 g

- Phenol red: 0.05 g
- Agar: 18 g
- Distilled water: 1000 mL
- pH: 7.2

Simmons Citrate Agar

- Ammonium dihydrogen phosphate: 1 g
- Dipotassium phosphate: 5 g
- Sodium chloride: 2 g
- Sodium citrate: 2 g
- Magnesium sulfate: 0.2 g
- Bromothymol blue: 0.08 g
- Agar: 18 g
- Distilled water: 1000 mL
- pH: 6.8

Mercury chloride

- $HgCl_{2:}$ 15 g
- HCL: 20 mL
- Distilled water:100 mL

الملخص

تعد النظم البيئية الساحلية الأرضية والتربة في الجزائر موطنا لمجموعة واسعة من الكائنات الحية الدقيقة التي يمكن أن توفر المواد النشطة بيولوجيا المستخدمة في قطاعات متعددة مثل صناعة الأدوية والزراعة العضوية والطب...

قمنا في دراستنا بتحليل الأنشطة المضادة للميكروبات ومضادات الأكسدة لسلالات الفطريات الشعاعية المعزولة من تربة الغابات والمناطق الجبلية الساحلية في منطقة المخلد.

مستخلصات سلالات الفطريات الشعاعية المكتسبة عن طريق التخمير في اوساط سائلة (وسط Benette وAF وGNG)

تم عزل ما مجموعه 11 سلالة باستخدام ISP2، وسط GNG، وسط TSA، Gauss، إلخ وتم تحديدها باستخدام وسائط مختلفة (الجيلاتين الجيلاتيني، أجار الكازين، TSI، مقايسة السيترات، مقايسة الكاتلاز، صبغة جرام...). ومن خلال تقييم النشاط المضاد للميكروبات باستخدام طريقة أسطوانة الأجار على 5 سلالات نقية، وجد أن E3.2 لديه منطقة تثبيط تبلغ حوالي 11 ملم ضد *باسيلوس سيروس* . و8 ملم ضد *كلابسيلا بنومونيا* أظهرت سلالات مثل E1.9 وE1.11 نشاطًا كبيرًا ضد *بسودوماناس ايروجينوزا* ، مع مناطق تثبيط تبلغ 9.75 و9.5 ملم، اما بالنسبة الى *كانديدا البيكانس* لم يسجل أي نشاط واضح. وفيما يتعلق بطريقة انتشار البئر، لم تظهر جميع السلالات منطقة تثبيط، ربما لأسباب مختلفة : عدم كفاية المستقلبات النشطة، أو المقاومة في الاختبارات البكتيرية، أو المشاكل التقنية مثل عدم كفاية قطر العينة أو عدم كفاية وقت ما قبل الانتشار قبل الحضانة. وفيما يتعلق بالنشاط المضاد للأكسدة الذي تم تحقيقه باستخدام المستخلصات التي تم الحصول عليها بعد التخمير، أظهرت النتائج أن السلالة E1.9 أظهرت أفضل قدرة مضادة للأكسدة إجمالية، بقيمة قصوى تبلغ 8.5 ملغ EAA/جم من المستخلص الجاف على وسط بينيت، بالإضافة إلى أعلى نشاط لاحتجاز جذر DPPH، حيث وصل التثبيط إلى 88٪. أظهرت سلالة E3.2 المزروعة على وسط AF أيضًا نشاطًا مضادًا للأكسدة ملحوظًا، مع قدرة إجمالية مضادة للأكسدة تبلغ 6.7 مجم EAA/جم مستخلص جاف وتثبيط DPPH بحوالي 59% . وهذا يدل على وجود سلالات أقوى مقارنة بالسلالات الأخرى، ويعتمد ذلك على طبيعة البيئات وتكوينها والسلالات المزروعة.

الى جانب ذلك، فإن تنوع التربة الساحلية في الجزائر و اختلاف كائناتها الحية تتمتع بإمكانات بيولوجية كبيرة كمضاد للميكروبات ومضاد للأكسدة ويستحق المزيد من الاستكشاف والقراءة.

الكلمات المفتاحية: التربة الساحلية، سلالات الأكتينوميسيتات، وسائط الزراعة والبكتيريا المختبرة, النشاط المضاد للميكروبات، النشاط المضاد للأكسدة.

Abstract

Algerian coastal terrestrial ecosystems and soil include a large diversity of microorganisms that can provide a remarkable origin of biologically active substances used in multiple sectors such as pharmaceuticals, industry, biological agriculture, and medicine.

In our study, we analyzed the antimicrobial and antioxidant activities of actinomycete strains isolated from the soil of forest and mountainous coastal areas of Mkhalled. Extracts from these actinomycete strains were acquired from fermentation in liquid media (Benett, AF, and GNG medium). A total of 11 strains were isolated using ISP2, GNG medium, TSA medium, Gauss and others and identified using different media (gelatin agar, casein agar, TSI, citrate test, catalase test, gram staining, etc.). Evaluating the antimicrobial activity using the cylinder agar method on 5 purified strains showed that E3.2 exhibits an inhibition zone approximately 11 mm against *Bacillus cereus* and 8 mm against *Klebsiella pneumoniae*, strains such as E1.9 and E1.11 showed remarkable activity against *Pseudomonas aeruginosa*, with inhibition zones of 9.75 and 9.5 mm, and no activity was on *Candida albicans*. Moreover, the well diffusion method indicates that all strains did not show any inhibition zone, possibly due to different reasons: insufficient active metabolites, resistance of the bacteria tests, or technical problems such as unsuitable well diameter or insufficient pre-diffusion time before incubation. Regarding the antioxidant activity achieved with the extracts obtained after fermentation, the results show that strain E1.9 presented the best total antioxidant capacity, with a maximum value of 8.5 mg AAE/g of dry extract on Bennett medium, as well as the highest activity of trapping the DPPH radical, reaching 88% inhibition. The E3.2 strain grown on AF medium also showed remarkable antioxidant activity, with a total antioxidant capacity of 6.7 mg EAA/g dry extract and an inhibition of DPPH of about 59%. This indicates that there are strains that are more potent compared to the others, which depend on nature, the composition of the media, and the cultivated strains.

Besides, the diversity and differentiation of Algerian coastal soils submit a large biological potential as antimicrobial and antioxidant to be more explored and more discovered.

Keywords: actinomycete strains, Mkhalled coastal soil, culture mediums, bacteria tests, antimicrobial activity, antioxidant activity.

Résumé

Les écosystèmes terrestres côtiers et le sol algérien abritent une grande diversité de micro-organismes qui peuvent fournir une origine reconnaissable à des substances actives biologiques utilisées dans de multiples secteurs, tels que l'industrie pharmaceutique, l'agriculture biologique, la médecine...

Dans notre étude, nous avons analysé les activités antimicrobiennes et antioxydantes de souches d'actinomycètes isolées du sol des zones forestières et côtières montagneuses de Mkhalled. Extraits de ces souches d'actinomycètes acquis par fermentation en milieu liquide (Benette, milieu AF et GNG). Un total de 11 souches ont été isolées à l'aide d'ISP2, du milieu GNG, du milieu TSA, de Gauss et autres et identifiées en utilisant différents milieux (gélatine gélatineuse, gélose caséine, TSI, test au citrate, test à la catalase, coloration de Gram...). et en évaluant l'activité antimicrobienne à l'aide de la méthode de cylindre d'agar sur 5 souches purifiées, il a été montré que E3.2 présente une zone d'inhibition d'environ 11 mm contre *Bacillus cereus* et de 8 mm contre *Klebsiella pneumoniae*, les souches telles que E1.9 et E1.11 ont montré une activité remarquable contre *Pseudomonas aeruginosa*, avec des zones d'inhibition de 9.75 et 9.5 mm, et aucune activité était sur *candida albicans*. Concernant la méthode de diffusion en puits, toutes les souches n'ont pas montré de zone d'inhibition, peut-être pour différentes raisons : métabolites actifs insuffisants ou des problèmes techniques tels qu'un diamètre de puits inadapté ou un temps de prédiffusion insuffisant avant l'incubation, concernant l'activité antioxydante obtenue avec les extraits obtenus après fermentation, les résultats montrent que la souche E1.9 présentait la meilleure capacité antioxydante totale, avec une valeur maximale de 8,5 mg EAA/g d'extrait sec sur milieu Bennett, ainsi que la plus forte activité de piégeage du radical DPPH, atteignant 88% d'inhibition. La souche E3.2 cultivée en milieu AF a également montré une activité antioxydante remarquable, avec une capacité antioxydante totale de 6,7 mg d'EAA/g d'extrait sec et une inhibition du DPPH d'environ 59%. Cela indique qu'il y'a des souches plus puissantes par rapport aux autres, dépendant de nature, de la composition des milieux, et les souches cultivées.

En outre, la diversité et la décomposition des sols côtiers algériens présentent un potentiel biologique important en tant qu'antimicrobien et antioxydant qui mérite d'être davantage exploré et découvert.

Mots clés : actinomycètes, souches, sol côtier de Mkhalled, milieux de culture, bactéries tests, activité antimicrobienne, activité antioxydante.