PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA

MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH

LARBI TBESSI UNIVERSITY

FACULTY OF EXACT SCIENCES AND SCIENCE OF NATURE AND LIFE

Department: Applied biology

Speciality : Applied Microbiology

Graduation note Submitted to the Department of Applied Biology

in Partial Fulfillment of the Requirements for a Master's Degree

MASTER MEMORY

Bioactive molecules producing Xerophilic Actinomycetes

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11_06_2024


بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

Acknowledgments

*Above all, we thank **"ALLAH "** the Almighty for the courage, will and passion he gave us to carry out this work.*

*We would like to express all our gratitude to **Dr.Mabrouka benhadj,** for having supervised and directed this work, and for her availability, her patience, her competence, her valuable advice, the trust she has placed in us and for her regular monitoring at the development of this dissertation. Thank you sir for the guidance and encouragement that allowed us to progress and broaden our field of vision of the research work*

*Thank you to all the jury members **Dr.Metrouh.R** and **Dr.Toumi.N***

having agreed to review and evaluate this work

*We also thank **Mrs. Hmimed.R** Thank you for standing with us and supporting us in this work. We wish her every success and excellence.*

We thank all our dear teachers that we were able to meet during our university career.

Thank you to all the people who in various ways and at different times helped us.

Dedication

« وَآخِرُ دَعْوَاهُمْ أَنِ الْحَمْدُ لِلَّهِ رَبِّ الْعَالَمِين »

I dedicate this work to:

First, ***my dear mother*** and all ***my brothers,***

especially my brother ***Noureddine, Bouzid, Yassin*** and ***Haydar***

who stood by me throughout my academic journey

And secondly, to all my friends, especially

Ikram, Nour El-Houda , Khawla, Marwa, Hayat, Noudjoud,

Chaima ,Kaouthar, Aya, Louisa , Akhila , Bassma

and ***Aicha***

Ibtissem

Dedication

Praise be to allah , love, thanks and gratitude for the start and conclusion, quot;And the last of their claim is that Praise be to God, Lord of the Worlds.

After a fatigue and hardship that lasted five years for the sake of dream and science, I carried in its folds the wishes of the nights and became my sons today for the eye Korra, here I am today standing on the threshold of my graduation I pick the fruits of my tiredness and raise my hat with pride. May God be praise to you before you are satisfied and praise be to you if you satisfied and praise after satisfaction,because you have succeeded me to complete this success and fulfill my dream.……

And with all love, I dedicate the fruit of my success and graduation

To the one who decorated my name with the most beautiful titles, who supported me without limits and gave me without charge, to the one who taught me that the world is a struggle and its weapon is science and knowledge, my first supporter in my career and my bond and my strength and my refuge after God is my pride and my pride

My father

To the one who made God paradise under her feet, and her heart embraced me before her hands and facilitated me adversity with her prayers, to the affectionate heart and the candle that I had in the dark nights, the secret of my strength and my success is my paradise

My mother

To the one who supported me with all love when I was weak and removed from my way the trouble paved the way for me, from planting confidence and determination inside me to the one who pulled God with him muscular, so he was a certain good.

My brother: Oussama, Ibrahim, Fateh

To that star who always illuminates my path, to my constant rib that does not tend, to those who have had a bond with her and my first and last refuge.

My sister : Oumaima

To all those with whom I searched the cup of love, brotherhood and friendship and I had with them the most precious memories and the most beautiful moments and my girlfriends: Ibtissam, Zainabe, Noussaiba,, Manar, Imane, Hadile,, Ryan ,nour , nodjoude, kawther. To everyone my pen forget then and my heart did not forget them, especially my payment of a 2024 microbiological batch

Aya

Abstract

Actinomycetes are gram-positive filamentous bacteria that receive great attention as producers of biomolecules and are distributed in various environmental media.

The goal of this work revolves around searching for biomolecules produced by actinomycetes that love harsh and dry conditions and studying their physical and chemical properties. Through this discussion. We are interested in proving that 7 of the Actinomyces strains have the ability to produce antibiotics against 4 targe bacterial strains of *klebsilla, staphilococcus* and *bacillus* and are capable of producing antifungals against 5 strains of yeasts of *candida, saccharomycs, rhodotorula* and kluveromycs and against 6 strains. of the filamentous fungi of the type *Fusarium, Lichthermia, Corylinifica, Rhizopus* and *Ac.fleww.* We used the double layer technique in ISP2 culture media in only 10 days and an agar cylinder on different days (7,10) in different culture media ISP2, GYEA.

Its production of special enzymes was studied, as well as the physiological conditions of pH. Salinity and temperature.

Through the results obtained, we found that the majority of the 07 isolated strains present antibacterial and antifungal activity, with diameters of inhibition reaching 74.5 mm against bacteria, 77.5 mm against yeasts, and 73.7 mm against fungi. Based on the results of physiological conditions, all isolated strains grow at 25°C and are salt-loving. As for the study of the metabolic profiles of these strains, it showed that these strains have an important diversity in enzymatic activity.

In conclusion, actinomycetes can be considered specialized in producing important biomolecules in the medical field, distinguished by their metabolic capacity and their distribution in various media.

Key words: actinomycetes. Antifungals. Antibiotics. Biomolecules. Bacteria. Yeasts. Filamentous fungi.

Résume

Les actinomycètes sont des bactéries filamenteuses à Gram positif qui font l'objet d'une grande attention en tant que productrices de biomolécules et sont distribuées dans divers milieux environnementaux.

L'objectif de ces travaux s'articule autour de la recherche de biomolécules produites par des actinomycètes aimant les conditions rudes et sèches et de l'étude de leurs propriétés physiques et chimiques. A travers cette discussion. Nous souhaitons prouver que 7 des souches d'Actinomyces ont la capacité de produire des antibiotiques contre 4 souches bactériennes cibles de *klebsilla*, *staphilococcus* et *bacillus* et sont capables de produire des antifongiques contre 5 souches de levures de *candida, saccharomycs, rhodotorula* et *kluveromycs* et contre 6 souches. des champignons filamenteux du type *Fusarium, Lichthermia, Corylinifica, Rhizopus* et *Ac.fleww.* Nous avons utilisé la technique double couche dans des milieux de culture ISP2 en seulement 10 jours et un cylindre de gélose à différents jours (7,10) dans différents milieux de culture ISP2, GYEA.

Sa production d'enzymes spéciales a été étudiée, ainsi que les conditions physiologiques de pH. Salinité et température.

A travers les résultats obtenus, nous avons constaté que la majorité des 07 souches isolées présentent une activité antibactérienne et antifongique, avec des diamètres d'inhibition atteignant 74,5 mm contre les bactéries, 77,5 mm contre les levures et 73,7 mm contre les champignons. Sur la base des résultats des conditions physiologiques, toutes les souches isolées poussent à 25°C et aiment le sel. Quant à l'étude des profils métaboliques de ces souches, elle a montré que ces souches présentent une diversité importante d'activité enzymatique.

En conclusion, les actinomycètes peuvent être considérés comme spécialisés dans la production de biomolécules importantes dans le domaine médical, se distinguant par leur capacité métabolique et leur distribution dans divers milieux.

Mots clés : actinomycètes. Antifongiques. Antibiotiques. Biomolécules. Bactéries. Levures. Champignons filamenteux.

الملخص

الاكتينوميسات بكتيريا خيطية موجبة الغرام تتلقى اهتماما كبيرا كونها منتجة للجزيئات الحيوية و تتوزع هذه البكتيريا في أوساط بيئية مختلفة.

الهدف من هذا العمل يتمحور حول البحث عن الجزيئات الحيوية التي تنتجها الأكتينوميسات المحبة لظروف القاسية و الجافة و دراسة خصائصها الفيزيائية والكيميائية . من خلال هذه المناقشة . نحن مهتمون باثبات ان 07 من سلالات الاكتنوميسات لها القدرة علي انتاج المضادات الحيوية ضد 4 سلالات بكتيرية مستهدفة من نوع *staphilococcus, klebsilla* و *bacillus* و قادرة على انتاج مضادات الفطريات ضد 5 سلالات من الخمائر من نوع *rodotorula, saccharomycs, candida* و *kluveromycs* و ضد 6 سلالات من الفطريات الخيطية من نوع *rhizopus, corylinifica, lichthermia, fusarium* و *Ac.fleww* . استخدمنا تقنية الطبقة المزدوجة في وسط الزرع ISP2 في 10 أيام فقط و أسطوانة اجار في ايام مختلفة (7,10) في أوساط زرع مختلفةISP2 , GYEA.

تمت دراسة انتاجها للانزيمات الخاصة و دراسة كذلك الظروف الفيزيويوجية من درجة الحموضة . الملوحة و درجة الحرارة .

من خلال النتائج المتحصل عليها وجدنا ان اغلبية السلالات 07 المعزولة تقدم نشاط مضاد للبكتيريا و مضاد للفطريات تصل اقطار التثبيط الي 74.5ملم ضد البكتيريا و 77.5 ملم ضد الخمائر و73.7 ملم ضد الفطريات . و من خلال نتائج الظروف الفيزيولوجية تنموا جميع سلالات معزولة عند 25°C وتكون محبة للملوحة . واما بالنسبة لدراسة الصيفات الايضية لهذه السلالات بينت ان هذه السلالات لديها تنوع مهم في النشاط الانزيمي .

وفي الختام يمكن اعتبار الاكتينوميسات متخصصة في انتاج الجزيئات الحيوية المهمة في المجال الطبي , وتميزها بالقدرة الايضية وتوزعها في مختلف الأوساط .

الكلمات المفتاحية : الاكتينوميسات. مضادات الفطريات . مضادات الحيوية . الجزيئات الحيوية . البكتيريا . الخمائر . الفطريات الخيطية .

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Introduction

Although antibiotics have saved millions of lives over the past 70 years, their indiscriminate use has led to the emergence of antibiotic-resistant organisms. This concerns many medical experts who caution that we may soon return to the pre-antibiotic era.**(Singer, 2003; Talbot , 2006).**

Actinomycetes, which occur in both terrestrial and aquatic habitats, are among the most common groups of gram-positive microorganisms in nature. Actinomycetes decompose organic matter and display antagonism against other bacteria and fungi, with which they compete for nutrients. Actinomycetes have incredible abilities to survive under extreme conditions in their natural environment and have long been the focus of scholarly attention and have been harnessed as valuable sources of natural compounds, such as antibiotics, enzymes, and vitamins. More than 90 percent of chemotherapeutic antibiotics have been isolated from actinomycetes . **(Newman , 2007 ; Demain , 1999).**

Actinomycetes represent one of the largest and most diverse bacterial groups described **(Ludwig , 2012)**.They are Gram-positive bacteria belonging to the order Actinomycetales and the phylum Actinobacteria, being cosmopolitan microorganisms with habitats in both terrestrial and marine aquatic and freshwater environments **(Bergeijk ,2020)**. Its genome is enriched in G+C content. Its morphology varies from cocci and rods to complex multicellular structures similar to the mycelium of filamentous fungi, . The mycelial structures can be aerial or adhered to the substrate **(Cimmino , 2016 ; Wu ,2020).** This characteristic, together with the fact that some individuals produce spores as a form of asexual reproduction, has already led actinomycetes to be considered as transitional microorganisms between bacteria and fungi. However, actinomycetes' genetic and cellular profiles are characteristic of bacterial attributes, such as a chromosome wrapped in a prokaryotic nucleoid and a cell wall composed of peptidoglycans **(Barka , 2016).**

Actinomycetes are recognized as among the most valuable prokaryotic microorganisms in biotechnology. They are renowned for their production of antibiotics and other bioactive compounds, which have demonstrated effectiveness against a range of pathogens. These bioactive molecules include streptomycin, tetracycline, and chloramphenicol for antibacterial purposes, nystatin for combating fungi, tunicamycin for antiviral activity, and avermectin for addressing parasitic infections. Their significance lies in their diverse capabilities, making them essential in various fields like medicine and agriculture. **(Chaudhary , 2013) .**Actinomycetes are known as versatile producers of antimicrobials active metabolites **(Larpent , 1989).**

Based on the above. This study aims to search for biologically active molecules (antibiotics and antifungals) from some Actinomycetes strains and characterize them.

- **in this subject**

The part One

The theoretical part revolves around general information about actinomycetes, their classification, types, importance, and an overview of the biomolecules which they produce.

The second part

The applied part includes the materials and methods used to conduct this study and the results obtained and discussed.

Bibiographic part

I. Actinomycetes

1. Historical

The characterization, discovery, naming and use of *Actinobacteria* can be divided into different periods **.** divides the history of Actinomycetes into four major periods **(Waksman, 1959).**

- The first (1874-1900), is that of the discovery of their roles in pathology **(Cohn, 1872)** Cohn discovered the first actinomycete which he called *Streptothrix foeresteri*: **(Harz, 1877)** Harz isolated the agent responsible for *Actinomycosis* cattle and named it Actinomyces bovis.
- Second period (1900-1919) relates to the identification and study of soil Actinomycetes with the work of Orla Yensen (1909) who created the *Actinomycetaceae* family which includes a single genus Actinomyces, subsequently, many telluric species were isolated. Buchanan created the order *Actinomycetales* **(Buchanan , 1917).**
- Third period (1919-1940): during which a better knowledge of germs was acquired, thanks to the research of Orskov in (1923) who created the genus *Micromonospora* **(Sveshnikova , 1969)**. This genus includes actinomycetes which do not produce no aerial mycelium, Actinomycetes whose substrate mycelium fragments include the genus *Paraactinomyces* (currently *Nocardia*).
- Fourth period begins in 1940, and corresponds to the era of antibiotics produced by actinomycetes, with the creation of the genus *Streptomyces* (by combining the names of the genera *Streptothrix* and *Actinomyces*) **(Waksman et Henricib;1943)** which brings together the actinomycetes don' t aerial mycelium produces chains of spores carried by sporophores. In 1958 Pridham proposed a classification system for *Streptomyces* based on the morphology of the spore chains and the color of the aerial mycelium **(Pridham et al ;1958)**, introduced an important criterion in the differentiation of species: the production of melanoid pigments.

2.Definition of Actinomycetes

Actinomycetes are a diverse group ofmicrobes having filamentous mycelia. They are among the biggest taxonomic ranking between the main descendants identified nowadays within bacterial domain. Actinomycetes are Gram-positive aerobic bacteria that have a high percentage of Guanine and Cytosine (GC) in their DNA. Currently, they are characterized by Polyphasic method that uses morphological, chemotaxonomic, and molecular data to describe various strains . Actinomycetes Are abundant in natural settings and play a key role in a variety of metabolic and biological activities including the synthesis of extracellular enzymes. Moreover, actinomycetes produce nearly Two-thirds of all naturally produced antibiotics employed in different fields (agriculture, medicine, and veterinary

practice). Amidst them, the *Streptomyces* genus produces the bulk therapeutic molecules recording almost 70% of total Antimicrobials secreted by actinomycetes, where 75% of them areclinically available as antibiotics to treat several human infections **(Bhatti , 2017).**

3. General characteristics

3.1. Morphological characteristics

Actinomycetes possess broad morphological varieties, majorly with regards to the absence or presence of aerial or substrate Mycelia, their colors, the secretion of melanoid pigments, and the shape and form of their spores. The differentiation between the substrate and aerial mycelia is possible due to their dissimilarities in function and morphology. The substrate mycelium has a major role in absorbing nutrients from the medium to allow the growth of actinomycetes. It also has an extensive variety of colors. On the contrary, aerial mycelium Is the hyphae produced by vegetative mycelium under stressful conditions. The main function of such mycelium is reproduction, In which spores develop on aerial hyphae. The hypha looks phasebright, refractive, and coarse (**Chaudhary , 2013)**

•Mycelial structure

Actinomycetes mycelia are similar to that of fungi, but smaller, and range from 0.4 m to 1.2 m in size. They can have a coccoid structure (like *Micrococcus*), rod-coccoid (like *Arthrobacter*), or a Fragmenting hyphal form (like *Nocardia spp*.). Some Actinomycetes form permanent and well differentiated branched mycelia (such *as Frankia a*nd *StreptomycesSpecies*), others grow extended filaments on the surface of the Medium, but do not develop genuine mycelia like *Rhodococcus,* while Corynebacterium does not develop any mycelia. In general, most *Rhodococcus* and *Mycobacterium* species do not possess aerial mycelia **(Rayan , 2022)**

•Types of spores

different morphological features of spores can be used to classify different actinomycetes species. Spores can have various shapes and surface characteristics. Spore shapes Include rod-shaped, globose, reniform, doliform, allantoid, and ovoid forms. also, surface ornamentation of spores varies to include parallel or irregular rugose, smooth, verrucose, Hairy, warty, and spiny textures . **(Zahr et al , 2022).** Spores can either form on aerial and/or substrate mycelium as sole spores, or chains of spores of varying lengths. In some Instances, spores can be formed in unique vesicles called Sporangium and supplied by a flagellum.Monospore type is present in several genera Including *Micromonospora*, *Saccharomonospora*, *Thermoactinomyces*, and *Thermomonospora.* Regarding spore Chains, they can be divided into di-sporous, oligo-sporous, and Poly-sporous chain.

•Melanoid pigments:

Melanins, also referred to as melanoid pigments, are Polymers of various molecular structures with a crucial function in improving the competitiveness and survival of organisms. They also possess antioxidant activities and protect against ultraviolet Radiations harmful to living beings. Melanoids are usually utilized in the fields of medicine, pharmacology and cosmetics. Depending on the strain, age of the culture, and the medium, Actinomycetes produce different colors. It was shown that when same isolates were grown on different media (casein starch Agar, glycerol asparagine agar, yeast extract-malt , extract agar, starch Yeast extract agar, tyrosine agar, and glycerol yeast extract agar), Actinomycetes produced different pigments. On the other hand, When different isolates were grown on same media (casein starch Agar), the colors of colonies varied.**(Ghorbani-Nasrabadi et al , 2013).**

3.2.Chemotaxonomic characteristics

Chemotaxonomy is utilizing the arrangement of chemical entities to categorize organisms based on the resemblance of their cellular chemistries. several characteristics related to the composition and structure of peptidoglycans have been Used to discriminate between actinomycetes species. these include the amino acid type in location three of the side chain of a tetrapeptide, the sugar content of peptidoglycans, and the existence of glycine in interpeptide bonds. Another important chemotaxonomic characteristic is the occurrence or lack of optical Isomers of the amino acid 2,6-Diaminopimelic acid (DAP). DAP Analysis is essential in studying the taxonomy of actinomycetes. Based on major amino acids and sugar patterns, the cell wall can be split into four types. type I is characterized by Having L-DAP, glycine and no sugar as major constituents. Type II cell wall is comprised of meso-DAP, xylose, glycine and arabinose. Type III is composed of meso-DAP, and 3-O-methyl-D-galactose. and Type IV that have meso-DAP, galactose, and arabinose. different groups may share a common DAP profile. Since the four groups of Actinomycetes present different morphological characteristics, they belong to different families. thus, when classifying Actinomycetes, it is not enough to depend on DAP profiling but on genotypic or phenotypic criteria as well **(Jakubiec-Krzesniak et al , 2018).**

3.3.Molecular characteristics

Currently it is no longer possible to propose the creation of a new one species without going through their genetic analysis. These genetic studies have allowed to trace any actinomycetes phylogenie,

grouping or separating species between them or merge genres between them **(Zitouni, 2005).** •DNA/DNA hybridization: the studies of DNA/DNA reassociations are also used in the classification of actinomycets to determine which ones species. **(Boubetra et Biskri , 2013).** •The coefficient of Chargaff or GC% : is an important criterion not only in gender identification but also familiars of actinomycets whose DNA contains a percentage of G+C above 55% (Badji 2006). •Sequence Analysis of the DNA codant pourL ARNr 16S: is today widely used to establish phylogenic relations between two groups bacterials and determine the taxonomic positions of many organism **(Boubetra et Biskri , 2013).**

4.Classification of actinomycetes

•Kigdom: Bacteria – As members of the Bacteria Empire, Actinomycetes are one-celled organisms that are distinguished by their simple cell structure. Although they are present in different environments around the world, some species may cause disease in human beings. Gram-positive bacteria also possess a layer of peptidoglycan in their cell wall.

•Phylum: According to the *Phylum Actinobteria,* Actinomycetes are Gram-positive bacteria that are distinguished by their high level of G+C in their DNA. They meet in terrestrial and aquatic environments where they present a great nutritional variety. Members of this group also generate mycelium.

•Subclass*:* *Actinobacteridae* subclass is varied and includes a great diversity of organisms that can meet in different habitats. As a subclass of the phylum *Actinobacteria,* the members of this subclass produce myceliums.

•Order: *Actinomycetales* - Members of the class Actinomycetales are called Actinomycetes. They are varied in nature and meet in the aquatic and terrestrial environments. This is Gram-positive and aerobic bacteria (some family members are anaerobic). They are also distinguished by a filamentous pattern of growth. **(Jakubiec-Krzesniak et al , 2018).**

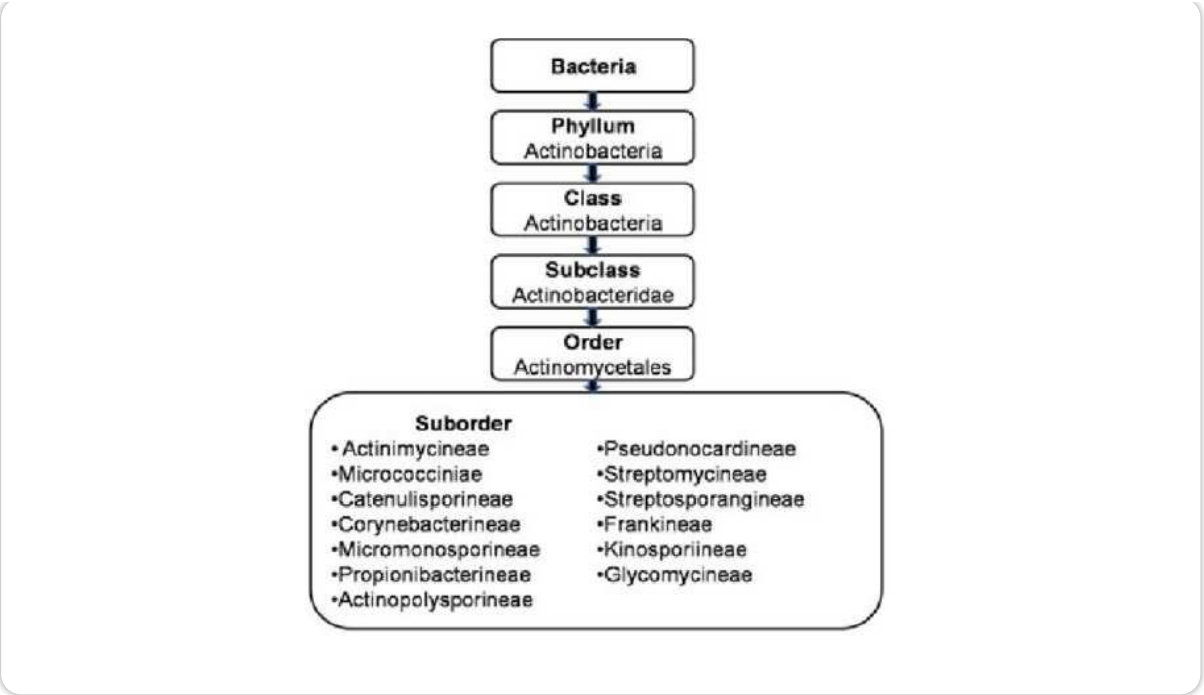

Figure 01 : Systemic classification of Actinomycetes **(Kerbab , 2010)**

5. Taxonomy of actinomycetes

According to the first edition of Bergey's Manual of Systematic Bacteriology, *Actinobacteria* belonged to the order *Actinomycetales* and was subdivided into 4 families Streptomycetaceae, *Actinomycetaceae*, *Actinoplanaceae*, and *Mycobacteriaceae*. The taxonomy of *Actinobacteria* has evolved considerably over time with the buildup of information. In the second edition of Bergey's Manual of Systematic Bacteriology, *Actinobacteria* were included separately in the fifth volume. Phylum Actinobacteria is separated into 6 classes: *Actinobacteria, Acidimicrobiia, Coriobacteriia*, *Nitriliruptoria, Rubrobacteria*, and *Thermoleophilia*. Class *Actinobacteria* is subdivided into 16 orders: *Actinomycetales, Actinopolysporales, Bifidobacteriales, Catenulisporales, Corynebacteriales*, *Frankiales, Glycomycetales, Jiangellales, Kineosporiales, Micrococcales, Micromonosporales, Propionibacteriales, Pseudonocardiales, Streptomycetales, Streptosporangiales,* and Incertae sedis. Bergey's Manual of Systematics of Archaea and Bacteria showed that phylum *Actinobacteria* includes 5 classes, 19 orders, 50 families, and 221 genera. However, many novel taxa continue to be discovered, so this listing is certainly unfinished. The class *Actinobacteria* and fundamental taxonomic ranks above the genus level were proposed exclusively on the basis of 16S rRNA gene sequence-based groups and taxon-specific 16S rRNA gene sequences. This classification represented an obvious change in the classification of *Actinobacteria* above the genus level as it showed that previous classifications based on the form and function did not reflect natural relationships. *Actinobacteria* have been assigned the

rank of a phylum as the phylogenetic depth signified by the lineage resembles that of existing phyla on the basis of its branching position in 16S rRNA gene trees **(Barka et al , 2016).**

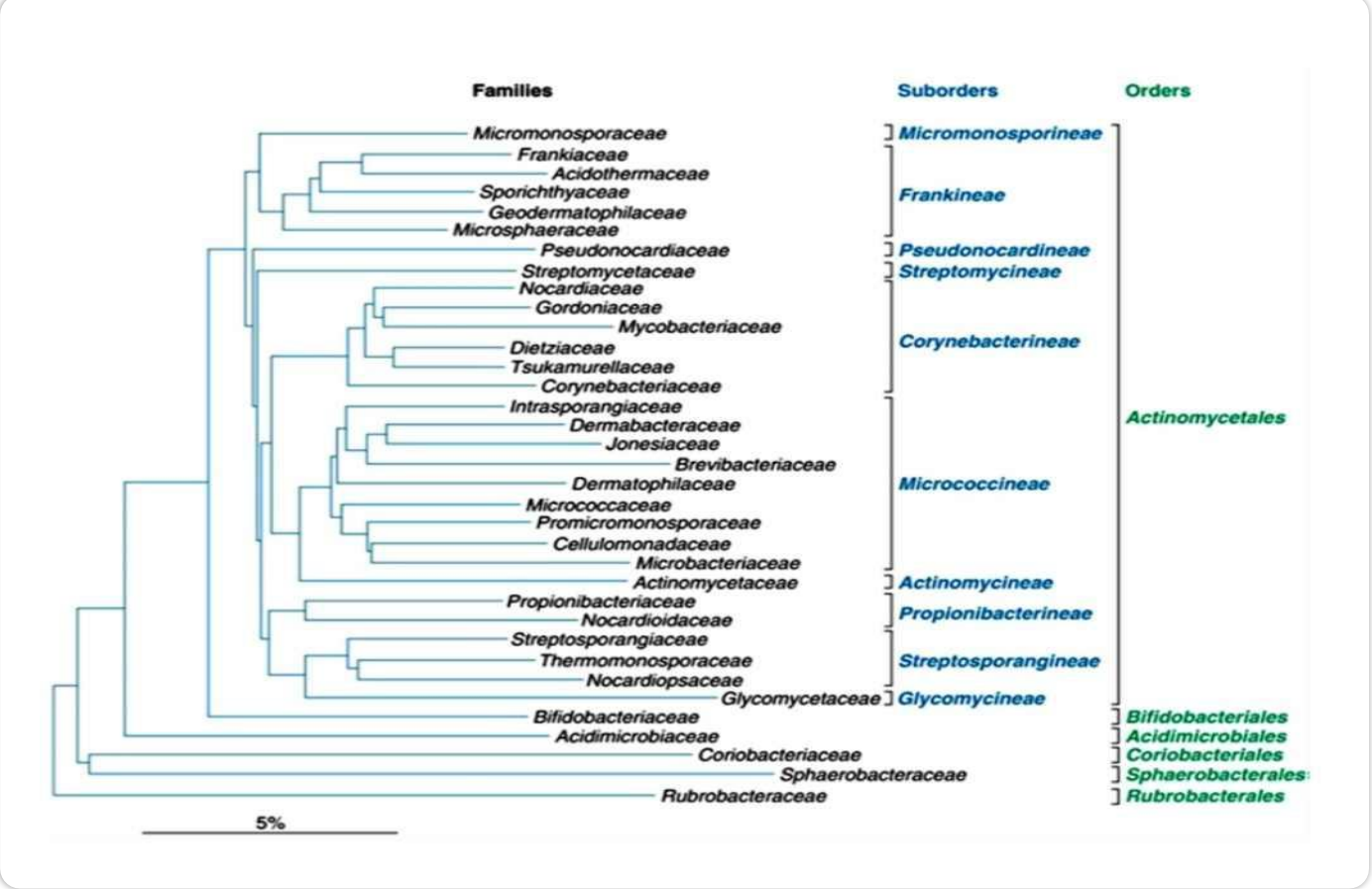

Figure 02: Phylogenetic tree of actinomycets **(Prescott et al. 2003)**

7. The importance of actinomycetes

Actinomycetes are biotechnologically valuable bacteria which are well exploited for secondary metabolites **(Balagurunathan et Radhakrishnan , 2010).** Among various genera of *Actinomycetes; Streptomyces, Saccharopolyspora, Amycolatopsis, Micromonospora* and *Actinoplanes* are the major producers of commercially important biomolecules **(Solanki et al , 2008).** and are prolific producers of secondary metabolites, many of which have commercial importance as antibiotics, antiparasitics and antifungal agents, herbicides, pesticides, anticancer or immunosuppressive agents as well as industrially important enzymes **(Takahashi et Omura, 2003 ; Atta et Ahmad, 2009)**

The actinomycetes are important not only in the field of pharmaceutical industries and also in the agriculture. Actinomycetes have the potential to inhibit the growth of several plant pathogens e.g. *Erwinia amylovora* (bacteria that cause fireblight to apple), *Agrobacterium tumefaciens* (casual agent of Crown Gall disease) …etc **(oskay et al , 2004 ; Jeffrey et al , 2007; Jeffrey, 2008).**

Actinomycetes have several key roles in their ecosystems. Scientists have long known that actinomycetes keep soil bacteria populations in balance. They have the ability to break down various organic materials in soil as well as their ability to produce a range of bioactive molecules, including antibiotics and various kinds of enzymes. With this, about 15% of the world's nitrogen fixed naturally is from symbiotic relationships between various species of the *Frankia* family of *actinobacteria* and their host plants. Their role in the decomposition of plant and other material especially in the degradation of complex and relatively recalcitrant polymers is hugely important. Lignin, cellulose and lignocellulose are all examples of what they degrade. There is evidence that actinomycetes are involved in the degradation of many other naturally occurring polymers in soil such as hemicellulose, pectin, keratin, chitin and fungal cell wall material **(Dilip , 2013)**. Given that they help recycle materials that can be used by plants, it is beneficial in agriculture practices. They also produce a variety of enzymes that are useful in various industries, such as the medical industry. As they are known for their ability to produce various antibiotics, the actinomycetes are widely explored by various research groups in search of novel drug molecules. Another significance of these bacteria is that from the rhizosphere, they suppress the growth of pathogens. Since they produce various bioactive metabolites that are used to produce various drugs (antifungal, anti-parasitic and antibiotics etc). Actinomycetes are an ecologically important group, which play a crucial role in several biological processes such as biogeochemical cycles, bioremediation, bio-weathering and plant growth improvement **(Bawazir , 2018).**

II. Extremophilic actinomycetes

Extremophiles are organisms that live in extreme habitats. They often have unique survival mechanisms to withstand harsh conditions such as high temperature, extreme pH, salinity, pressure, and aridity **(Kohli , 2020 ; Merino , 2019).**

organisms requiring one or more extreme growth conditions. In comparison, extremotolerant organisms are those that are able to tolerate one or more physicochemical parameters **(Rampelotto , 2013)**. Extremophile-the suffix '-phile' originated from the Greek word 'philos', which conveys the meaning of 'love' and 'preference' of extreme environments **(Giddings , 2015 ; Rothschild , 2001) .**

1. Types of Extremophiles

Some examples of different types of extremophiles are listed in the following :

1.1. Thermophile

An organism that grows best at high temperatures and is commonly found in hot places such as the desert. .**(Prieur, 2011 ; Pikuta , 2007)** Thermophiles can be found in extremely hot environments such as thermal vents with temperature reaching as high as 464°C **. (coker , 2016 ; pikuta , 2007).**

Microorganisms can maintain a constant pH or salt gradient across the membrane, but cannot isolate themselves from their warm, watery environment. To thrive in such environments, thermophiles have undergone numerous physiological and biochemical adaptations that maintain the integrity and function of their cells **(Ferrera et Reysenbach , 2007).**

1.2. Psychrophile

Psychrophile produce enzymes that function optimally at low temperatures and are often denatured or inactivated at higher temperatures, even moderate ones. The molecular bases are not fully explained but, in terms of their secondary structure, these enzymes contain a greater proportion of helices and fewer sheets compared to enzymes inactive at low temperatures. As sheets tend to form a more rigid structure, the higher proportion of helices in enzymes adapted to operation in a cold environment allows more flexibility in these conditions **(Michael et John, 2007).** These same enzymes also contain more polar amino acids and fewer hydrophobic amino acids than their mesophilic or thermophilic counterparts. This also contributes to the flexibility of these enzymes (and therefore to their activity) at low temperatures. Finally, proteins from cold-adapted organisms have fewer weak bonds and fewer interactions between their different domains compared to proteins from organisms growing at higher

temperatures. These modifications probably promote their flexibility **(Michael et John, 2007)** , an organism that grows best at low temperatures. .**(Prieur, 2011 ; Pikuta, 2007)**

One of the characteristics of the psychrophiles concerns the transportation process that has a low temperature. This indicates that the structure of cytoplasmic membranes and psychrophiles is adapted to a function at a lower temperature. These membranes contain more saturated acids, which favors the fluid in the fried area (the membranes contain more saturated fats and non-functional ones at a lower temperature). The lipids of certain psychrophiles contain large amounts of polygenic acids and long hydrocarbon chains that contain a number of double liaisons. Ainsi, a new hydrocarbon content of double liaisons (C319) 8 liters of lipids from antarctica bacteria and bacteria of the type Psychroflexus contents of acids gras with four cinq double liaisons. Grass acids are more fluid at higher temperatures, such as saturates or monosaturates **(Michael et John, 2007)**

1.3. Halophile

an organism that thrives in habitats with high salt concentrations, such as sea and salt lakes. **(Prieur, 2011 ; Pikuta, 2007)** Halophiles can tolerate environments with very high salinity such as salt lakes, salterns and brine pools on the ocean bottom. Such environments are saltier than sea water by about 30% and can reach up to 440% sodium chloride concentration. **(coker j, 2016.pikuta ,2007).**

1.4. Alkaliphile

An organism that grows best in an alkaline environment. .**(Prieur, 2011 ; Pikuta, 2007).** Alkaliphiles lives in extremely basic environment with pH ranging from 8.5–11 while **(coker , 2016 ; pikuta , 2007).**

Under alkaline conditions, hydrogen ion concentrations are very low and cells have difficulty using ATP synthase to produce energy and other essential ions, such as magnesium and calcium, which precipitate in the absence of water in the form of salts therefore are only available in the lowest levels and the basic microbes like to bypass in this level. These problems actively pump in these ions and export other ions to maintain their interior at near-neutrality **(Suchita et al., 2006).** Alkalophile peptidoglycan and cell wall-associated polymers have a negative charge to reduce the charge density on the cell surface and contribute to the stabilization of the cell membrane **(Ramírez et al , 2006).**

1.5. Acidophile

An organism that grows best in an acidic environment. **(Prieur, 2011 ; Pikuta , 2007)** Acidophiles can tolerate environment with pH 2.0 and below.**(coker , 2016 ; pikuta , 2007)**

Low pH conditions in environments are considered a challenge to cellular biochemistry since extreme acidity leads to protein denaturation. But acidophilic actinomycetes protect their proteins by the inclusion of several amino acids with neutral side groups and by actively pumping protons outside the cell to maintain constant intracellular pH levels **(Suchita et al , 2006).** Also, the membrane surface of acidophiles are positively charged with a high capacity for internal regulation **(Ramirez et al, 2006).**

1.7. Xerophile

Xerophylic actinomycetes thrive in dry conditions, like deserts, due to their ability to withstand low moisture levels. They play crucial roles in soil ecology Growing in dry conditions, with low water availability **(Rothschild , 2001).** He is an organism that grows best in an extremely arid area such.**(Prieur, 2011 ; Pikuta , 2007) .** the family *Geodermatophilaceae* was first proposed (but invalidly named) only by **(Normand et al, 1996),** and for- mally described a decade later by **(Normand, 2006).** Besides the type genus *Geodermatophilus*, the *Geodermatophila- ceae* contain only the genera *Blastococcus* and *Modestob- acter*. Since they share the harsh (extreme) conditions of low availability of water and nutrients, these two genera are known inhabitants of rock surfaces, whereas Geodermatophilus prefers arid soils as natural habitats **(Urzi et al, 2001).**

2.Distribution in the environment

Actinobacteria have very wide distribution in nature: sol, the air, the gentle waters, seawater, compost, plant debris, pollen, bees melifers, plants (endophytes), lichens and several other substrates **(Benzekhroufa, 2018).** Actinomycetes also have great ecological importance. They can degrade a number and a huge variety of organic compounds and they are extremely important for organic matter mineralization. Although a lot of assignycets be microorganisms living freely, a few are pathogens at the man's home; with other animals and with certain plants **(Prescottetal , 2010).** Most actinomycetes behave like mesophilic bacteria with optimal growth located between 25° and 30°C. However, there are strains thermophiles isolated at a temperature located between 50° and 60°**C (Edwards, 1993) .** As far as PH is concerned, most Actinomycets behave like pHs neutrophile bacteria. Their growth is better at PH between 6 and 9, with a maximum around neutrality. However, some strains of *Streptomycets* were isolated from acid soil samples (PH 3.5) **(Loqman, 2009).**

III. Bioactive molecules and their production by actinomycetes

1. Secondary Metabolites Production by Actinomycetes

Actinomycetes are the most economically and biotechnologically valuable prokaryotes able to produce wide range of bioactive secondary metabolites, such as antibiotics, antitumor agents, immunosuppressive agents and enzymes. These metabolites are known to possess antibacterial, antifungal, neuritogenic, anticancer, antialgal, antimalarial and anti-inflammatory activities (**Uthiraselvam , 2001).**

2. Antimicrobial activity of Actinomycetes

The ability to produce microbial bioactive compounds makes *Actinobacteria* one of the most explored microbes among prokaryotes. The secondary metabolites of *Actinobacteria* are known for their role in various physiological, cellular, and biological processes . Bioactive metabolite producing Actinomycetes were recovered from soil and tested against human pathogenic bacteria and fungiand found to have *Antibacterial* and antifungal property.(**Binod, 2018)**

Extracts derived from extremophilic Actinomycetes have shown significant inhibitory activity against conditionally pathogenic bacteria. Some of the extracts exhibited a broad spectrum of activity inhibiting growth of both Gram - positive and Gram-negative test bacterial pathogens chosen for our study. On the other hand there were extracts that only inhibited growth of either gram positive or gram negative pathogens. moreover, there was a difference in activity of the extractsobtained from strains grown in different growth media**. (Ainur , 2020).**

Actinomycin as the first antibiotic isolated from an Actinomycete, these bacteria remain an invaluable source for discovering new antibiotics **(Mast et Stegmann , 2019).**

1.1. Antibacterial activity

Actinomycetes are renowned for their capacity to produce antibiotics that have the ability to inhibit or restrict the growth of other bacteria. This is the antibacterial activity **(Muiru et al , 2008 ; Meurant, 2012)**

Additionally, antibiotics can be generated by a diverse range of microorganisms. About 17% of them are synthesized by non-mycelial bacteria, 38% by fungi, and about 45% by Actinomycetes. Among the latter, approximately 75% of metabolites are produced by species belonging to the genus *Streptomyces* **(Solecka et al, 2012)**

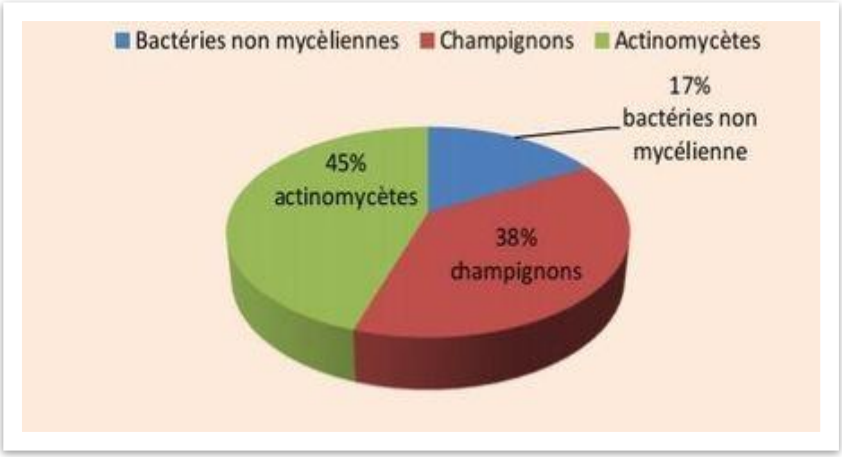

Figure 03. origin of antibiotics **(Solecka et al , 2012)**

Actinomycetes are well known as an inexhaustible source for antibiotics. Most of the known antimicrobials today were originally isolated from Actinomycetes, especially from the genus *Streptomyces.* The produced substances include all important drug classes used in clinics today, such as β-lactams, tetracyclines, macrolides, aminoglycosides, or glycopeptides**. (Bentley et al , 2002).** Disclosure of new antibiotics secreted by *Streptomycetes* continue for instance; mediomycins A, B, and clethramycin, were isolated from *Streptomyces* mediocidicus ATCC23936 and *Streptomyces* malaysiensis DSM4137 respectively show a wide spectrum of antifungal activity **(Hussain , 2002).** Polyketides are very important natural products because of their pharmaceutical applications. Instances of such polyketides are erythromycin (antibacterial), nystatin (antifungal), furthermore avermectin (antiparasitic). All the previous antibiotics have been produced by *Streptomyces sp.* which are considered as the principle producers of antibiotics **(Table) (Hasani , 2014)**

Table 01. List of some antibiotics produced by *Streptomyces sp* **(Hasani , 2014)**

Streptomyces sp	**Antibiotic**	**Streptomyces sp**	**Antibiotic**
S.orchidaccus	Cycloserin n	*S.erythraeus*	Erythromycin
S.oriantalis	Vancomycin	*S.vensuella*	Chlortetracycline,
S.fradiae	Neomycin, actinomycin, fosfomycin, Dekamycin	*S.aureofaciens*	Chloramphenico. dimethylchlo
S.nodosus	Amphotricin B	*S.ambofaciens*	Spiramycin
S.noursei	Nistatin	*S.avermitilis*	Avermicin
S.mediterranei	Rifampin	*S.alboniger*	Puromycin
S.griseus	Streptomycin	*S.niveus*	Novobicin
S.knanamyceticus	Kanamycin	*S.platensis*	Platenmycin
S.tenebrarius	Tobramycin	*S.roseosporus*	Daptomycin
S.spectabilis	Spectinomycin	*S.ribosidificus*	Ribostamycin
S.viridifaciens	Tetracycline	*S.garyphalus*	Cycloserine
S.lincolensis	Lincomycin, clindamycin	*S.vinaceus*	Viomycin
S.rimosus	Oxytetracyclin	*S.clavuligerus*	Cephalosporin

2.2 . Antifungal activity:

Numerous Actinomyces species, particularly those belonging to the genus Streptomyces, are known as antifungal biocontrol agents, inhibiting several fungal plant pathogens . The antagonistic activity of Streptomyces species against fungal pathogens is usually related to the production of antifungal compounds and extracellular hydrolytic enzymes **(Keikha et al , 2015).** Among the novel antifungal secondary metabolites produced by Actinomycetes is sceliphrolactam. **(Oh et al , 2011).**

These antifungal constitute a very small but significant group of drugs and play an important role in controlling mycotic diseases **(Bharti et al , 2010).** Given the limited number of molecules used in fungicide treatment and the emergence of resistance to multiple drugs, the pharmaceutical industry and researchers have called for the search for new antifungals that are more effective and less aggressive for the body **(Badji et al , 2005)**

Table 02 . Some clinically important antifungal from Actinomycetes

Antibiotic	***Produced by***	**Activity**	**Reference**
Libramycin	*Streptomyces sp.*	Antifungal	**(Yahagi et al.,1974)**
Candihexin)	*Streptomyces viridoflavus*	Antifungal	**(Martin et McDaniel.1974)**
Nanaomycin	*Streptomyces rosa*	Antifungal	**(Omura et al, 1974)**
Purpuromycin	*Actinoplanes ianthinogenes*	Antifungal	**(Coronelli et al, 1974)**
Zorbonomycin	*Streptomyces bikiniensis*	Antifungal	**(Argoudelis et al, 1971)**
Validamycin	*Streptomyces ,5008*	Antifungal	**(Wu et al, 2012)**
Azalomycin	*Streptomyces hygroscopicus*	Antifunga	**(Arai et Hamano, 1970)**
Azalomycin	*Streptomyces malaysiensis*	Antifungal	**(Cheng et al, 2010)**
Antimycin	*Streptomyces antibioticus*	Antifungal	**(Xu et al, 2011)**
Antimycin	*Streptomyces lucitanusus*	Antifungal	**(Han et al, 2012)**
Lomofungin	*Streptomyces lomondensis*	Antifungal	**(Bergy, 1969; Das et al., 2012)**
Sclerothricin	*Streptomyces scleogranulatus*	Antifungal	**(Kono et al, 1969).**

Experimental part

Material and Method

I. Objective

Many antibiotics have been isolated in a variety of microorganisms And have been employed in a lot of areas: the industry, agriculture ,Scienceets Pharmaceutical **(Oskay et al, 2004)**. The Actinomycetes represent the main sources of secondary metabolites have an activity antimicrobial .this group produces more than 3,000 antibacterial antifungal activity antibiotics **(Belyagoubi, 2014).**

•This study " the research of bioactive molecules produced by some Xerophyle Actinomycetes" leads to a main goal:

•At first, we were interested in merging the activity *Antibacterials* and the Antifungal activity.

•In second stage we sum ourselves focused on the physiological and biochemical stady of Xerophyles Actinomycetes.

II. Study framework

This work was carried out within the microbiology laboratory of faculty of the brain exact sciences and natural and life sciences of Larbi Tébessi University Tébessa, under the direction of Dr. Benhadj M. during the period ranging from the 24/02/2024 to 31/05/2024.

III. Materials used

1. Non-biological materials

1.1. Great materials

- ✓ Agitator
- ✓ Autoclave
- ✓ Bain marie
- ✓ Balance
- ✓ Etuve
- ✓ Bacteriological hut
- ✓ Optical microscope
- ✓ Vortex

1.2.Small Material

- ✓ Platinum Anse
- ✓ Magnetic bars
- ✓ Bec bunsen
- ✓ Petri dish
- ✓ Doors
- ✓ Funnel
- ✓ Ladies
- ✓ Micropipette
- ✓ Spatula
- ✓ Virreris: graduated beams, graduated test specimens, sterile test tubes, vials.

2.Biological materials

2.1. The actinomycetes

The 07 studied Actinomycetes *(Streptomycetes)* isolates have been provided by our Ms.Framer Dr.Benhadj M :

- ✓ S.ATSP5
- ✓ S.ATSP25
- ✓ S.ATSP28
- ✓ S.ATSP8
- ✓ S.ATSP79
- ✓ S.ATP49
- ✓ S.TN8`

1.2. The indictor strains

*Bacteria:

- *Bacillus subtilis ATCC 6633(PCM 482)*
- *Staphylococcus aureus(INC3)*
- *Staphyloccus epidermidis(CCM 801)*
- *Klebsilla pneumonae(348 LH)*

* Yeasts

- *Candida glabrata(ICF20)*
- *Candida .parapsilosis*
- *Saccharomyces cerivisiae(ICF43)*
- *Rodotorula mucilaginosa(LRM)*
- *Kluveromyces marxianus(ICF44)*

*Filamemteus Fungi

- *Fusarium.oxysporum (F.Foxy)*
- *Lichthermia corylinifica(St87)*
- *Fusarium solani(FfSO)*
- *Rhizopus.oryzea (FRHory)*
- *Aspergillus flavus (ATCC XXXXX)*
- *Ac .Fleww Atcc*

3. Midium used

- ✓ GYEA
- ✓ ISP2
- ✓ Liquid LB
- ✓ Moll LB
- ✓ Solid LB
- ✓ Mac conkey
- ✓ Moll ISP2
- ✓ Liquid ISP2
- ✓ Nutritif église
- ✓ Sabouraud
- ✓ Nutritif Bollion

* The composition of each medium is present in the annex part.

4.The solutions and dyes used

- ✓ Sterile distilled water
- ✓ Sterile physiological water
- ✓ Gentiane purple
- ✓ Lugol

- ✓ Alcohol
- ✓ Fuchsine
- ✓ Immersion oil
- ✓ Methanol

IV. Working method

1. Origins of strains

The 7 strains of Actinomycetes studied in this work stem from a collection of strains of Actinomycetes isolated from a system Xerophylic (Sahara of Algeria Oued sof and Adrar).

2. Retacking and purification of isolation Actinomycetes

The first step in our work is the purification of strains of Actinomycete Studied. This step is very essential, it allows to give isolated and pure colonies.Stem repealing is done by the stride sowing method in petri dish containing the ISP2 medium, these petri dish are incubated at a temperature 30°C for 7 days, as the Actinomycets have slow growth **(Eriskon, 1949**)

3.Study morphological of Actinomycetes

3.1 Macromorphological studies

Ihe study of macromorphological characters of the Actinomycetes allows to determine the presence or absence of air mycelium and its color, also color of substrate mycelium, these features were noted on the ISP2 medium by the methode of incubation seedling by strie of exhaustion, and on settings (ISP2) by the tightened streak seedling method after 7 days of incubation.**(Shirling et Gottlieb, 1966).**

3.2. Gram Staining Technique

Gram Staining is differential unecoloration that allows the distinction of the Gram+ and Gram- bacteria on chemical composition difference labase and ultra Structure of cell walls. It is observed on ISP2 environment, using it of optical microscope. This technique involves seeing .

- ✓ Actinomycetes are Gram+ bacteria.
- ✓ The spore morphology.
- ✓ Spect of filaments **(Cross, 1989; Wink, 2001).**

It is done by the following steps:

- ✓ Prepare smear.
- ✓ Fix smear to heat.
- ✓ Cover gentiane's purple blade for 1 minute, wash with water distilled.
- ✓ Cover lugol for 1 minute.
- ✓ fade into alcohol and then rinse with water.
- ✓ Cover diluted fuchsine blade for 30 seconds then water lava.

- ✓ Dry by blotting paper.
- ✓ Magnification observeral (X100) of an optical microscope **(Funke, 2003)**

4. The antimicrobial activity of the strains of Actinomycetes

4.1. Study of Antibacterial Activity

✓Preparation of growing testing bacteria in solid medium for antibacterial activity test of Actinomycetes against 4 bacterias Following *" B.subtilis ATCC 6633 PCM 482 , S.aureus INC3 , S.epidermidis CCM 801, K.pneumonae 348 LH "* . These bacteria are encemented by the method of strie dulling on the solid LB, and Mac conkey, nutritif eglise then incubated at 30°C and 37°C according to the bacterium For 24h to check their purity.

✓ Preparation for inoculum : After the 24h incubation on the previously cited environments, an inoculum is prepared feach test bacteria in the medium Luria Bertani (LB) liquid and then incubate them for 24h.

4.1.1.Conventional agar cylinders method (Tortorant et al, 1979)

This method consists of sowing the 05 strains of Actinomycetes to test on the (ISP2, GYEA) in tight streaks. In the 7 days of incubation,white the aide of sterile blue cones, the cylinders of agar bearing colonies of actinomycetes , from 07 mm in diameter are cut out from these environments, and deposited at the surface of the molle LB medium previously sown with the defferent 4 bacterias *" B.subtilis ATCC 6633 PCM 482 , S.aureus INC3 , S.epidermidis CCM 801, K.pneumonae 348 LH "* . from the inoculume to the micropipett assistance is added to the 10ml molle LB medium .The sown boxes are kept at the refrigerator for 30 min prior to being incubated, to allow the diffusion of the active substances while preventing the growth of bacteria and then incubated at 30°C and 37°C (according to the bacteria) for 24h **(Kitouni, 2007).**This test was repeated for crops of 10j of incubation. The diameters of areasd of inhibition apparues are measured around agar cilyndres (**Shomura et al , 1979; Saadoun et al moumani, 1997; Petrosyan et al ,2003; Boudjelal et al , 2011)**.

4.1.2.Double layer method

✓The isolats of Actinomycetes test are seeded at the center of the petri box previously sunk by the middle ISP2 then incubate at 30°C for 7 days.

✓Deposited at the surface of the petri boites the molle LB medium previously sown with the defferent 4 bacterias *"B.subtilis ATCC 6633 PCM 482 , S.aureus INC3 , S.epidermidis CCM 801, K.pneumonae 348 LH "* from the inoculume to the micropipett assistance is added To the 10ml molle LB medium.

.The incubation is done at 30C° 24h .The Results in presence or absence of growth of the test strain around the Colony of actinomycetes.

4.2. Study of Antifungal Activity

✓Preparation of yeast cultivation and filamentous fungi test in the solide midium: For the antifungal test of isolation Actinomycetes about the 5 Yeasts *" C.glabrata ICF20, C.parapsilosis, S.cerivisiae ICF43 , R.mucilaginosa LRM , k.marxianus ICF44"*. and 06 filamentous fungi *" Foxysporum F.Foxy, L.corylinifica.St87, F.solani Ffso , R.oryzea FRHory , A.flavus ATCC XXXXX , Ac .Fleww Atcc "*

These target germs are seeded by the method of epuisement strits on the Sabouraud at the aide of platinum anse then incubate them at 25°C For yeast and 30°C for filamentous fungi.

✓Preparation of suspension of target germs :

•After the incubation of filamentous fungi on the solide médium Filamentous fungi colonies, they are put into 500µl from the water Sterile physiology,then put into ISP2 molle medium.

•After the incubation of yeast, they are put in ISP2 liquid medium then incubated at 30°C for24h .

4.2.1.Double layer method

✓The isolation of actinomycetes Test are seeded at the center of the petri box previously seeded by the midium ISP2 then incubate at 30°C for 7 days.

✓Deposited at the surface of the petrie boites the molle ISP2 medium previously sown with the defferent 4 yeast *"C.parapsilosis, S.cerivisiae ICF43 , R.mucilaginosa LRM , k.marxianus ICF44"*

and 4 filamentous fungi *" Foxysporum F.Foxy , L.corylinifica.St87, R.oryzea FRHory , A.flavus ATCC XXXXX "*from the inoculum to the micropipett assistance is added to the 10 ml molle ISP2 medium.The incubation is done at 30C° 24h.The results in presence or absence of growth of the test strain around the colony of Actinomycetes.

4.2.2.Conventional agar cylinders method (Tortorant et al, 1979)

✓Sowing the 05 strains of Actinomycetes to test on the (ISP2 , GYEA) in tight streaks. In the 7 days of incubation,White the aide of sterile blue cones, the cylinders of agar bearing colonies of

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and defferent 06 filamenteus fungi *" F.oxysporum F.Foxy, L.corylinifica.St87, F.solani Ffso , R.oryzea FRHory , A.flavus ATCC XXXXX , Ac .Fleww Atcc "*From the inoculume to the micropipett assistance is added to the 10ml molle ISP2 medium" .The sown boxes are kept at the Refrigerator for 30 min prior to being incubated, to allow the diffusion of the Active substances while preventing the growth of bacteria and then incubated at 30°C for 24h (**Kitouni, 2007).**This test was repeated for crops of 10j of incubation. The diameters of areasd of inhibition apparues are measured around agar cilyndres.

5.Physiological and biochemical study of Actinomycetes strains

5.1. Study of strain growth of actinomycets in different Temperature

The 07 strain of actinomycets are encemented in the midium ISP2 whithe an sterile platinum ance by the method of sires streaks. Then are incubated in a different temperature:in 4°C, in 25°C, and in 45°C for 7 days in order to know the temperature suitable for optimal growth for each strain of Actinomycetes.

5.2. Study of strain growth of actinomycets in different concentration of NaCl

The 07 strain of actinomycetes are seeded whithe an sterile platinum ance by the method of sires streaks in the midium ISP2 whithe different concentration of NACL: in ISP2 2,5% NaCl (ISP2+2,5 grams of NaCl), in ISP2 5% (ISP2+5 grams of NaCl) in ISP210% NaCl (ISP2+10grams of NaCl).Then are incubated in30°C for 7days In order to know which concentration of NACL are most suitable growth for each strain of Actinomycetes.

5.3.Study of strain growth of Actinomycetes in different pH

The 07 strain of actinomycets are seeded whithe an sterile platinum ance in the midium ISP2 whithe different PH : in ISP2 pH=3/ in ISP2 PH=5/ in ISP2 PH=11.Then are incubated in 30°C for 7 days , In order to know the ph suitable for optimal growth for each strain of Actinomycetes.

V. Biochemical stady of actinomycets strains:

•Api20E system

✓Taking a single isolated settlement (from a pure culture) from each strain of the 7 Actinomycetes and making a bacterial suspension in sterile distilled water.

✓Reunite background and cover of an incubation box and spread water into the alveoli to create a wet atmosphere. Then sternly drop the gallery into the incubation box.

✓Taking a pastor pipette and fill these compartments with the bacterials suspensions .

✓Theses galeries are incubated at a temperature 30°C for 7 days .

Results and discussion

1. Purification and preservation of Actinomyces strains

Pure colonies of the isolates are obtained after successive subculturing on ISP_2 medium by the exhaustion method . The way each strain grows is different from the other. Strains Tn8', ATSP 79, and ATSP5 are characterized by rapid growth, unlike strains ATP49, ATSP 25, ATSP 28, and ATSP8.

Strains 07 of Actinomycetes characterized by Aerial mycelium and Substrate mycelium and Isolated strains ATSP28, ATSP5, ATSP25 and ATSP8 produce pigments Spread in ISP2 culture medium. At ATSP5, the pigment is black and spreads over the culture medium ISP2, at ATSP28 it is yellow, at ATSP25 it is beige and at ATSP8 it is green. After purifying the 7 strains of Actinomycetes using culture medium for ISP2, they are stored in tubes inoculated with ISP2 after incubation for between 07 and 21 days, as shown in the picture.

Figure 04. Conservation of Actinomyces strains in the tubes

2.Morphological study of Actinomycetes isolates

2.1.Macroscopic Characterization

The isolated actinomycetes were observed for aerial mycelium, submerged mycelium, color, and diffusible pigments . The table (table 3) shows the most important phenotypic characteristics obtained after cultivation on ISP2 medium and incubation for a period of more than 7 days.

Actinomycetes 07 strains are characterized by the presence of Substrate mycelium and Aerial mycelium, each of which has a distinct color (as shown in the table 3 and figure 4), where the Aerial mycelium at S.Tn8', S.ATSP5, S.ATP49, and S.ATSP25 are white, at S.ATSP 28 mixed between yellow and white, and at S.ATSP8 and S.ATSP79 In grey. In two strains, S.ATSP79 and S.ATSP8, the substrate mycelium is green, S.ATSP5, S.ATSP28 is black, S.Tn8' is yellow, and S.ATSP25 and S.ATSP49 are beige.

Table 3. The morphological characters of the 20 isolates on ISP2 medium

Strain actinomycetes	Fashion growth	Aerial mycelium	Substrate mycelium	pigmentation
S.Tn8'	+++	whait	Yellew	-
S.ATSP79	+++	grey	Green	-
S.ATSP25	+	whait	Beige	+
S.ATSP5	+++	whait	Black	+
S.ATSP28	++	Yellow and whait	Black	+
S.ATP49	++	whait	beige	-
S.ATSP8	+	grey	green	+

+++ : Abundant growth

++ : Average growth

+ : Slow growth

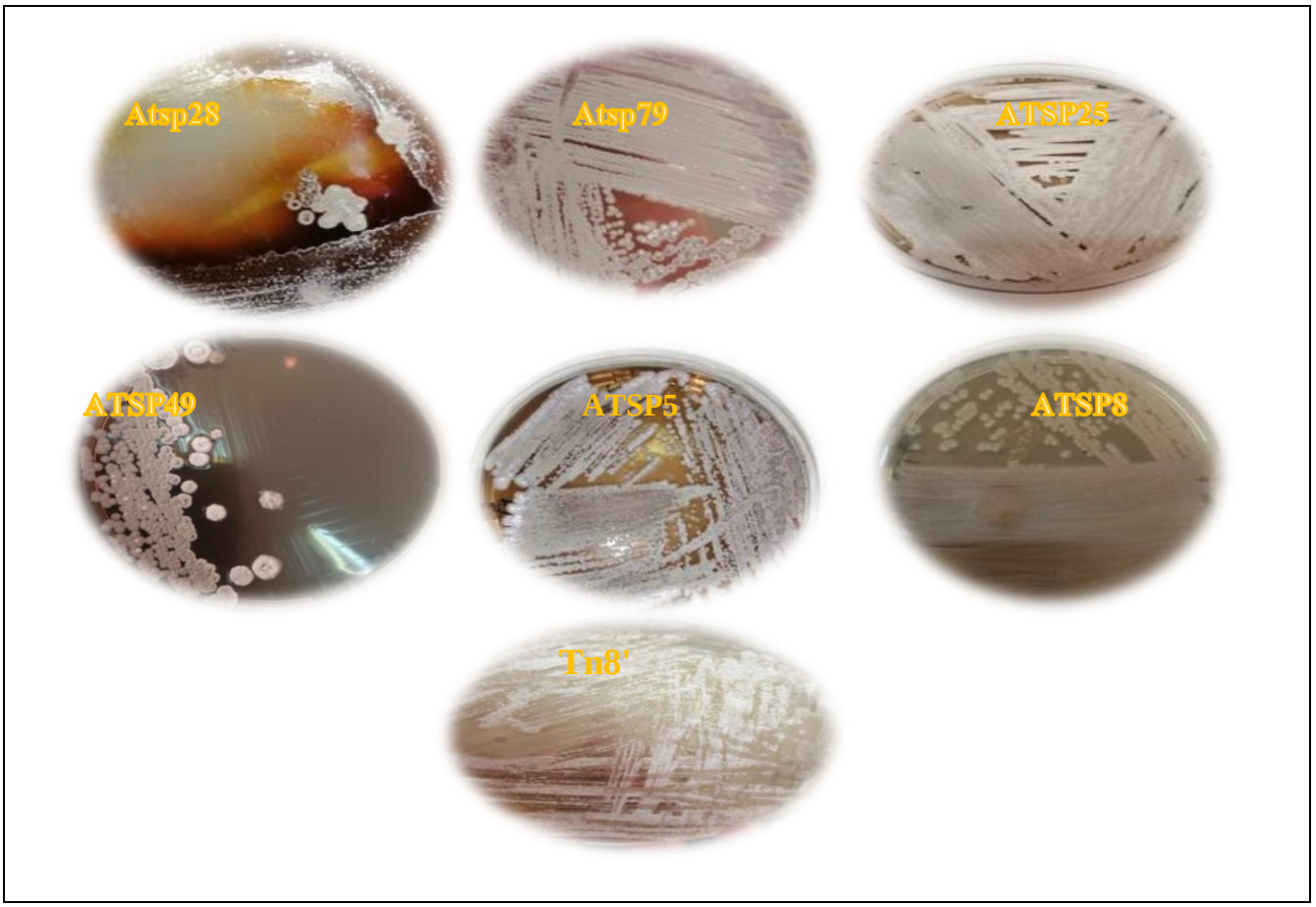


Figure 05. Pictures represent phenotypic characteristics of 07 Actinomyces strains on ISP2 medium

2.2.Microscopic Observation

According to the results obtained after direct observation and Gram staining, we summarize that actinomycetes are Gram-positive, aerobic mycelial bacteria with the presence of spores. They present certain analogies with mushrooms: mycelial structure presenting ramifications

The microscopic study shows that all the isolates present a filamentous appearance with the presence of isolated spores or in clusters which are sometimes short or long chains, which confirms their belonging to the major group of Actinomycetes.

This study makes it possible to determine the characteristics and chains of spores (number, shape and morphology) by direct examination at X100 magnification . Results shown in **(table 4)** and (**figure 5)**

Table 04. Result of the micromorphological characteristics of insulators

Actinomycetes strains	Microscopic observation after Gram stain
S.ATSP25	Long fine filament, linear and grouped with isolated spores
S.ATP49	Long fine filament, linear and grouped with isolated spores
S.ATSP79	Long fragment of linear filament, with isolated spores
S.ATSP5	Long fine filament, linear and grouped with isolated spores
S.Tn8'	Short filament, with spores grouped into cluster
S.ATSP28	Absence of filament, with spores grouped in clusters, and with a long chain
S.ATSP8	Short filament, with spores grouped into cluster

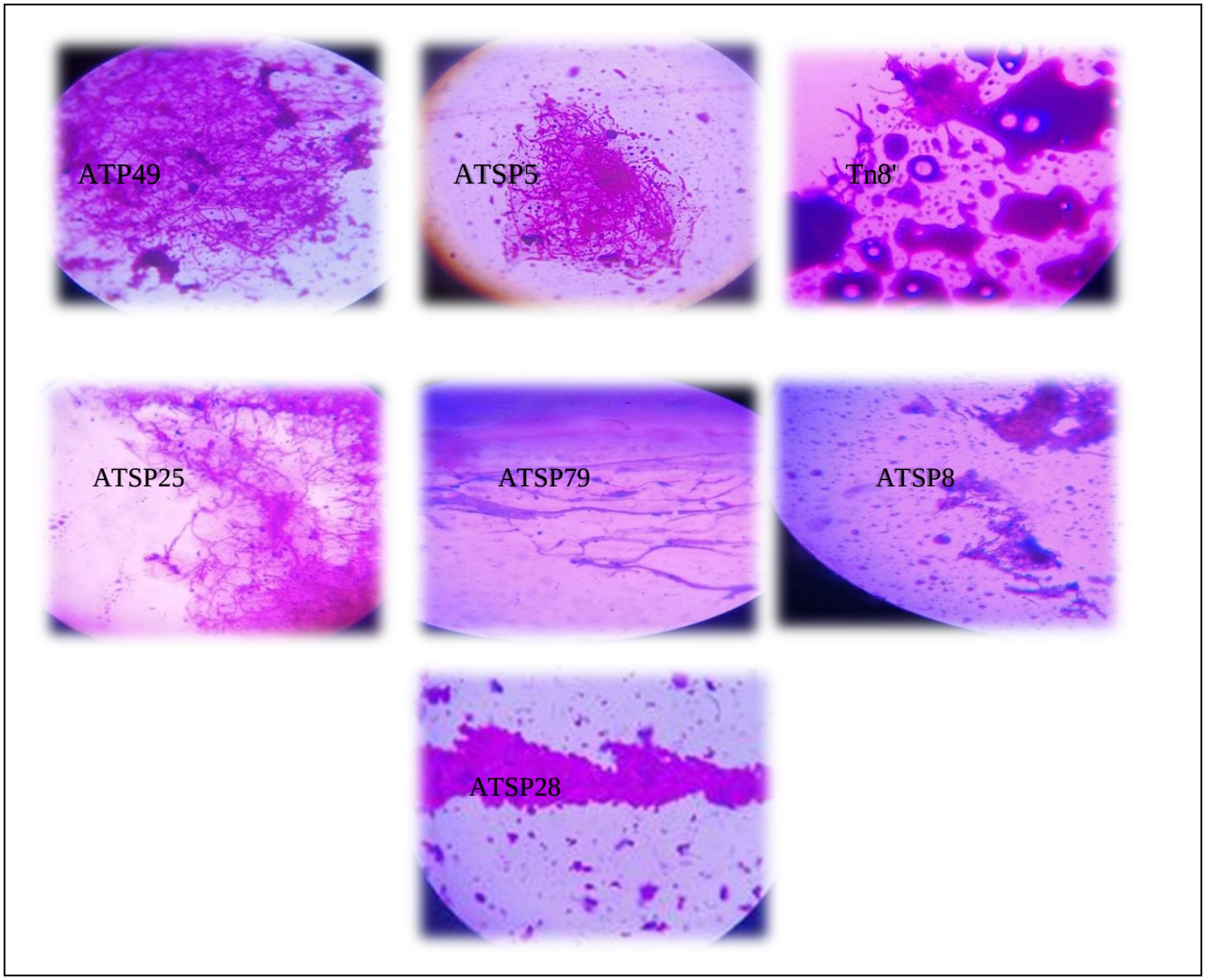


Figure 06: Photos of the results of the Microscopic observations after Gram stainin

3.Investigation of the antimicrobial activity of Actinomycetes

3.1.Study of antibacterial activity by double layer

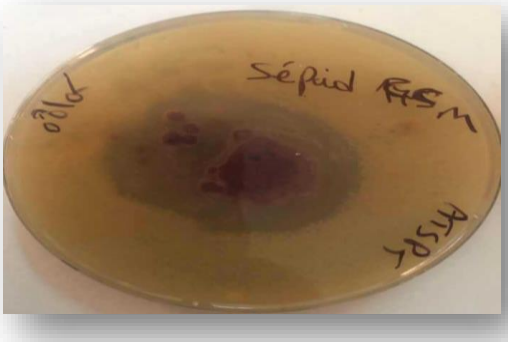
• In order to confirm the antimicrobial activity of our isolates, we used a more valuable technical that of the double layer, which gives more precision on the antimicrobial activity of Actinomycetes isolates.

• The antibacterial activity of the test isolates was varied. Actinomycetes isolates were shown to have very potent in vitro antibacterial activity against both phytopathogenic and other G (+) and G(-) bacteria.The results of the antibacterial activity of active isolates are given in Table 6. We found S.ATP49 against the Gram negatif indicator strain *348 LH* of genus *Klebsilla pneumonae* (74.5mm).

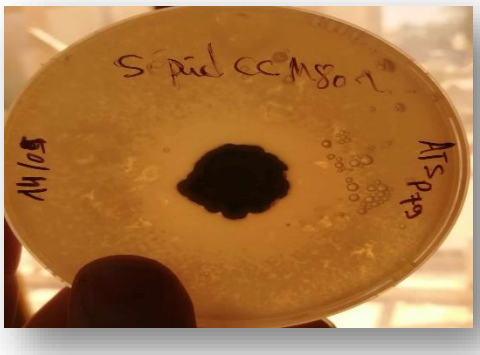
S. ATSP8 against the Gram positif indicator strain *CCM 801* of genus *Staphyloccus epidermidis* (68.7mm).

Table 5. Results of antibacterial activity tests by double layer (Zones of inhibition mm)

Bacterial strains	**S.ATSP5**	**S.ATSP25**	**S.ATSP79**	**S.ATP49**	**S.Tn8'**	**S.ATSP8**	**S.ATSP28**
PCM 482	55.1 58.9 55.2	-	-	-	53.1 51.7 56.0	-	56.1 59.7 58.4
INC3	-	-	-	-	-	-	-
CCM 801	42.5 45.5 57.5	56.1 68.6 61.3	43.0 44.1 39.1	66.4 55.8 67.5	-	62.3 68.7 61.5	-
348 LH	-	-	-	69.7 74.5 66.1	-	-	44.7 44.6 38.2



Positive S.ATSP5 against *sp.epidermidis*



Positive S.ATSP79 against *sp.epidermidis*

Figure 07 : Results of studying the antibacterial activity of the double layer

3.2. Study of antibacterial activity by agar cylinders

- The Anti-Bacterial Spectacular Activity of the strains of Actinomycetes Withheld, has been Researched by the method of cylindres agar vis 3strains of Gram+ bacteria *(ATCC6633 PCM 482* of genus *Bacillus subtilis, INC3* of genus *Staphylococcus aureus,CCM 801* of genus *Staphyloccus epidermidis)* and one strain of Gram -bacteria *(348 LH* of genus *Klebsilla pneumonae*)
- The results of this study show that the activity is different according to the results Bacteries Actinomycetales (different result of a strain to another) and the test bacterium has a one effect (bacteria Gram positive or negative), and the Result is ditferent from one day to another (7or 10 day), and the mediums utilised (ISP2 ,GYEA) are influence on results of the anti-bacterial activity test
- From the 7day 5strains of Actinomycetes that means75% haves an antibacterial activity versus all the 4 bacteria in the midium ISP2,and the abcence of antibacterial activity versus all the 4 bacteria in the midium GYEA
- Absence of antibacterial activity in (10day) from all the strains of bacteria (Gram+, Gram-).(The abcence of this activity can be explained by the development of a resistance against bioactive molecules (Antibacterials substances).
- The persontage of the antibacterial activity are increase from the cylinder agar of the medium ISP2 ,to reverse the activity antibacterial in the midium GYEA that is descrease .This result confirms that the best cultivation environments of Actinomycetes To find a good result are ISP2,our results are confirme to the resultFound by the SP2 medium is better than ather midiums for the Production of antibiotics. **(Boubetra-Biskeri, 2013).**
- Results of the anti-bacterial activity test explain the presence of bioactive molecules.

- The strain S.ATSP5 have a spectrum activity versus all strains of the Bacteria be tested ,in the day7,from the ISP2 medium.
- The S.ATSP25 have a spectrum activity versus the *bacteria:ATCC6633 PCM 482 of genus Bacillus subtilis, INC3 of genus Staphylococcus aureus,CCM 801 of genus Staphyloccus epidermidis* .have an antibacterial activity versus the Gram positise bacteria.
- The strain S.ATSP79 have an antibacterial activity versus the two bacteria :ATCC6633 PCM 482 of genus Bacillus subtilis, INC3 of genus Staphylococcus aureus (Gram positive bacteria)
- The strain S.ATP49 have an antibacterial activity very important versus the bacteria *CCM801 of* genus staphylococcus epidermidis (Gram positive bacteria),whith inhibition zone=20,6mm.
- Results confirm that Gram positive bacteria are senseble to the antibiotics Produced by Actinomycetes more than Gram- bacterial strains, results agree With de **(Boubetra-Biskri, 2013)** that find that the activity of molecules produced by the Strain Saccharothrix is mostly directed against Gram positiv ,and sometimes against the Fungi and rarely against Gram negative , this result is explained by composition Gram- membrane chemical by peptidoglycan and lack of layer Lipopolysaccharide (LPS) which is present in Gram negative bacteria this layer makes the Cell membrane waterproof to lipophilic substances **(Kim et al ,1994),** our results Are similarsces found by other authors reflecting on the sensitivity of bacteria to Gram Positive for secretions of actinobacteria compared to gram negative bacteria **(Sabaou et Al ,1998; Prescott et al , 2002)**

-The results confirm that the best day to find agood results from the test of antibacterial activity is the 7day

Table 6 .7. Results of antibacterial activity tests by agar cylinders (Zones of inhibition mm)

Table 6.

07 days	**ISP2**					**GYEA**				
Bacterial strains	**S.ATSP5**	**S.ATSP25**	**S.ATSP79**	**S.ATP49**	**S.Tn8'**	**S.ATSP5**	**S.ATSP25**	**S.ATSP79**	**S.ATP49**	**S.Tn8'**
PCM 482	11.1 11.8 12.6	13.1 12.4 13.0	14.0 10.5 11	-	-	-	-	-	-	-
INC3	14.0 12.2 11	13.4 11.0 12.0	13.7 12 15.2	-	-	-	-	-	-	-
epidermidis CCM 801	13.0 14.2 11.1	12.2 12.8 15.0	-	21 26.0 23.0	-	-	-	-	-	-
pneumonae 348 LH	17.0 14.2 19.0	-	-	-	-	-	-	-	-	

Table7.

10 days	ISP2					GYEA				
Bacterial strains	**S.ATSP5**	**S.ATSP25**	**S.ATSP79**	**S.ATP49**	**S.Tn8'**	**S.ATSP5**	**S.ATSP25**	**S.ATSP79**	**S.ATP49**	**S.Tn8'**
PCM 482	-	-	-	-	-	-	-	-	-	-
INC3	-	-	-	-	-	-	-	-	-	-
epidermidis CCM 801	-	-	-	-	-	-	-	-	-	-
pneumonae 348 LH	-	-	-	-	-	-	-	-	-	-

3.3. Study of antifungal activity by double layer

• We note that the results of this screening also show that the antimicrobial activity of the Actinomycetes strains tested depends on the method used **(Lemriss, 2003).** The inhibition zones obtained by the double layer technique are more important compared to that obtained by the agar cylinder technical ; it can reach (77.5mm) for the S.ATSP2 srain. Furthermore, the double layer technique is better suited to the study of antifungal activities **(Gandhimathi et al, 2008)** allows the diffusion of microorganisms seeded en masse in soft agar **(Bastide et al, 1989).**

- In the table 08 the absence of anti-yeast activity at S.ATSP8 and S. Tn8'. As well as the absence of Anti-Filamenteus Fungi activity at S.ATP49 and S.Tn8'.

- Figure 8 shows the inhibition zones (mm) resulting from S. ATP49 against *ICF44* of g*enus Kluveromyces marxianus* .

Table 8.9 . Results of antifungal activity tests by double layer. (Zones of inhibition mm).

Table 8.

Strains of filamentous fungi	S.ATSP5	S.ATSP25	S.ATSP79	S.ATP49	S.Tn8'	S.ATSP8	S.ATSP28
oxysporum F.Foxy	64.6 56.3 41.2	-	-	-	-	-	-
corylinifica. St87	-	-	-	-	-	-	73.5 73.7 69.3
flavus ATCC	-	-	-	-	-	67.6 66.2 67.3	-
oryzea FRHory	68.6 44.2 55.4	-	35.2 21.1 31.7	-	-	67.9 61.0 69.3	-

Table9.

Yeast strains	S.ATSP5	S.TSP25	S.ATSP79	S.ATP49	S.Tn8'	S.ATSP8	S.ATSP28
parapsilosis	-	25.1 26.6 36.2	-	-	-	-	77.5 71.6 71.5
ICF43	59.7 56.6 66.1	-	-	41.7 31.5 35.5	-	-	-
.mucilaginosa .LRM	63.4 66.6 55.6	33.6 55.7 44.1	-	44.8 57.8 44.3	-	-	-
ICF44	45.4 39.2 48.7	66.5 57.3 51.1	27.1 28.0 31.6	64.8 68.4 64.5	-	-	41.6 36.5 37.4

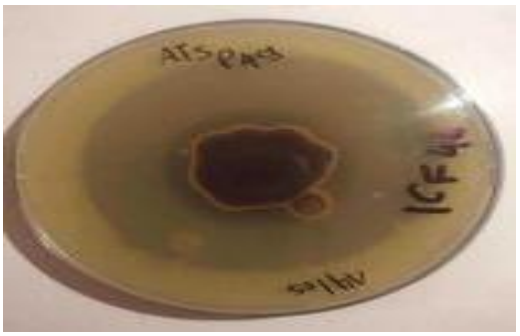


Figure 08: Antifungal activity results of ATP49 (inhibition zones)

3.4.Study of antifungal activity by agar cylinder

✓ Yeasts strains in 7 day:

About the ISP2 medium: The actinomycet strain S.ATP49 have an important activity in the day7 against 3 yeasts: ICF43 of genus *Saccharomyces cerivisiae,* ICF44 of genus *Kluveromyces marxianus,ICF20* of genus *Candida glabrata.*

-The Actinomycetes strains S.ATSP79, S.ATP49 and S.Tn8 ' having an activity versus the yeast: INC43 of genus Saccharomyces cerivisiae, whith defferent inhibition zones.

- About the GYEA medium ,the Actinomycetes strains:S.ATSP5,S.ATSP25 and S.ATSP79 having an activity antifungal against just of one yeast:*Candida* of genus *Candida parapsilosis,* whithe different inhibition zones.

- ✓ Yeasts strains in 10day:

-We observed the absence of the antifungal activity against of all the yeasts strains in the10day, about the ISP2 medium and the GYEA . This results are confirme that the best day to find a good prodection of antifungal substats is the 7day.

- ✓ Filamentous fungi in 7day:

-About the ISP2 medium:the actinomycet strain S.ATSP5have an important percentage of antifungal activity against the majority of filamentous fungi strains 90% *(F.Foxy of genus Fusarium.oxysporum,St87 of genus Lichthermia FRHory* of genus *Rhizopus.oryzea , ATCC XXXXX* of *genus Aspergillus flavus, Fleww Atcc* of genus *Aspergillus).*

-The strain S.ATSP25 have a very important antifungal activity against of 80% of filamentous fungi strains.

-The strain S.ATP49 have a good prodection of antifungal substrats against the filamentous fungi FRHory of genus *Rhizopus oryzea* ,whith inhibition zone=20,6cm.

-About the GYEA medium: we observed that the 3 Actinomycetes ; S.ATSP5, S.ATSP25, S.ATSP79 having antifungul activity against just of two filamenteus fungi strains *(ATCC XXXXX of* genus *Aspergillus flavus,St87* of genus *Lichthermia corylinifica).*

- ✓ Filamentous fugi in 10day:

-About the ISP2 medium: The strain S.Tn8' have a very important prodection of bioactive moleculs against of two filamenteus fungi: *St87* of genus *Lichthermia corylinifica* whithe inhibition zone=30,49mm,FRHory of genus *Rhizopus.Oryzea,* whithe inhibition zone=30mm.

-About the GYEA medium:we observed that the strain S.Tn8' having excellent antifungal activity against 03 filamentous fungi: St87 of genus *Lichthermia corylinifica.* whithe inhibition zone=20,50mm ,FRHory of genus *Rhizopus.oryzea* whithe inhibition zone=20,57mm.

Tables.10.11.12.13. Results of antifungal activity tests by agar cylinder (07and 10days)(Zones of inhibition mm).

Table 10.

07 Days	ISP2					GYEA				
Yeast strains	**S.ATSP5**	**S.ATSP25**	**S.ATSP79**	**S.ATP49**	**S.Tn8'**	**S.ATSP5**	**S.ATSP25**	**S.ATSP79**	**S.ATP49**	**S.Tn8'**
ICF20	-	-	-	14.9 13.7 15.5	-	-	-	-	-	-
parapsilosis	-	-	-	-	-	10.3 10 10.2	16.0 14.8 13.0	10 12.2 12.9	-	-
ICF43	-	-	12 11.1 12.0	12.4 12.0 11.2	13.0 12.8 12.0	-	-	-	-	
mucilaginosa LRM	-	-	-	-	-	-	-	-	-	-
ICF44	11.7 11.0 12.5	11.1 14.0 12.9	-	12.0 11.1 13.2	-	-	-	-	-	-

Table11.

07 Days	ISP2					GYEA				
Strains of filamentous fungi	**S.ATSP5**	**S.ATSP25**	**S.ATSP79**	**S.ATP49**	**S.Tn8'**	**S.ATSP5**	**S.ATSP25**	**S.ATSP79**	**S.ATP49**	**S.Tn8'**
oxysporum F.Foxy	10.8 10.2 10.7	18 18.2 17.9	-	-	-	-	-	-	-	-
corylinifica. St87	10.3 10 10.5	12 11.5 11	-	-	-	10 10.4 10.2	13.8 12.0 11.8	8 10.9 11.0	-	-
solani FfSO	-	-	-	15.5 16.0 14.4	-	-	-	-	-	-
oryzea FRHory	10.3 11.0 11.8	17.0 14.5 12.0	-	20 26 24.5	-	-	-	-	-	-
flavus ATCC	10.9 11.0 10.7	-	-	10 10.9 10.3	-	-	-	-	-	-
Fleww Atcc	11.2 11.0 11.8	11.0 10.8 11.4	-	-	-	10.2 12.5 10.5	13.6 13.0 13.4	14.2 13.9 13.0	-	-

Tabla12.

10 Days	ISP2					GYEA				
Yeast strains	S.ATSP5	S.ATSP25	S.ATSP79	S.ATP49	S.Tn8'	S.ATSP5	S.ATSP25	S.ATSP79	S.ATP49	S.Tn8'
ICF20	-	-	-	-	-	-	-	-	-	-
C.parapsilosis	-	-	-	-	-	-	-	-	-	-
ICF43	-	-	-	-	-	-	-	-	-	-
R.mucilaginosa	-	-	-	-	-	-	-	-	-	-
ICF44	-	-	-	-	-	-	-	-	-	-

Table 13.

10 Days	ISP2					GYEA				
Strainsof filamentous fungi	S.ATSP5	S.ATSP25	S.ATSP79	S.ATP49	S.Tn8'	S.ATSP5	S.ATSP25	S.ATSP79	S.ATP49	S.Tn8'
oxysporum F.Foxy	-	-	-	-	-	-	-	-	-	25.0 20.3 20
corylinifica. St87	11.7 11.1 13.6	12.5 15.6 11.4	10 10.5 10.3	15.2 13.4 15.5	24.8 34.9 32.7	10.5 10.8 10.3	13.7 11.9 11.1	-	10 10.9 11.2	24.8 25.2 23.0
solani FfSO	-	-	-	-	-	-	-	-	-	-
oryzea FRHory	13.0 15.9 12.5	14.2 13.6 13.3	10.7 11.2 10	15.8 12.2 14.9	32.2 31.4 30	-	11.8 11.5 10.9	-	-	25.7 25.2 25.0
flavus ATCC	-	-	-	-	-	-	-	-	-	-
Fleww Atcc	-	-	-	-	-	-	-	-	-	-

4. Physiological tests for actinomycetes

Results of a study of physiological characteristics in different media with tolerance to pH, temperature, and salinity (NaCl) in table 5

✓ **Grouth in different Ph midium**

- We observed that the majority strains of Actinomycetes are tolerant in PH=11.This results confirm that our strains of Actinomycetes are living in Alcalophilic environments.
- The strain S.ATSP28 are tolerant in PH=5 ,its capable to grouth in acidophilic environments.

✓ Grouth in different Nacl midium

- We observed that all the strains of Actinomycetes are tolerant in the concentration of Nacl=2,5%
- All the strains are tolerant in concentration of Nacl=5% and Nacl=10% just S.ATSP79 and S.ATP49.
- This results confirm that the majority of our actinomycetes strains are Halophilic.

✓ **Grouth in different temperatures medium**

- All the actinomycets strains are tolerant in temperature =25°C.This results confirm that the strains of Actinomycets are mesophilic.
- The strain S.ATSP28 and S.Tn8' are tolerant in temperature=45°C, the two strains capable to living in hight temperature.

Tables 14. results of characteristics Physiological tests for Actinomycetes.

Actinomycetes	S.Tn8'	S.ATSP79	S.ATP49	S.ATSP25	S.ATSP8	S.ATSP28	S.ATSP5
pH=3	-	-	-	-	+	+	-
pH=5	-	-	-	-	-	+	-
pH=11	-	+	+	+	-	-	+
NaCl=2.5%	+	+	+	+	+	+	+
NaCl=5%	+	-	-	+	+	+	+
NaCl=10%	+	-	-	+	+	+	+
T = 5°C	-	-	-	-	-	-	-
T = 25°C	+	+	+	+	+	+	+
T = 45°C	+	-	-	-	-	+	-

5. The Api 20E system

Enzymatic biodiversity tests and the assimilation of certain sugars are confirmed by the Api 20E system. The results of the biochemical gallery are reported in table 10 and figure 8

- These results show that all Actinomyces 07 strains are able to absorb and use ONPG, arginine, ornithine and citrate. They are unable to use na thiosulfate , tryptophan , na pyruvate , mannitol and inositol , sorbitol and sucrose (as shown in the table).
- Lysine is used by all strains other thanS.Tn8'.
- The production of other enzymes by actinomycetes varies, some of which are used and others not.

From this, we find that actinomycetes also produce specialized enzymes, not just antimicrobials .

Table 15. Results of API 20E for the enzymes produced by the 07 presenting strains

Actinomycetes	S.Tn8'	S.ATSP79	S.ATSP25	S.ATP49	S.ATSP28	S.ATSP8	S.ATSP5
ONPG	+	+	+	+	+	+	+
+ADH	+	+	+	+	+	+	+
LDC	-	+	+	+	+	+	+
ODC	+	+	+	+	+	+	+
CIT	+	+	+	+	+	+	+
H2S	-	-	-	-	-	-	-
URE	-	+	+	+	-	+	+
TDA	-	-	-	-	+	+	-
IND	-	-	-	-	-	-	-
VP	-	-	-	-	-	-	-
GEL	-	+	+	+	-	+	+
GLU	+	-	-	-	+	-	-
MAN	-	-	-	-	-	-	-
INO	-	-	-	-	-	-	-
SOR	-	-	-	-	-	-	-
RHA	-	-	-	-	+	+	-
SAC	-	-	-	-	-	-	-
MEL	-	+	+	+	-	+	+
AMY	+	-	-	+	+	-	-
ARA	-	+	-	+	+	-	+

Figure 09. Photos of API 20E system results of representative strains

Conclusion

Conclusion

Actinomycetes are characterized by their ability to produce antibacterial and antifungal in large quantities, as well as their ability to adapt to harsh environments.

The objectif of this study is to reserche of bioactive molecules prodected by 7 Actinomycetes Xyrophilic against of strains of bacteria, yeasts and filamentous fungi. In ours study ,two parts have been developed.

- In the first part, we did merging the activity Antibacterials and the Anti Fungal Activity.
- The second part is about the physiological and biochemical stady of xerophylic Actinomycetes.

-The results show that most strains of actinomycets are produced antibacterial and Antifungal activity against at least one of the indicater strain. The bigger inhibition zone was Observed by the Strain *S.ATP49* against *INC3* of genus *Staphylococcus aureus* (60,75mm), And against *348 LH* of genus *Klebsilla pneumonae* (70,45mm). About the antifungal activity the bugger inhibition zone was observed the Strain *S. ATSP28* against *Candida* of genus *Candida parapsilosis*(70 mm) anout yeasts,and against *St87* of genus *Lichthermia corylinifica* 70,37mm) about filamenteus fungi.

- These results help to confirm that the two strains of Actinomycete *S.ATSP28* and *S.ATP49 a*re the best producers of bioactive molecules. In this work, it can be Said that our Actinomycete strains have importantes production of bioactive metabolites (Antibacterial or antifungal).

- Based on the agar cylinder technique, we determined that ISP2 culture medium is better than GYEA.

- The production of biomolecules on day 7 is more abundant than on day 10.

- Seven strains of Actinomycetes grow at 25°C and are all halophilic

- Actinomyces strains are distinguished by their ability to use various enzymes, which allows them to live in harsh conditions.

Perspectives

✓The study of antimicrobien activity on a wide range of tests bacteria, yeasts and fungi.

✓Extracting biomolecules with multiple solvents.

✓Sudy of antimicrobial activity against Pathogens strain.

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Annex

1. Composition of mediums utilised

ISP2

Extracted from Malt……10 g

Yeast extract……..……4 g

Glucose……4 g

Agar ……..…..15 g

Destilled water ……1000 ml

PH =7

GYEA

Yeast Extract……..…..10 g

Glucose……...10 g

Agar…….... 18 g

Distilled water…….....1000 ml

ISP2 molle

Glucose……4 g

Yeast extract……..……..…….. 4 g

Extract from malt……..…... 10 g

Agar……...10 g

Distilled water……..…1000 ml

ISP2 liquid

Glucose……..……..……4 g

Yeast extract……4 g

Extract from malt……..……10 g

Distilled water……..……1000 ml

Solid LB

Treptone……10 g

Yeast extract……..……..…..5 g

NaCl……..……10g

Agar …… 20 g

Distilled water…… 1000 ml

Moll LB

Treptone……..……4 g

Yeast extract……..…… …2 g

Na Cl……..……2 g

Distilled water……..……800 ml

PH=7,2

Liquid LB

Treptone……..……10 g

Yeast extract……5 g

Na Cl ……..……10 g

Distilled water……1000 ml

PH=7,2

Nutritif eglais

Peptone ……..……... 5 g

Extracts from malt ……..……..……1 g

Yeast Extracts…….....2 g

Sodium Chloride……..……..5 g

Agar …….....15 g

Distilled water……..……1000 ml

pH = 7.4

Sabouraud

Peptone ……..……..……10 g

Glucose ……..……..…… 20 g

Agar-agar ……..……..... 15 g

Destilled water……..…… 1000 ml

pH = 6,0

Mac conckey:

Peptone .....20 g

Lactose ……..10 g

Bile salts ……1,5 g

Purple Crystal…….... 0.001 g

Neutral Red ……..0.05 g

Sodium Chloride…….... 5 g

Agar ……..…15 g

Destilled water……1000 ml

pH = 7.1

Nutritif bouillon

Nutritif bouillon……..21g

Distilled H2O …… ……1000ml

pH: 7.2 to 7.2

2. Solutions

Physiological water

Na Cl……..9g

Distilled water……1000 ml