People's democratic republic of Algeria
Ministry of higher education and scientific research
Ziane Achour University–Djelfa
Department of Biological Sciences
Field of Natural and Life Sciences

Thesis presented for obtaining the Master's degree

Specialty : Applied Microbiology

Study of exopolysaccharids production and antimicrobial activity in *Actinobacteria* strains isolated from saline soils

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2022/2023

Acknowledgments.

First of all, we thank "**Allah**" the Almighty, the Merciful, for giving us the courage, strength, health, and persistence to complete this work.

We would like to express our gratitude to our supervisor Mr. Mostefaoui Abdellah, whose kind encouragement and advice enabled us to successfully carry out this work.

Our heartfelt gratitude to the jury members, starting with Mr. Boutaiba Saad, who presided over this committee and stands as a cornerstone of the University of Djelfa. His unwavering commitment to excellence has shaped our academic journey. We also extend our thanks to Ms. Rachedi Fatma Zohra, our examiner and invaluable guide from the University of Djelfa. Her insightful evaluation and guidance have greatly enriched the quality of our work.

We extend our sincere thanks to the amazing lab staff for their invaluable support and kindness throughout this journey. Their guidance and assistance have played a vital role in the success of this project, and we are truly thankful for their contributions.

Dedications

I dedicate this humble work, the result of years of study, to my beloved **Dad.** Your unwavering support, endless encouragement, and countless sacrifices have been the foundation of my success. Your love, guidance, and wisdom have shaped me into the person I am today, and for that, I am forever grateful.

May Allah bless you with happiness, health, and long life, and protect you from all harm.

To my dearest **Mom**,
No matter how expressive words may be, they cannot convey the extent of love and affection I feel for you. You have showered me with tenderness and affection throughout my journey. You have never ceased to support and encourage me during all the years of my studies. May Allah protect you and grant you health, happiness, and a long life.

To my dearest sisters **Thiziri** and **Hadil**, You have been my confidants, supporters, and friendsthroughout my life. Your unwavering love, guidance and encouragement have been a constant source of strength for me. I cannot thank you enough for always being there for me,

Through thick and thin.
May Allah bless you with happiness, health, And a long life and protect you from all harm.

Ould Mohamed imane

To my dear brothers **Mouloud** and **Mohamed**, whose unwavering support and encouragement have been instrumental in my success. I am truly blessed to have you both in my life, and I cherish the moments we have shared together. I am forever grateful for all that you have done for me, and for the impact you have had on my life.

May Allah bless you both with Happiness, health, and long life, and protect you from all harm.

I'm deeply grateful to all my friends Your unwavering support, understanding, and laughter have added immeasurable joy to every step of this journey.

To **Assia**, a friend, and a colleague with heartfelt thanks for allowing me to join you on this journey.

Dedications

After the name of God, the Most Merciful

Hello to the reader of this page, a dedication page for this work, which we can say is a first step towards an interesting journey waiting for us, it was an enjoyable experience, with a companion worth praise, because with all honesty, love and gratitude she is the owner of the search and the insistence **'Imane'** I present this work as a gift for myself **'Assia'**, as it was my first experience with my partner in that adventure, my partner ”eyes of the sea"

In dedication, my emotion defeats me like every time in expression

To the friend of the soul, my **mother**, my first supporter and my guideline in life after God Almighty to the shield my **father** “My eldest Son”

Serbah Assia

To my sweeties, my life'scandy **"Basma and Qamar "**My brothers "my legends” **Walid, ilyes, Omar ibn Al -Khattab, kais.**To my brother's wifeour new sister **“nor"**And to the first granddaughter, piece of sugar **" mallouka"** At last and not least, my besties the companions of the path **: Imane, Wiam, denia, chaima, Nour Al –Imène** …

And for those who live in the heart in word and act...

Wrote with love

ASSOUSS

List of abbreviations

EPS :	Exopolysaccharides.
MS :	Substrate Mycelium.
MA :	Aerial Mycelium.
OD :	Optical Density.
SCA :	Starch casein Agar.
SCDA :	Soybean-casein digest Agar.
ISP :	International Streptomyces Project Medium.
MH :	Mueller Hinton.
NT :	Total Bacteria Count.
NTA :	Expected *Actinobacteria* Count.
SA :	*Staphylococcus aureus*.
EC :	*Escherichia coli*.
SC :	*Saccharomyces cerevisiae*.

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Abstract

This study aimed to isolate and characterize *Actinobacteria* strains from saline soils, focusing on their exopolysaccharide (EPS) production and antimicrobial activity against four microorganism, including *Escherichia coli* and *Staphylococcus aureus*, as well as fungal (mold) strains such as *Penicillium.sp* and the yeast strain *Saccharomyces cerevisiae*. The research was conducted at the microbiology laboratory of Zian Achour University of Djelfa, using standard methods for *Actinobacteria* isolation, characterization, and antimicrobial testing. Soil samples were collected from a saline site 'sabkha' area in Mosran, Djelfa, and *Actinobacteria* were isolated using a dilution series and selective media. The isolated strains were purified and subjected to salt, temperature, and pH tolerance tests. Cultural and morphological characteristics were analyzed, and the antibacterial activity of the strains was determined.

One of the key objectives of this study was to investigate the production of extracellular polysaccharides (EPS) in the chosen isolated *Actinobacteria* strains H3, H8, and H13. EPS production is a crucial trait exhibited by many bacteria, including *Actinobacteria*, and it serves various biological functions. To assess EPS production, the *Actinobacteria* strains were cultivated in specific media (ISP2 liquid media) known to promote EPS production, and the amount of EPS produced was quantified. The yield of EPS production for all three samples (H3, H8, and H13) was approximately 0.202%.

In terms of antimicrobial activity, the isolated *Actinobacteria* strains were evaluated against four microorganism. Antimicrobial testing was performed using the perpendicular streak method and the agar well diffusion method. The results revealed different degrees of antimicrobial activity exhibited by the *Actinobacteria* strains. Some strains demonstrated antimicrobial activity against all four tested microorganisms, inhibiting the growth of pathogens, while other strains showed activity specific to certain microorganisms. The antimicrobial activity of the *Actinobacteria* strains was observed through the formation of inhibition zones around the perpendicular streaks and agar wells.

The findings of this study shed light on the potential of *Actinobacteria* from saline soils for biotechnological applications. By elucidating their antimicrobial activity against four microorganisms, the *Actinobacteria* show promise as a source of novel antimicrobial agents. Furthermore, the investigation of EPS production in the *Actinobacteria* strains contributes to our understanding of their biological activities and opens avenues for further research in the field of microbial biotechnology.

This research emphasizes the significance of studying *Actinobacteria* from saline soils, which represent a unique ecological niche with untapped microbial diversity. The implications of this study extend to various fields, including biotechnology, agriculture, and pharmaceutical sciences, where the search for novel bioactive compound and antimicrobial agents is of paramount importance.

Keywords : *Actinobacteria*, Saline soils, Exopolysaccharides, Antimicrobial activity.

Resumé

Cette étude avait pour objectif d'isoler et de caractériser des souches *d'Actinobactéries* à partir de sols salins, en se concentrant sur leur production d'exopolysaccharides (EPS) et leur activité antimicrobienne contre quatre microorganismes, telles qu'*Escherichia coli* et *Staphylococcus aureus*, ainsi que des souches fongiques (moisissures) telles que *Penicillium.sp* et la souche de levure *Saccharomyces cerevisiae*. La recherche a été menée au laboratoire de microbiologie de l'Université Zian Achour de Djelfa, en utilisant des méthodes standard pour l'isolement, la caractérisation des *Actinobactéries* et les tests antimicrobiens. Des échantillons de sol ont été prélevés sur un site salin "sabkha " à Mosran, Djelfa, et les *Actinobactéries* ont été isolées en utilisant une série de dilutions et des milieux sélectifs. Les souches isolées ont été purifiées et soumises à des tests de tolérance au sel, à la température et au pH. Les caractéristiques culturelles et morphologiques ont été analysées, et l'activité antibactérienne des souches a été déterminée.

Un des objectifs clés de cette étude était d'investiguer la production de polysaccharides extracellulaires (EPS) dans les souches *d'Actinobactéries* isolées choisies, à savoir H3, H8 et H13. La production d'EPS est une caractéristique cruciale exhibée par de nombreuses bactéries, y compris les *Actinobactéries*, et elle remplit diverses fonctions biologiques. Pour évaluer la production d'EPS, les souches *d'Actinobactéries* ont été cultivées dans des milieux spécifiques (milieu liquide ISP2) connus pour favoriser la production d'EPS, et la quantité d'EPS produite a été quantifiée. Le rendement de la production d'EPS pour les trois échantillons (H3, H8 et H13) était d'environ 0,202 %.

En ce qui concerne l'activité antimicrobienne, les souches *d'Actinobactéries* isolées ont été évaluées contre quatre microorganismes. Les tests antimicrobiens ont été réalisés en utilisant la méthode de l'ensemencement en quadrillage et la méthode de la diffusion en puits d'agar. Les résultats ont révélé des degrés variables d'activité antimicrobienne exhibés par les souches *d'Actinobactéries*. Certaines souches ont montré une activité antimicrobienne, inhibant la croissance des agents pathogènes, tandis que d'autres ont montré une activité spécifique contre micro-organismes. L'activité antimicrobienne des souches *d'Actinobactéries* a été observée par la formation de zones d'inhibition autour des ensemencements en quadrillage et des puits d'agar.

Les conclusions de cette étude mettent en lumière le potentiel des *Actinobactéries* des sols salins pour des applications biotechnologiques. Les *Actinobactéries* montrent un potentiel en tant que source de nouveaux agents antimicrobiens. De plus, l'étude de la production d'EPS dans les souches *d'Actinobactéries* contribue à notre compréhension de leurs activités biologiques et ouvre des perspectives pour de futures recherches dans le domaine de la biotechnologie microbienne.

Cette recherche met en avant l'importance de l'étude des *Actinobactéries* des sols salins, qui représentent une niche écologique unique avec une diversité microbienne inexploitée. Les implications de cette étude s'étendent à divers domaines, notamment la biotechnologie, l'agriculture et les sciences pharmaceutiques, où la recherche de nouveaux composés bioactifs et d'agents antimicrobiens est d'une importance primordiale.

Mots-clés : *Actinobactéries*, Sols salins, Exopolysaccharides, Activité antimicrobienne.

ملخص

تهدف هذه الدراسة إلى عزل سلالات *الأكتينوبكتيريا* من التربة المالحة، مع التركيز على إنتاج البوليساكاريدات الخارجية (EPS) والنشاط المضاد للميكروبات ضد أربعة كائنات دقيقة، بما في ذلك *Escherichia coli*،*Staphylococcus aureus* ، بالإضافة إلى سلالات فطرية (عفن) مثل *Penicillium*.sp. وسلالة الخميرة *Saccharomyces cerevisiae*،تمت الأبحاث في مختبر علم الأحياء الدقيقة بجامعة زيان عاشور في الجلفة، باستخدام طرق لعزل *الأكتينوبكتيريا* ، و تحديد خصائصها ، واختبارها المضاد للميكروبات. تم جمع عينات التربة من موقع مالح من منطقة "الصبخة" في مصران، الجلفة، وتم عزل *الأكتينوبكتيريا* باستخدام سلسلة من التخفيف ووسائط زراعة. تم تحديد السلالات المعزولة وتعريضها لاختبارات تحمل الملوحة والحرارة ودرجة الحموضة. تم تحليل خصائص *الأكتينوبكتيريا* ومورفولوجيتها، وتم تحديد النشاط المضاد للبكتيريا للسلالات.

واحدة من الأهداف الرئيسية لهذه الدراسة كانت دراسة إنتاج البوليساكاريدات الخارجية (EPS) في سلالات *الأكتينوبكتيريا* المعزولة H3 وH8 وH13. إن إنتاج EPS هو خاصية حيوية تظهر في العديد من البكتيريا، بما في ذلك *الأكتينوبكتيريا* ، وهو يؤدي وظائف حيوية متنوعة. لتقييم إنتاج EPS، تمت زراعة سلالات *الأكتينوبكتيريا* في وسط محدد (وسط سائل ISP2) المعروف بتعزيز إنتاج EPS، وتم قياس كمية الEPS المنتج. كانت نسبة إنتاج EPS للعينات الثلاثة (H3 وH8 وH13) تقدر بحوالي 0.202%.

فيما يتعلق بالنشاط المضاد للميكروبات، تم اختبار سلالات *الأكتينوبكتيريا* المعزولة ضد أربعة كائنات دقيقة. تم إجراء اختبار المضادات الميكروبية باستخدام طريقة الخط المستقيم المتعامد وطريقة انتشار الآبار في الأجار. أظهرت النتائج درجات متفاوتة من النشاط المضاد للميكروبات الذي تظهره سلالات *الأكتينوبكتيريا*. بعض السلالات أظهرت نشاطًا مضادًا للميكروبات ذو طيف واسع، مثبطة لنمو الكائنات الممرضة، بينما أظهرت أخرى نشاطًا محددًا ضد بعض الميكروبات. تم ملاحظة النشاط المضاد للميكروبات لدى سلالات *الأكتينوبكتيريا* من خلال تكوين مناطق تثبيط حول الخطوط المستقيمة المتعامدة والآبار في الأجار.

تسلط نتائج هذه الدراسة الضوء على إمكانات *الأكتينوبكتيريا* من التربة المالحة للتطبيقات البيوتكنولوجية. من خلال توضيح نشاطها المضاد للميكروبات ضد أربعة كائنات دقيقة ، توضح *الأكتينوبكتيريا* إمكانية أن تكون مصدرًا لمضادات ميكروبية جديدة. علاوة على ذلك، يساهم استكشاف إنتاج EPS في سلالات *الأكتينوبكتيريا* في فهمنا لأنشطتها البيولوجية ويفتح آفاقًا للبحوث المستقبلية في مجال التكنولوجيا الحيوية المجهرية.

يؤكد هذا البحث على أهمية دراسة *الأكتينوبكتيريا* من التربة المالحة، والتي تمثل مكانة بيئية فريدة مع التنوع الميكروبي غير المستغل. تمتد آثار هذه الدراسة إلى مجالات مختلفة، بما في ذلك التكنولوجيا الحيوية والزراعة والعلوم الصيدلانية، حيث يكون البحث عن مركب حيوي جديد وعوامل مضادة للميكروبات ذا أهمية.

الكلمات المفتاحية: *الأكتينوبكتيريا* ، التربة المالحة،عديدات السكر الخارجية، النشاط المضاد للميكروبات.

Introduction

Introduction

In recent years, there has been a growing interest in the isolation and characterization of *Actinobacteria* from extreme environments, such as saline soils, due to their potential for producing unique bioactive compounds. This is particularly relevant to the field of biotechnology, as *Actinobacteria* are known for their capability to produce a wide range of secondary metabolites, including antibiotics, enzymes, and other bioactive compounds. Additionally, there has been a surge of interest in the study and utilization of plant-derived compounds, including those with strong biological activity, for industrial purposes. Therefore, the study of EPS production and antimicrobial activity in *Actinobacteria* strains isolated from saline soils holds great promise for advancing our understanding of these microorganisms and their potential for biotechnological applications.

Saline soils are known for their harsh conditions, including high salt concentration and low moisture content, which can pose a challenge for microorganisms that inhabit these environments **(Mokrane *et al.*, 2013; Lubsanova *et al.*, 2014; Binayke *et al.*, 2018).** However, some bacteria have adapted to survive and even thrive in these conditions, including *Actinobacteria*.

Actinobacteria are a group of gram-positive, filamentous bacteria known for their capability to produce a wide range of secondary metabolites, including antibiotics, enzymes, and other bioactive compounds. Many *Actinobacteria* are found in soil environments, where they play important roles in nutrient cycling and soil structure formation **(Malviya *et al.*, 2012)**. However, some *Actinobacteria* have also been isolated from extreme environments, such as saline soils, where they have adapted to survive under high salinity conditions **(Trenozhnikova &Azizan, 2018).**

Actinobacteria can be difficult to isolate, but their morphological and physiological characteristics can provide useful clues for their identification and characterization. The growth of *Actinobacteria* is from the hyphal, which is similar to filamentous fungi, and they can form complicated structures such as spores **(Malviya *et al.*, 2012; Li *et al.*, 2016).**

Actinobacteria, as remarkable microorganisms, have demonstrated their ability to adapt and thrive in saline soils despite the challenging circumstances. These environments present several obstacles that test the resilience of microorganisms. The high salt concentration in saline soils imposes osmotic stress on cells, disrupting their internal balance and compromising essential cellular processes. Furthermore, the limited moisture content creates water scarcity, which further challenges the survival of microorganisms **(Rammali *et al.*, 2022).**

Recently, there has been a growing interest in the study of *Actinobacteria* isolated from saline soils, as they are considered to be an underexplored source of novel bioactive compounds

(**Basilio *et al*., 2003**). In particular, the study of extracellular polysaccharides (EPS) production in these microorganisms has received attention due to their potential for biotechnological applications **(Lubsanova *et al.*, 2014).**

Exopolysaccharides (EPS) are complex polysaccharides that are produced by many bacteria, including *Actinobacteria*. EPS have a wide range of functions, including protection against environmental stresses, biofilm formation, and cell-cell communication **(Kumar *et al*., 2011).** They have also been shown to have important biological activities, including antimicrobial and immunomodulatory properties **(Li *et al*., 2016).**

EPS production in *Actinobacteria* is essential for environmental protection, biofilm formation, and intercellular communication. These complex polysaccharides exhibit antimicrobial and immunomodulatory properties, making them valuable resources for biotechnological applications. By studying EPS production in *Actinobacteria*, researchers can uncover the functional significance of these molecules and harness their potential in various industrial and therapeutic applications **(Qi *et al*., 2022).**

As mentioned earlier, there has been a growing interest in recent years in the isolation and characterization of *Actinobacteria* from saline soils due to their potential for producing unique bioactive compounds **(Basilio *et al*., 2003; Djebaili *et al*., 2018).** This focus on *Actinobacteria* has emerged due to their remarkable ability to yield bioactive substances with diverse applications. Numerous research studies have explored the effectiveness of *Actinobacteria* that were isolated from various regions and grown in different conditions of salinity and pH levels in the medium **(Basilio *et al*., 2003).**

Actinobacteria grow actively in saline soils, and the length of their growth cycle is shorter than that of other bacteria **(Djebaili *et al*., 2020).** In one study, the isolation and identification of EPS-producing bacteria from polluted soil samples and their antimicrobial activity were investigated **(Sarika *et al*., 2021).** In a study conducted in northeast India, *Actinobacteria* were obtained from soil samples collected from agricultural sites after fire operations. The *Actinobacteria* isolates showed diversity in their cell morphology, carbon source utilization, sensitivity to antibiotics, and tolerance to salt. **(Malviya *et al*., 2012)**

Several studies have investigated the antimicrobial activity of *Actinobacteria* and their potential as sources of new antimicrobial agents **(Xie *et al*., 2018; Sengupta *et al*., 2015).** By characterizing the EPS production and antimicrobial activity of *Actinobacteria* strains isolated from saline soils, researchers hope to gain insight into their potential as sources of new antimicrobial

agents, as well as their potential for biotechnological applications **(Sarika *et al.*, 2021).** *Actinobacteria* have been found to produce bioactive compounds with antimicrobial and immunomodulatory properties, and their isolation and characterization from various environments, including extreme conditions, have gained interest in recent years **(Malviya *et al.*, 2012; Kumar *et al.*, 2011).**

The amount of EPS produced by different bacterial strains can vary considerably, and further studies are needed to evaluate the potential of the isolated polysaccharide for biotechnological applications **(Kumar *et al.*, 2011).**

To consolidate information, this study investigates the isolation and characterization of *Actinobacteria* strains from saline soils. Special emphasis is placed on their EPS production, antimicrobial activity, and tolerance to environmental stressors like salinity, pH, and temperature. By examining these traits, we can gain insights into the potential biotechnological applications of *Actinobacteria* from saline soils.

In this study, we hypothesize that *Actinobacteria* isolated from saline soils will exhibit significant EPS production and antimicrobial activity. Furthermore, we expect these strains to display unique adaptations to extreme conditions, reflecting their specialized niche in saline soils. By exploring these hypotheses, we aim to contribute to the growing body of knowledge surrounding *Actinobacteria* and their potential for biotechnological advancements.

Chapter 01
Material and Methods

The aim of this study is to isolate and characterize strains of *Actinobacteria* from saline soil samples, and to evaluate their exopolysaccharide (EPS) production and antimicrobial activity against pathogenic Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) strains, as well as non-pathogenic microorganisms strains such as fungi-mold (*Penicilium.sp*) and the yeast (*Saccharomyces cerevisiae*). This study was conducted in the microbiology laboratory of Zian Achour University of Djelfa, using standardized methods for the isolation and characterization of *Actinobacteria*, as well as antimicrobial tests to assess their therapeutic potential. The results of this study could have important implications for research on *Actinobacteria* and their potential use in biotechnology. Including exopolysaccharide (EPS) production.

Materials

1. Laboratory material

1.1. Equipment and glassware

Tab. 1 : Laboratory Equipment and Glassware

Apparatus	**Glassware**	**Others**
Autoclave Magnetic stirrer with hot plate. pH meter Vortex Spectrophotometer Water bath Precision balance Incubator (30, 35, 40,45°C). Bunsen burner and a lighter. Refrigerator 4°C. Optical microscope. Screw cap tubes. Balance Heating mantle. Centrifuge machine.	Erlenmeyer flask Beakers : 200, 250, 300ml. Bottles Pasteur pipette Petri dishes Test tube rack Pipette tips Centrifuge tubes (14, 15 ml) Watch glasses Graduated cylinders Platinum Loop Magnetic Stir Bars	Micropipette : 1000, 100 µl. Spatula, wash bottle, droppers Slides and coverslips Ruler Aluminum foil, Parafilm. Blue and yellow tips. Spectrophotometry cuvettes. Sterile swabs Cork borers Needle-like instrument. Flat-ended needle holder.

1.2. Culture media

SCA and SCDA, Used for Isolation of *Actinobacteria*.

ISP1, ISP2, ISP3, ISP4 : Used for morphological study of *Actinobacteria*.

MH, Used for antimicrobial Activity test.

ISP2, Used for EPS production.

(See appendix 01 for the composition of media)

1.3. Chemical products and Solvents

Sodium chloride (NaCl), Used for preparing saline solution, and culture media.

Agar-agar, Used for solid media.

Sodium hydroxide (NaOH), Used for pH ajustement.

Ethanol (99.8%), Used for EPS extraction and precipitation.

Distilled water, Used for media preparation.

2. Biological material

Actinobacteria strains, isolated from saline soils.

Microbial strains, used for antimicrobial activity test ;

Bacteria : *Staphylococcus aureus* (G+), *Escherichia coli* (G-).

Fungi : *Saccharomyces cerevisiae* (yeast), *Penicillium*.sp.

Methods

1. Isolation

1.1.Sampling

Sampling Method and Collection of Soil Samples were collected from a site called 'sabkha' in Mosran, located in the Djelfa region (see Figure 1). Three samples were taken, with each one located 15 meters apart from the previous one. To collect the samples, the first few centimeters of the soil surface were scraped, and each sample was taken from a depth of 10 cm below the surface using a sterile spatula. Approximately 200 g of saline soil were collected from each sample and placed into sterile polyethylene bags, which were sealed and transported to the laboratory. The soil sampling method was adapted from previous studies **(Sapkota *et al*., 2020; Rai *et al*., 2018)** with slight modifications.

Fig 1 : Sampling site for saline soil in Mosran, Djelfa : Sabkha area (original picture, Geographical location Hassi Bahbah 22JV+6WF).

1.2. Isolation method

The process of isolating microorganisms from soil involves preparing dilutions of soil suspensions from a given sample, following the protocol described by **(El Karkouri *et al*., 2019).** To ensure aseptic conditions for the experiment, the work area and all equipment used were sterilized, using two Bunsen burners for 30 minutes. We poured the media into petri dishes and allowed it to solidify. To increase the chances of obtaining *Actinobacteria*, three replicates were performed for each soil sample.

First, we prepared one liter of soybean-casein digest agar (SCDA) with 10% NaCl and one liter of Starch Casein Agar (SCA) with 10% NaCl and without NaCl. The media were poured into petri dishes and allowed to solidify.

Next, the three samples were labeled E1, E2, and E3, and 10 g of sample E1 was weighed. Then it was mixed with 100 ml of physiological water using a small flask with a magnetic stirrer for up to 15 minutes.

Three dilutions (-1, -2, and -3) were prepared for the first sample, E1, labeling them as E1-1, E1-2, and E1-3, respectively. To prepare E1-1, 1 ml of the first sample was taken and added to a tube containing 9 ml of physiological water. Then it was mixed well using a vortex for less than a minute and named E1-1. The process was repeated for E1-2 and E1-3, using the appropriate dilutions.

Then, 0.1 ml of each of the three tubes (E1-1, E1-2, and E1-3) was taken and added to separate Petri dishes containing already-prepared SCDA (with 10% NaCl) and SCA antifungal solution (with and without NaCl). The dishes were labeled as follows : E1-1 SCDA, E1-2 SCDA, E1-3 SCDA (dated 7/3); E1-1 SCA antifungal solution, and SCA NaCl antifungal solution,
E1-2 SCA antifungal solution and SCA NaCl antifungal solution, E1-3 SCA antifungal solution, and SCA NaCl antifungal solution were labeled (dated 15/3) and subjected to incubation at two different temperatures : 30°C and 40°C.

The same steps were repeated for E2 and E3, resulting in a total of 54 Petri dishes. Specifically, 18 Petri dishes were prepared for SCDA (9 at 30°C and 9 at 40°C), and 36 Petri dishes were prepared for SCA (18 with antifungal NaCl and 18 without antifungal NaCl), with 9 Petri dishes for each combination of temperature. Each Petri dish was labeled with the appropriate names. Finally, the growth of total results and suspected *Actinobacteria* on the Petri dishes (54) was observed after the appropriate incubation time for the three samples : 5-6 days at 30°C and 40°C, and 7-8 days at 30°C and 40°C (see Figure 2 and Figure 3).

Fig 2 : Laboratory work for isolation of *Actinobacteria* from soil samples : Preparation of agar media in Petri dishes and soil dilution series, followed by inoculation of Petri dishes.

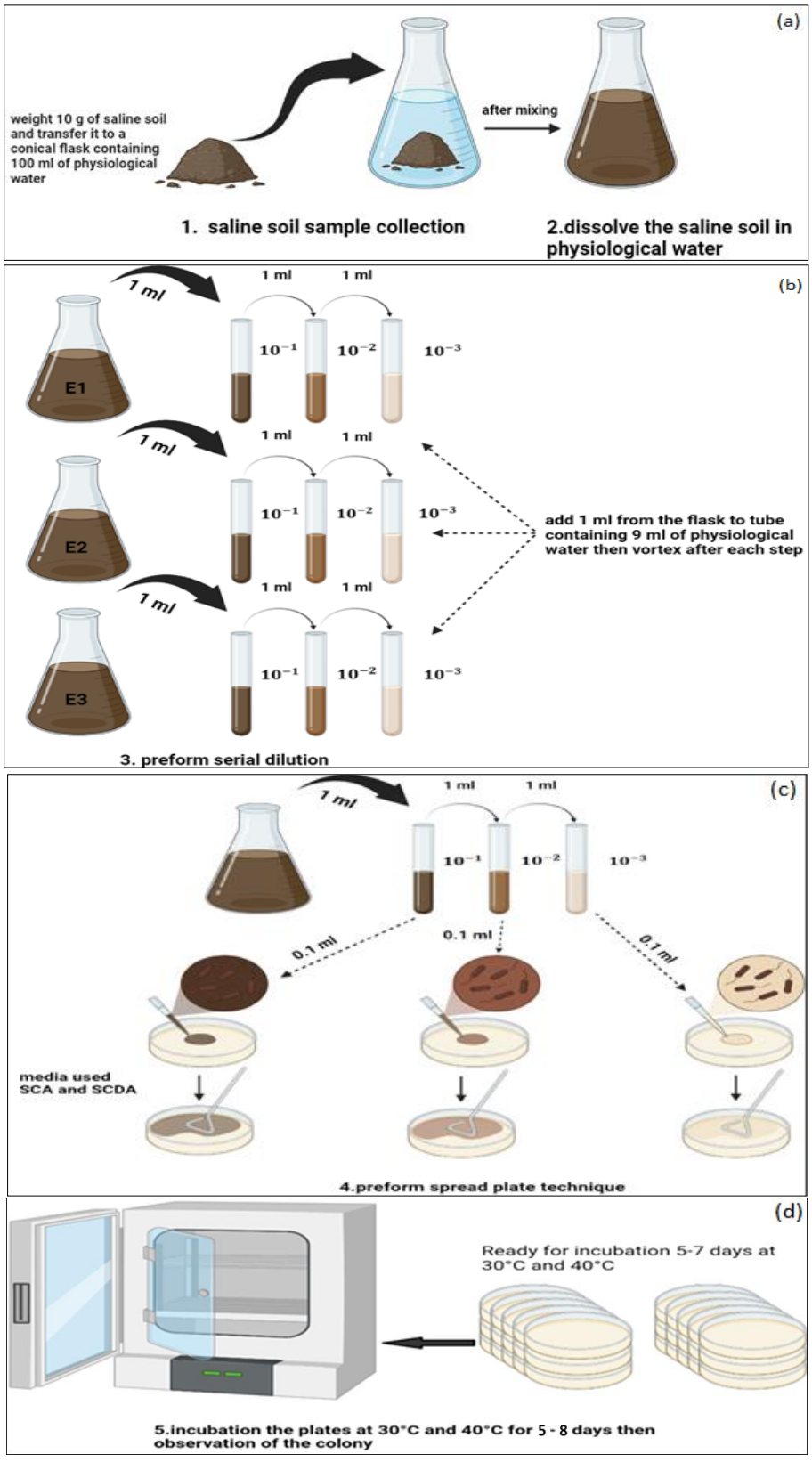

Fig 3 : Serial dilution method protocol (a, b, c, d)**.**

2. Purification

F1 (*Penicillium*.sp), a halophilic fungus, was subcultured by plating it on SCA and SCDA media (with/without) NaCl at 30 and 40°C. The streak method (zigzag pattern) was employed on the same media to isolate and obtain a pure culture of the fungus for further testing. The colonies were purified using the zigzag streak method on the appropriate media. Following this, we labeled and plated the halophilic *Actinobacteria* as H1 to H25 and the fungi as F1 (*Penicillium*.sp). The zigzag streak method was employed for each of these microorganisms.

3. Preservation

The strains were maintained by subculturing them onto the same agar medium used for their initial isolation. This involved transferring a small portion of each strain onto fresh agar plates of the corresponding medium. The plates were then incubated at the same temperature and duration as mentioned before. After incubation, the agar plates were stored at 4°C to preserve the strains for future use. This storage method ensures the viability and long-term maintenance of the microbial cultures.

4. Cultural and morphological characteristics

The cultural and morphological attributes of the *Actinobacteria* strains were investigated using a method based on **Djebbaha *et al.* (2021)** and the criteria outlined in 'Bergey's Manual' (1994). To differentiate between groups of *Actinobacteria*, various cultural characteristics were examined, including the production of aerial mycelium (MA), the presence of substrate mycelium (MS), and the determination of the color of both MA and MS, as well as the presence of diffusible pigments in the culture medium. After the initial isolation and purification, the *Actinobacteria* strains were subcultured onto four distinct culture media : ISP1, ISP2, ISP3, and ISP4. Subsequently, these subcultures were incubated at optimal temperatures tailored to each strain's requirements for a duration of 5 to 7 days.

4.1. Macroscopic examination

The colony morphology and pigmentation of the *Actinobacteria* strains were observed macroscopically in ISP media **(**1, 2, 3 &4**).**

A first morphological characterization is the observation of the macroscopic aspect of the colonies, with a possible orientation of the results during the identification. According to the authors **(Djebbaha *et al.*, 2021; Shirling & Gottlieb, 1966).**

4.2. Microscopic examination

The microscopic characteristics of the *Actinobacteria* strains were studied, including the observation of spore chains, mycelium fragmentation, and branching patterns, under the microscope using the transplantation embedding method (see Figure 4).These microscopic observations allowed for the assessment of various micromorphological criteria, such as the presence of sporanges on aerial mycelium (MA) or substrate mycelium (MS), the form and size of sporanges, the number of spores per sporangium, and the length of sporangiophores. Additionally, the formation of spores on MA and/or MS, their shape, size, and arrangement, as well as the mode of sporulation (spores carried by sporophores or aerial mycelium fragmenting into spores in an anarchic manner) and the formation of specific structures like synnemata and false sporanges were examined **(Li *et al.*, 2016).**

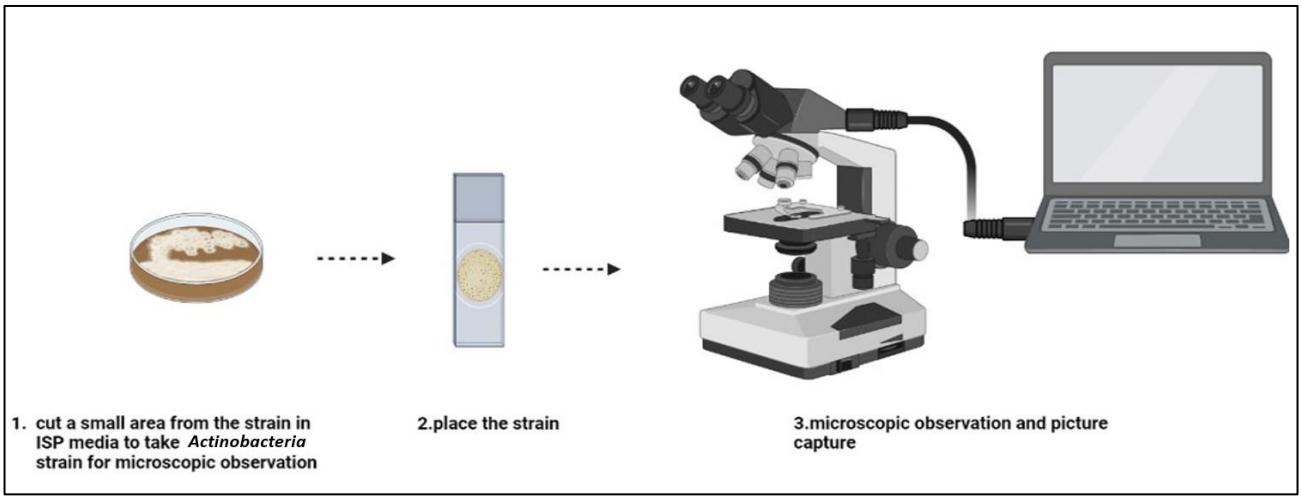

Fig 4 : Microscopic examination steps with transplantation embedding method **(Li *et al.*, 2016).**

5. Physiological tests

Following the methodologies outlined by **Rammali *et al.*, 2022,** we conducted a series of physiological tests to assess the cultural, micro-morphological, biochemical, and physiological characteristics of the *Actinobacteria* isolates.

5.1. Salinity test

To determine the halotolerance and halophilic nature of the *Actinobacteria*, two tests were conducted. In the halotolerance test, the *Actinobacteria* that grew in SCA medium containing 10% NaCl were transferred to SCA medium without NaCl to assess their ability to tolerate low salt concentrations. This test aimed to evaluate their tolerance to a range of salt levels, including the absence of salt.In the halophilic test, the *Actinobacteria* that grew on SCA medium without NaCl

were transferred to SCA medium supplemented with 10% NaCl to determine their ability to grow in high salt concentrations. This test aimed to identify *Actinobacteria* that thrive specifically in saline environments.

Furthermore, the halophilic and halotolerant *Actinobacteria* that exhibited growth in the previous tests were subjected to additional evaluations. They were tested on SCA media containing 15% and 20% NaCl to further assess their resistance to higher salt concentrations.

These tests provided valuable insights into the halotolerance, halophilic, and salt resistance capabilities of the *Actinobacteria*, enhancing our understanding of their adaptation and survival mechanisms in different saline conditions.

5.2. PH test

The pH tolerance of the *Actinobacteria* was assessed by subjecting them to SCA antifungal medium at two different pH levels : pH 9 and pH 5. This testing was performed to evaluate their ability to thrive and exhibit growth across a wide pH range. The *Actinobacteria* were inoculated onto plates containing SCA antifungal medium adjusted to pH 9 and pH 5. By observing their growth and response to these different pH conditions, we gained insights into their pH tolerance capabilities.

By observing the growth and viability of the *Actinobacteria* on these plates, we were able to gain insights into their pH tolerance and their ability to adapt and survive in different pH environments. This information is valuable in understanding the ecological niches and potential applications of these *Actinobacteria* in various pH conditions.

5.3. Temperature test

To comprehensively investigate the impact of temperature on the growth and characteristics of the *Actinobacteria* strains, a multifaceted temperature experiment was conducted. This experiment consisted of two main components : switching the incubation temperatures of selected strains and subjecting all strains to additional temperature tests.

In the first part, a temperature switch was performed for specific strains. The strains originally incubated at 40°C were transferred to new agar plates and incubated at 30°C. Similarly, the strains initially incubated at 30°C were transferred to new agar plates and incubated at 40°C.

Furthermore, additional temperature tests were conducted on all strains. In addition to their original incubation temperatures, the strains were subjected to two additional temperature conditions : 37°C and 45°C. By exposing the strains to these varied temperature settings, the aim

was to assess their adaptability and performance across a wider temperature range.

Through careful observation and analysis of the growth patterns, characteristics, and responses of the *Actinobacteria* strains under these varying temperature conditions, valuable insights were gained into the effects of temperature on their physiology and behavior. This information contributes to a comprehensive understanding of their ecological adaptations and potential applications in various fields.

6. Detection of antimicrobial activity

We conducted the assessment of antimicrobial activity following established methods **(Mohamed *et al.*, 2017; Sripreechasak & Athipornchai, 2019).**

6.1. Perpendicular streak method

The antimicrobial activity assessment of the *Actinobacteria* strains was conducted using the perpendicular streak method (See Figure 5). Initially, the strains were introduced onto MH medium using the spot inoculation technique. Following this, the plates were left to incubate for a period of 2 to 5 days, allowing the strains to attain optimal growth.

Subsequently, for the activity test, plates were preserved to evaluate their effect on specific target microorganisms, including Gram-positive bacteria (*Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*), yeast (*Saccharomyces cerevisiae*), and fungus-mold (*Penicillium sp*).

The process involved inoculating each target microorganism onto separate agar plates. Then, the preserved plates containing the *Actinobacteria* strains were retrieved and perpendicular streaks were applied onto the agar plates previously inoculated with the target microorganisms. These plates were subsequently incubated at an appropriate temperature (35°C) for a duration of 48 hours.

During this incubation period, observations were made and any indications of growth inhibition or zones of inhibition surrounding the perpendicular streaks were carefully recorded These observations served as valuable indicators of potential antimicrobial activity.

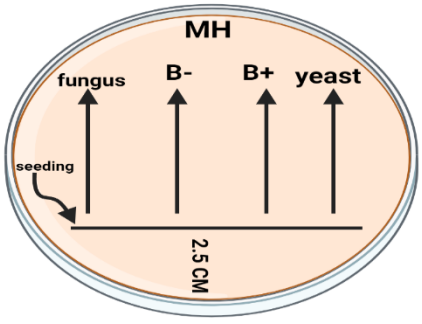

Fig 5 : Antimicrobial activity-perpendicular streak method

6.2. Agar well diffusion method

The procedure was carried out with strict adherence to sterile techniques, achieved by sterilizing both the work area and equipment using ethanol or alcohol. A series of four tubes, each containing 10 ml of physiological water, were prepared. Subsequently, the tubes were inoculated with the designated strains, namely *Escherichia coli*, *Staphylococcus aureus*, *Penicillium sp*, and *Saccharomyces cerevisiae*.

To attain the desired optical density (OD) range of 0.5 to 1, the suspensions' OD was measured at a wavelength of 600 nm using a spectrophotometer, with measurement facilitated by a cuvette. The resulting OD values for each strain were recorded. Following this, MH agar plates were inoculated with the corresponding suspensions using sterile swabs.

To facilitate precise identification, the MH plates were labeled accordingly. Using a sterile cork borer, agar wells were meticulously created on the labeled plates. Agar discs, sourced from SCA plates containing *Actinobacteria* strains, were then carefully transferred into these wells. The plates were subsequently subjected to an incubation period of 1 hour at 4°C, allowing for the optimal diffusion of antimicrobial substances.

For the subsequent phase, the plates were moved to an incubator set at 37°C for a duration of 48 hours (see Figure 6). To ensure the proper disposal of materials and maintain sterilization standards, appropriate sterilization and disposal procedures for used materials were diligently followed.

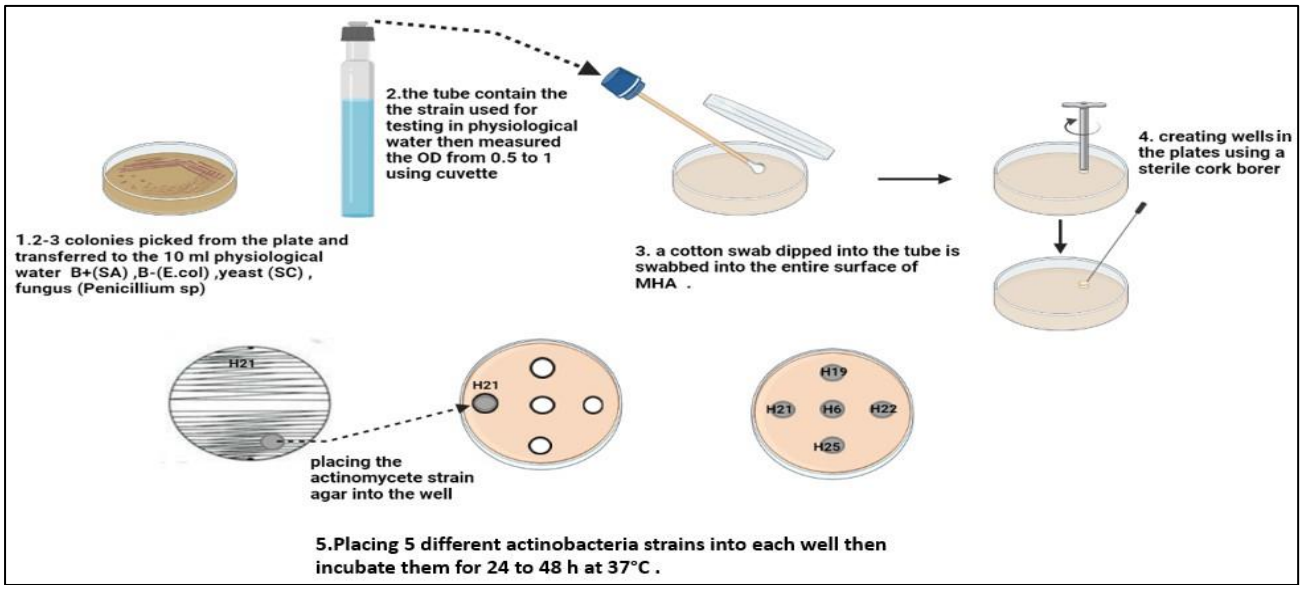

Fig 6 : Antimicrobial activity-agar well diffusion method.

7. Production of EPS

The EPS production from *Actinobacteria* obtained from saline soil was estimated using the method described by **Sivaperumal *et al.* (2018),** with slight modifications

The study encompassed the process of exopolysaccharide (EPS) production, carried out in a retrospective manner to depict the conducted actions. The initial step involved selecting three strains for EPS assessment, specifically H3, H8, and H13. To prepare for EPS testing, pre-cultures were meticulously generated in tubes containing 10 ml of ISP2 liquid media. After a period of three to five days, the Optical Density (OD) of these pre-cultures was gauged. A 1 ml was used from the 10 ml pre-culture tube to facilitate the measurement. Subsequently, the phase of inoculation and incubation commenced. The flasks, each containing 90 ml of ISP liquid media, were inoculated with the pre-cultures derived from the 9 ml tubes. These flasks were then subjected to an incubation period extending a minimum of five days. Regular agitation, occurring three to four times daily, was carried out to ensure proper mixing.

Following incubation, the focus shifted to the inactivation of cells and enzymes. Achieved by heating the flasks at 100°C for 10 minutes, this step served to render them inactive. The subsequent stage involved centrifugation, executed at 5000 G for 20 minutes at 4°C. This centrifugation facilitated the separation of biomass, with the aqueous phase (supernatant) retained while the pellet was discarded.

The process continued with ethanol precipitation, encompassing a sequence of actions :

- Adding 100 ml of pure ethanol to the aqueous phase and ensuring thorough mixing.
- Allowing the mixture to rest overnight at 4°C.
- Conducting centrifugation at 1000 G for 20 minutes at 4°C to retrieve the EPS.
- Disposing of the aqueous phase and retaining the resulting pellet.

For the final step of yield estimation, the pellet was weighed to determine the EPS yield. This comprehensive process reflects the detailed methodology employed in EPS production and assessment (see Figure 7).

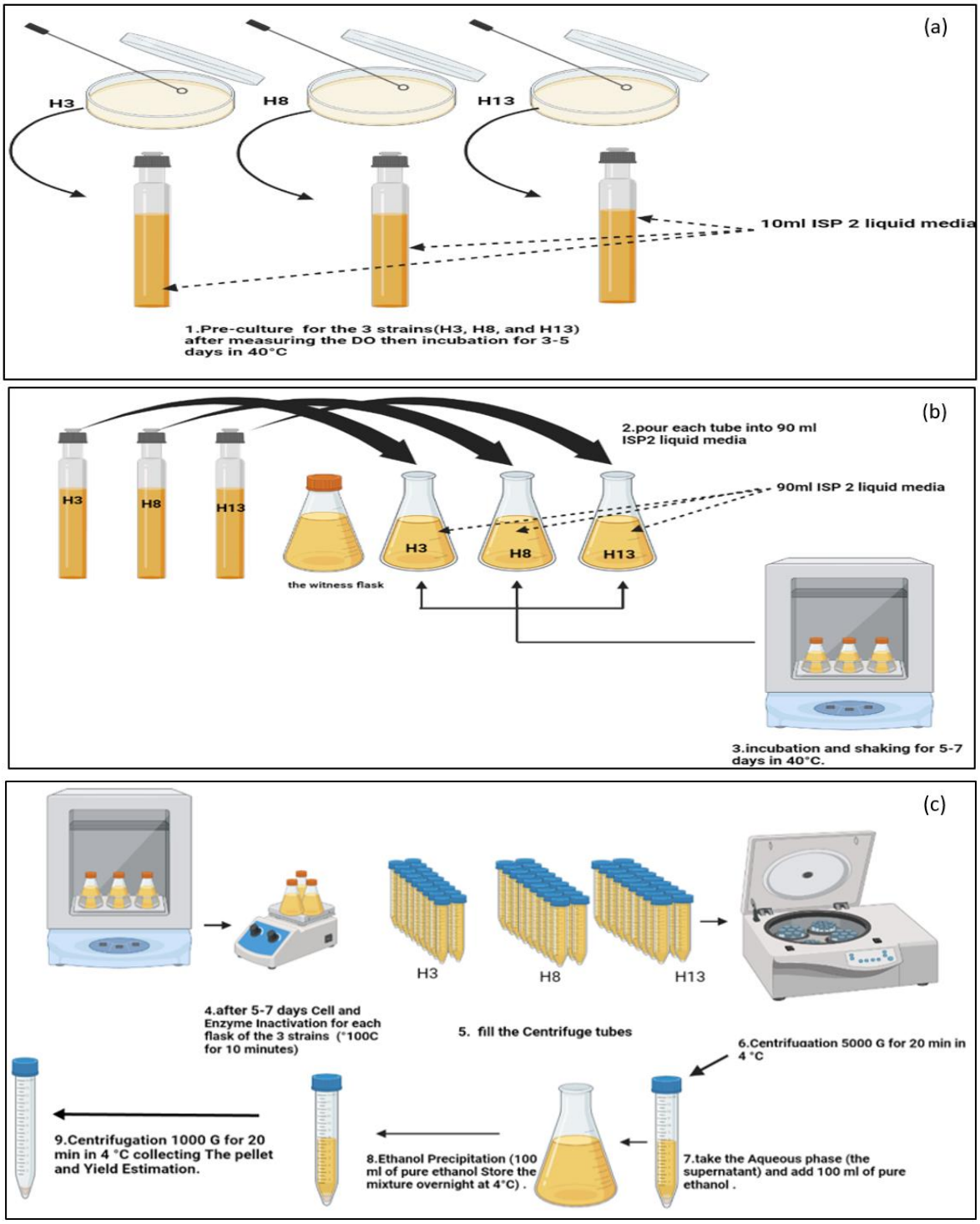

Fig 7 : Protocol of EPS Production (a,b,c) .

Chapter 02

Results and Discussion

1. Isolation results

Tab. 2 : Number of *Actinobacteria* isolates per soil sample

Soil Sample	Number of Isolates
E1	13
E2	5
E3	2
Total	20

From **3** saline soil samples, a total of 20 *Actinobacteria* isolates were obtained on SCA and SCDA media with or without salt. All these isolates displayed **mycelial** characteristics, were aerobic, and exhibited Gram-positive staining (Table 3, Figure 8). These isolates were further purified through subculturing on solid medium and subsequently identified using conventional phenotypic methods.

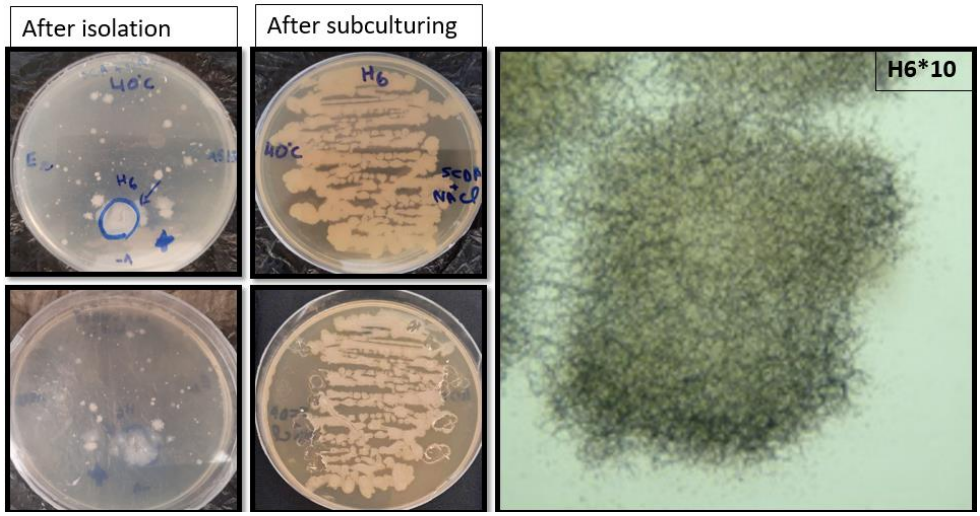

Fig 8 : *Actinobacteria* colonies in SCA and SCDA media

Tab. 3 : Results of total *Actinobacteria* isolates.

***Actinobacteria* Isolates**	**T °C**	**Samples dilution**	**Media**
H3	40 °C	E1 (-2)	SCA+ Antifungal
H4	40 °C	E1 (-2)	SCA+ Antifungal
H5	40 °C	E1 (-2)	SCA+ Antifungal
H6	40 °C	E2 (-1)	SCA+Nacl
H7	40 °C	E2 (-2)	SCA+ Antifungal
H8	40 °C	E2 (-2)	SCA+ Antifungal
H9	40 °C	E1 (-1)	SCA+ Antifungal
H10	40 °C	E1 (-1)	SCA+ Antifungal
H11	40 °C	E1 (-1)	SCA+ Antifungal
H12	40 °C	E1 (-1)	SCA+ Antifungal
H13	40 °C	E1 (-1)	SCA+ Antifungal
H14	40 °C	E1 (-1)	SCA+ Antifungal
H17	40 °C	E1 (-3)	SCA+ Antifungal
H18	40 °C	E2 (-1)	SCA+ Antifungal
H19	30 °C	E2 (-2)	SCA+Antifungal+Nacl
H21	40 °C	E3 (-1)	SCA+Antifungal+Nacl
H22	40 °C	E3 (-1)	SCA+Antifungal+Nacl
H23	30 °C	E1 (-1)	SCA+ Antifungal
H24	30 °C	E1 (-1)	SCA+ Antifungal
H25	30 °C	E1 (-1)	SCA+Antifungal+Nacl

2. Cultural caracterisation

2.1. Macromorphological study

The lecture for the substrate mycelium and aerial mycelium color.

The morphological and cultural characters were observed on the media (SCA, SCDA, ISP1, ISP2, ISP3, ISP4),

In this section, we present the results of our cultural characterization of *Actinobacteria* strains cultivated on ISP1 medium. Table 2 provides a summary of the mycelium color characteristics (MS and MA) for various strains. We chose to use ISP1 as an example because it yielded the most favorable results in terms of both growth and morphology.

For a more detailed exploration of the cultural characteristics and additional images, please refer to the appendix (02).

Table 4 : Cultural caracteristics of *Actinobacteria* strains in ISP1 medium.

Strains	**Media**	**Mycelium color**	
		MS	MA
H3	ISP1	Marron	White
H4	ISP1	Marron in the edge, beige in center	White
H5	ISP1	Beige	White
H6	ISP1	Orange	White
H7	ISP1	Beige	white
H8	ISP1	Marron	white
H9	ISP1	Beige	White
H10	ISP1	Marron	White
H11	ISP1	Beige	White
H12	ISP1	Orange	White
H13	ISP1	Orange	White
H14	ISP1	Orange	White
H17	ISP1	Orange	White
H18	ISP1	Marron in the edge, beige in center	White
H19	ISP1	Marron in the edge, beige in center	White
H21	ISP1	Marron	White
H22	ISP1	/	/
H23	ISP1	Marron in the edge, beige in center	White
H24	ISP1	Marron in the edge, beige in center	White
H25	ISP1	Beige	White

Note :

"/" Indicates no growth

MS Substrate mycelium

MA Aerial mycelium

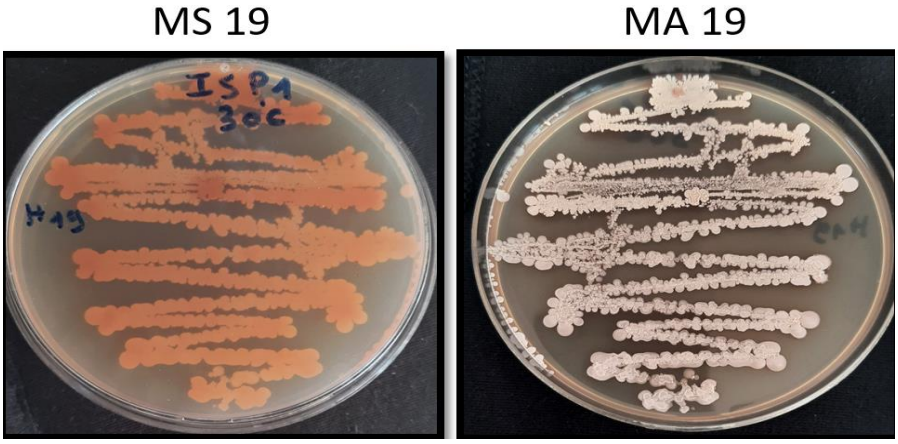

Fig 9 : MA and MS for H19 in ISP1 medium.

2.2. Micromorphological study

The preliminary identification of the proposed genera of the collected isolates was determined by morphological features, including microscopic examination (see Figure 10). The morphological and cultural characters were permitted to classify our isolates into six (6) groups as below :

Group A1, cultivated at a temperature of 40°C, displayed distinctive morphological characteristics. Samples H3, H9, and H10 exhibited a fragmented pattern, while samples H11, H12, H13, and H14 showcased a non-fragmented pattern. Furthermore, the mycelium Arial staining was positively observed for Group A1.

Group A2, cultivated at a temperature of 40°C, displayed distinctive morphological characteristics. Sample H4 exhibited a fragmented pattern Furthermore, the mycelium Arial staining was positively observed for Group A2.

Group A3, cultivated at a temperature of 40°C, displayed distinctive morphological characteristics. Sample H5 exhibited a non-fragmented pattern. Furthermore, the mycelium Arial staining was positively observed for Group A3.

Group A4, cultivated at a temperature of 40°C, displayed distinctive morphological characteristics. Samples H6, H7, and H8 exhibited a fragmented pattern, while sample H18 showcased a non-fragmented pattern. Furthermore, the mycelium Arial staining was positively observed for Group A4.

Group A5, cultivated at a temperature of 30°C, displayed distinctive morphological characteristics. Samples H19, H23, and H25 exhibited non-fragmented pattern. Furthermore, the mycelium Arial staining was positively observed for Group A5.

Group A6, cultivated at a temperature of 30°for H24 and 40°C for H21, H22, displayed distinctive morphological characteristics. Samples H21, H22, and H24 exhibited a non-fragmented pattern. Furthermore, the mycelium Arial staining was positively observed for Group A6.

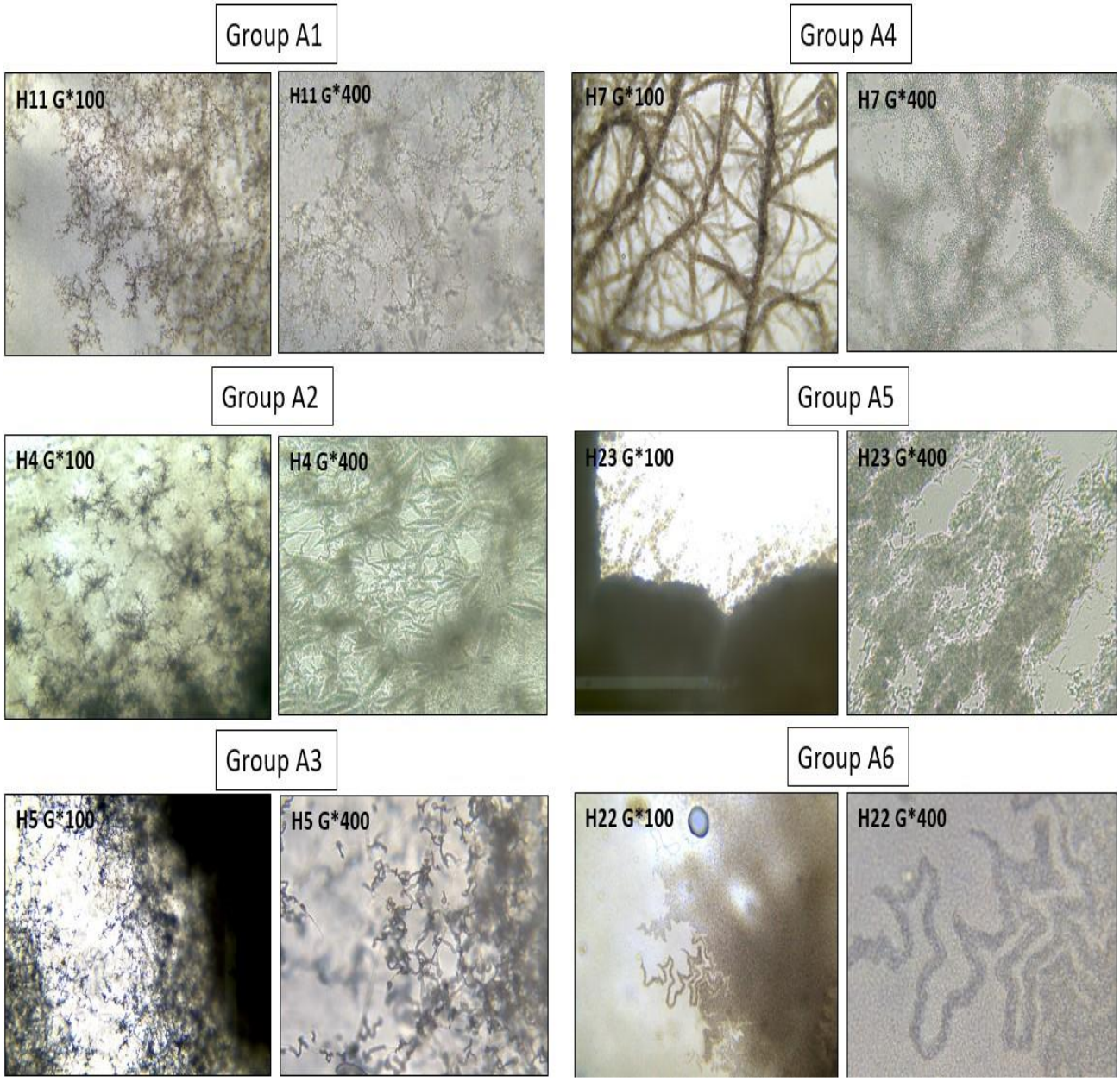

Fig 10 : Microscopic examination and morphological characteristics of *Actinobacteria* groups A1**(**H11**)**, A2**(**H4**)**, A3**(**H5**)**, A4**(**H7**)**, A5**(**H23**)**, A6**(**H22**)**.

3. Physiological tests

In this section, a series of physiological tests were conducted to explore the adaptability and behaviors of the *Actinobacteria* strains (see Table 5). The pH test unveiled their responses to varying pH levels, offering insights into potential environmental preferences. The temperature test employed a combination of a temperature switch experiment and additional temperature assessments, resulting in a comprehensive comprehension of how temperature impacts growth and traits. These insights extended to growth patterns, antimicrobial activity, and their capacity to thrive under diverse temperature conditions. The salt (NaCl) test concentrated on salt concentrations of 15% and 20% within SCA antifungal media. This examination delved into their navigational skills within high salinity environments, spotlighting their adaptability to challenging habitats. Collectively, these tests unveiled a holistic understanding of the *Actinobacteria* adaptability and potential applications across various contexts.

Halotolerant strains : H3, H4, H7, H8, H12, H13, H14, H18, H22, H23, H24

Halophilic strains : H6, H19, H21, H25.

Table 5 : Growth results of *Actinobacteria* strains under varied conditions.

	NaCl		pH		Temperature			
Strains	15%	20%	5	9	30°C	37°C	40°C	45°C
H3	-	-	+	+++	+++	++	++	++
H4	-	-	+	++	+++	++	++	++
H6	+	-	+	++	+++	++	++	++
H7	-	-	+	++	++	++	++	++
H8	-	-	++	++	++	++	++	++
H12	-	-	+	+++	++	++	++	++
H13	-	-	+	+++	+++	+++	++	++
H14	-	-	++	++	++	++	++	+++
H18	-	-	+	++	++	++	++	+++
H19	-	-	++	+	+++	+++	+++	+++
H21	+	-	+++	+	++	+++	+++	+++
H22	-	-	++	+	++	++	++	+
H23	+	-	+	++	+++	++	++	-
H24	+	-	++	+++	+++	++	++	+
H25	+	-	+	+	+++	++	++	++

Note :

"-" indicates no growth
"+" indicates low growth
"++" Indicates moderate growth
"+++" Indicates good growth
"++++" Indicates very good growth

Each strain's growth response was observed under two conditions : SCA without NaCl and SCA with NaCl. Strains that were initially tested in SCA NaCl media were subsequently evaluated without NaCl, and conversely, strains previously tested without NaCl were assessed in the presence of NaCl.

Halophilic strains (H6, H19, and H25) main media was with NaCl exhibited very good growth ("++++") in theabsence of NaCl. H21 exhibited good growth ("+++") in theabsence of NaCl.

Additionally, H22 exhibited no growth ("-") when tested under theabsence of NaCl.

In the tested strains, specific strains exhibited halotolerant characteristics, showcasing varying degrees of salt (NaCl) tolerance :

- Strain H3's main media was without NaCl and it was tested with NaCl, exhibiting moderate growth ("++") with NaCl.
- Strain H4's main media was without NaCl and displayed moderate growth ("++") with NaCl.
- Strain H7's main media was without NaCl and showed limited growth ("+") in the presence of NaCl.
- Strain H8's main media was without NaCl and displayed limited growth ("+") with NaCl.
- Strain H12's main media was without NaCl and exhibited limited growth ("+") with NaCl.
- Strain H13's main media was without NaCl and demonstrated limited growth ("+") with NaCl.
- Strain H14's main media was without NaCl and exhibited good growth ("+++") with NaCl.
- Strain H18's main media was without NaCl and showed very good growth ("++++") without NaCl.
- Strain H23's main media was without NaCl and displayed limited growth ("+") with NaCl.
- Strain H24's main media was without NaCl and showed moderate growth ("++") with NaCl.
- Strains H5, H9, H10, H11, and H17, whose main media was without NaCl, displayed no growth ("-") in the presence of NaCl.

This detailed analysis underscores the diverse responses of these strains to different NaCl concentrations and highlights their halotolerant and halophilic characteristics.

4. Detection of antimicrobial activity

Another important aspect of this study was to evaluate the antimicrobial activity of the isolated *Actinobacteria* strains. The antimicrobial potential of *Actinobacteria* is well-documented, and their ability to produce bioactive compound with antimicrobial properties is of great interest for pharmaceutical and biotechnological applications. To assess the antimicrobial activity, the *Actinobacteria* strains were subjected to antimicrobial assays against four microorganisms, fungiand yeast. The results demonstrated a range of antimicrobial activities exhibited by the *Actinobacteria* strains. Some strains showed broad-spectrum antimicrobial activity, inhibiting the growth of multiple pathogens, while others exhibited more specific activity against certain microorganisms **(Mohamed *et al.*, 2017; Sripreechasak & Athipornchai, 2019).**

4.1. Antimicrobial activity - perpendicular streak method

The antimicrobial activity of the *Actinobacteria* strains was assessed using the perpendicular streak method, and the results were observed after 48 hours of incubation (see Table 6 and Figure 11). This method provided valuable insights into the inhibitory effects of the *Actinobacteria* against various microorganisms.

Table 6 : Antimicrobial activity of the isolate using perpendicular streak method.

	Antimicrobial activity inhibition zone (mm)			
Strain	***Penicilium.sp* (mold)**	***Staphylococcus aureus* (G+)**	***Escherichia coli* (G-)**	***Saccharomyces cerevisiae* (Yeast)**
H3	4	3	5	3
H4	0	4	0	0
H5	1	0	0	0
H7	2	0	0	0
H8	0	6	0	0
H9	0	0	0	2
H10	0	0	0	0
H11	0	7	4	1
H12	0	6	1	2
H13	4	6	5	2
H14	1	5	5	1
H17	2	1	1	1
H18	1	2	1	0
H23	1	0	1	0
H24	0	1	0	0

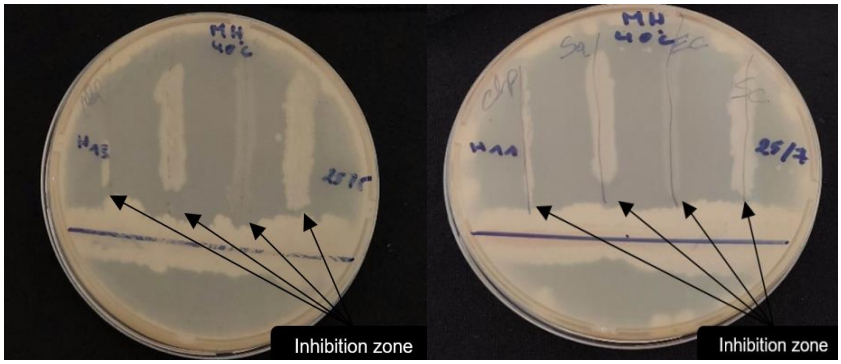

Fig 11 : Some result of antimicrobial activity - perpendicular streak method (H13 and H11).

4.2. Antimicrobial Activity - Agar Well Diffusion Method

In the assessment of antimicrobial activity using the agar well diffusion method, the optical density (OD) of the suspensions was adjusted to a range between 0.5 and 1, with OD measurements taken at a wavelength of 600 nm using a spectrophotometer cuvette. The recorded OD results for each strain were as follows :

Penicillium sp. OD = 0.760,

Staphylococcus aureus OD = 0.865,

Escherichia coli OD = 0.552,

Saccharomyces cerevisiae OD = 0.568.

After 48 hours, the results were examined, and the findings are presented in Table 7 and Figure 12.

Table 7 : Antimicrobial activity of the isolate using agar well diffusion method.

	Antimicrobial activity inhibition zone (mm)				**Well (mm)**
Strain	***Penicilium.sp* (mold)**	***Staphylococcus aureus* (G+)**	***Escherichia coli* (G-)**	***Saccharomyces cerevisiae* (Yeast)**	/
H6	8	9.72	12.28	8	8
H19	8	10.32	12.78	10.52	8
H21	8	13.12	10.92	8	8
H22	8	12.3	12.52	8	8
H25	8	8	12.62	8	8

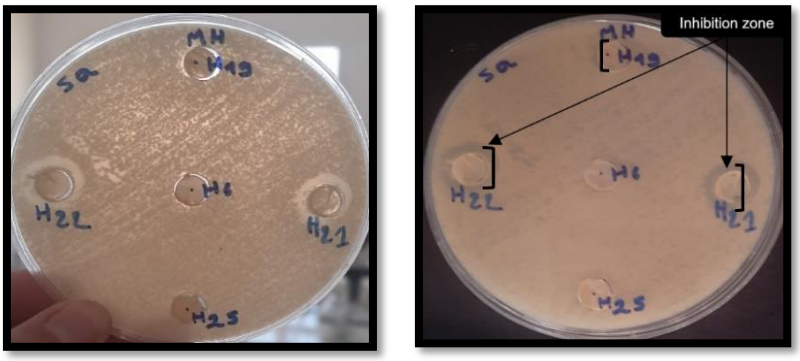

Fig 12 : Antimicrobial activity - agar well diffusion method against *Staphylococcus aureus*

5. Production of EPS

5.1. Experimental setup

In this study, the production of extracellular polysaccharides (EPS) in the isolated *Actinobacteria* strains (H3, H8, and H13) was evaluated. The *Actinobacteria* strains were cultivated in specific liquid media ISP 2 conducive to EPS production.

5.2. Measurement of EPS production

After three to five days of cultivation, pre-culture samples were taken, and the optical density (OD) of each sample was measured. A volume of 1 ml was withdrawn from each 10 ml pre-culture tube for the measurement. The OD values for each strain were recorded as follows :

H3 OD = 0.343, H8 OD = 0.871, H13 OD = 1.821

5.3. Quantification of EPS Yield

The yield of EPS production for all three samples (H3, H8, and H13) was calculated following the method outlined by **Lin *et al.* (2019),** which involves dividing the weight of EPS obtained (0.2 g) by the volume of the initial biomass (99 ml). The EPS yield was found to be approximately 0.202% for each strain.EPS yield (%) = (EPS obtained / initial biomass) * 100

EPS yield (%) = (0.2 g / 99 ml) * 100

EPS yield (%) = 0.202 %.

EPS yield (g/L) = (EPS obtained / initial biomass in liters)

Initial biomass in liters = 99 ml / 1000 = 0.099 L

The EPS yield :

EPS yield (g/L) = (0.2 g / 0.099 L) ≈ 2.02 g/L

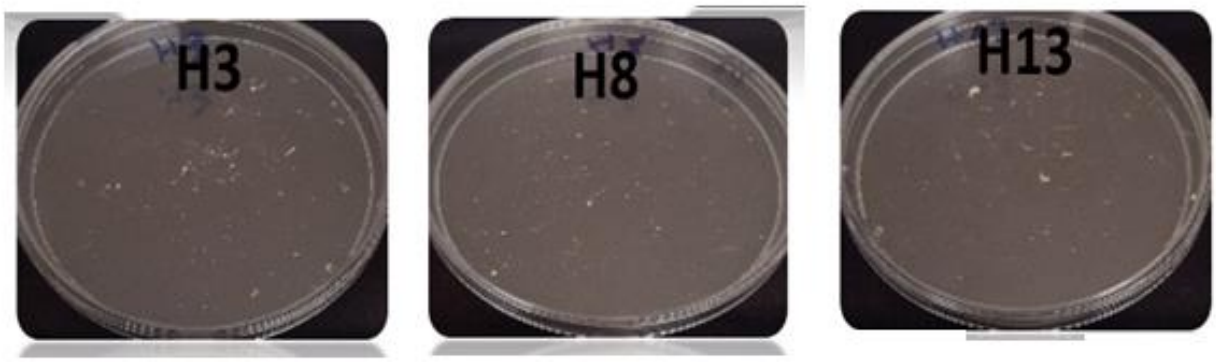

Fig 13 : EPS Powder of three *Actinobacteria* strains (H3, H8, H13).

Discussion

In this study, we successfully isolated *Actinobacteria* strains from saline soils using a methodology outlined in **Basilio *et al.*, (2003)** and **Sapkota *et al.*, (2020).** Employing a combination of selective media (SCA-SCDA), isolation techniques, and cultivation methods, a total of 20 strains were isolated. These isolated strains were subjected to thorough characterization based on their morphological, physiological, and biochemical characteristics. Notably, the isolated strains exhibited filamentous growth patterns, reminiscent of other *Actinobacteria*, with the formation of complex structures like spores. **(Basilio *et al.*, 2003; Sapkota *et al.*, 2020).**

In congruence with the investigations conducted by **Rammali *et al.*, (2022)** and **Mohamed *et al.*, (2017)**, we further characterized the isolated *Actinobacteria* strains. This extended characterization involved assessing their cultural traits, including colony morphology, pigmentation, and growth patterns across different culture media. Our observations were validated through microscopic examination, which confirmed the presence of filamentous hyphae and sporulation.

To discern the strains and illuminate their adaptability to saline environments, we performed physiological and biochemical tests. These assessments encompassed evaluating their resilience to salt concentrations, responses to varying pH levels and temperature conditions, as well as their antimicrobial potency against four microorganism. The outcomes of these analyses provided pivotal

insights into the metabolic capabilities and adaptive characteristics of these *Actinobacteria* strains. This, in turn, highlighted their promising potential for application in the realm of biotechnology.

The comprehensive characterization of these *Actinobacteria* strains, considering their cultural characteristics, environmental tolerance, and antimicrobial activity, significantly contributes to our understanding of their adaptability and potential applications in saline environments. This knowledge opens doors for further exploration of their bioactive compounds and their promising roles in biotechnology and pharmaceutical industries.

Furthermore, investigating the production of extracellular polysaccharides (EPS) in select strains shed light on their potential biotechnological applications, offering promising avenues for further research in microbial biotechnology and related fields. It is worth noting that several studies have reported the successful isolation and identification of *Actinobacteria* from various sources, including soil samples, the intestinal tract and feces of insects, and extreme biotopes , have been documented in several studies **(Sapkota *et al*., 2020; Santamaría *et al*., 2020).**These findings collectively contribute to the ongoing search for novel antimicrobial agents and extend our understanding of the diverse applications of *Actinobacteria* in different environments.

The salt (NaCl) test serves as a pivotal physiological assessment for *Actinobacteria*, offering valuable insights into their salt tolerance and halophilic/halotolerant attributes. In the scope of this study, the salt test was conducted on 20 *Actinobacteria* strains, following the methodologies outlined by **Jose &Jebakumar (2013)** and **Ara *et al*., (2013).**and the results showed that 5 strains were halophilic, while 10 strains were halotolerant The halophilic strains identified were H6, H19, H21, H22, and H25, while the halotolerant strains were H3, H4, H7, H8, H12, H13, H14, H18, H24, and H23. The ability of *Actinobacteria* to tolerate high salt concentrations is an important adaptation that allows them to survive in extreme environments, such as saline soils and hypersaline inland solar salterns **(Jose & Jebakumar, 2013; Ara *et al*., 2013).** The results of the NaCl test at 15% and 20% concentrations on SCA antifungal media provide insights into the salt tolerance and halophilic/halotolerant properties of *Actinobacteria*.

The pH test is an important physiological test that can provide insights into the acidophilic or alkaliphilic properties of *Actinobacteria*. In our study, we performed the pH test for the *Actinobacteria* strains. The results of the pH test showed that most of the *Actinobacteria* strains in our study were able to grow at pH 9, indicating their alkaliphilic properties, while some strains were able to grow at pH 5, indicating their acidophilic properties. These findings highlight the diversity

and adaptability of *Actinobacteria* in different environments The inspiration behind adopting the pH test in this study finds its roots in the works of **Poomthongdee *et al*., (2014)** and **Basilio *et al*., (2003),** who notably conducted similar assessments.

The temperature test is an important physiological test that can provide insights into the growth patterns, antimicrobial activity, and the ability of *Actinobacteria* to thrive and function under different temperature conditions. In the present study, the temperature test was performed for *Actinobacteria* strains, and the results showed that most of the strains were able to grow at 30°C and 37°C, while some strains were able to grow at 40°C and a few strains were able to grow at 45°C. These results suggest that some *Actinobacteria* strains may have thermophilic properties, which could be explored further for potential biotechnological applications.

The physiological tests performed in the present study, including the NaCl test, pH test, and temperature test, provide valuable insights into the diversity and adaptability of *Actinobacteria* in different environments. The optimization of process parameters, such as pH, temperature, and medium composition, can help to maximize EPS yield in *Actinobacteria*. The comparison of the results obtained in the present study with those reported in other studies can provide valuable insights into the diversity and adaptability of *Actinobacteria* and the optimization of process parameters for EPS production.

The cultural and morphological characteristics of *Actinobacteria* are important for their identification and classification. In the present study, the substrate mycelium and aerial mycelium color of *Actinobacteria* strains were observed on different ISP media. The results showed that most of the strains had beige or orange substrate mycelium and white aerial mycelium. However, some strains had different colors of substrate mycelium and aerial mycelium, such as marron, light orange, yellow, and greenish. These results suggest that the *Actinobacteria* strains have diverse cultural and morphological characteristics, which could be used for their identification and classification. Several studies have utilized ISP media for the isolation, identification, and characterization of *Actinobacteria*, focusing on their cultural and morphological characteristics **(Djebbah *et al*., 2021; Li *et al*., 2016; Sripreechasak &Athipornchai, 2019).**

Several other studies have contributed to the understanding of *Actinobacteria* ;

1. Study by **Basilio *et al.*, (2003)** :

A total of 609 *Actinobacteria* strains were isolated from 17 soil samples.The number of isolates recovered from each sample varied widely, ranging from 18 to 316.The highest number of strains was obtained under isolation conditions of pH 7 and 11, while the lowest was at high salt concentrations.

2. Study by **Sapkota *et al.*, (2020)** :

A number of (11) soil samples from Nepal were collected at varying altitudes.41 isolates were identified as *Streptomyces* spp., Nocardia spp., and *Micromonospora* spp.Different pigmentation was observed in most isolates.

3. Study by **Rammali *et al.*, (2022)** :

Soil samples from three different sites were analyzed for physico-chemical characteristics. *Actinobacteria* strains were isolated, with the highest number of isolates from site C.The strains showed different levels of salt tolerance.

4. Study by **Jose &Jebakumar, (2013) :**

The saltern soil sample had alkaline pH, high electric conductivity, and predominance of sodium and chloride.14 actinomycete isolates were obtained, with some slow-growing and rare actinobacteria among them.

5. Study by **Ara *et al.*, (2013) :**

Actinobacteria populations were observed to decrease at higher NaCl concentrations.Isolates were classified as halotolerant or moderately halophilic.

6. Study by **Poomthongdee *et al.*, (2014) :**

Actinobacteria were isolated from rice and rubber tree rhizosphere soils.Most isolates were assigned to streptomycetes, with some non-streptomycetes.Acidophilic and neutrophilic *Actinobacteria* were found in the soils.

7. Study by **Sripreechasak &Athipornchai, (2019) :**

Actinobacteria strains were isolated from Mongolian hypersaline soil.Strains under the genera *Nocardiopsis* were abundant in the soil, and there were possible novel species identified in *Nocardiopsis* and *Isoptericola* genera.

In this study, two different methods, the perpendicular streak method and the agar well diffusion method, were employed to evaluate the antimicrobial activity of the *Actinobacteria* strains. The results obtained from the perpendicular streak method revealed the antimicrobial activity of several strains against different microorganisms. Consistent with the findings of **(Mohamed *et al*., 2017; Sripreechasak & Athipornchai, 2019)**, strains H3, H13, and H14 exhibited significant antimicrobial activity against all tested microorganisms, including *Penicillium sp* (mold), *Staphylococcus aureus* (Sa), *Escherichia coli* (E. coli), and *Saccharomyces cerevisiae* (Sc). However, some strains in our research, such as H5, H7, H8, H10, H11, H12, H17, H18, H23, and H24, did not display any antimicrobial activity against the tested microorganisms. Notably, strains H4, H9, and H18 showed antimicrobial activity against specific microorganisms, indicating strain-specific variations in antimicrobial potential, which aligns with the observations made by **(Mohamed *et al*., 2017; Sripreechasak & Athipornchai, 2019).**

Additionally, the results of the agar well diffusion method, showed inhibitory zones formed around the wells for each strain. Strains H6, H19, H21, H22, and H25 exhibited significant antimicrobial activity against at least one of the tested microorganisms. However, similar to our perpendicular streak method results, other strains did not display antimicrobial activity under the conditions tested. These findings emphasize the importance of considering the method used for evaluating antimicrobial activity, as it can influence the observed results.

In the antimicrobial testing, our *Actinobacteria* strains displayed significant activity against prokaryotic microorganisms, including *Staphylococcus aureus* (a Gram-positive bacterium), and *Escherichia coli* (a Gram-negative bacterium). However, no antimicrobial activity was observed against eukaryotic microorganisms, such as *Penicilium.sp*, and *Saccharomyces cerevisiae* (a yeast strain). This indicates a preference for targeting prokaryotic pathogens over eukaryotic microorganisms.

To further contextualize our findings, we compared our results with those from other relevant studies exploring the antimicrobial potential of *Actinobacteria*. For instance, **Janardhan *et al*. (2014)** isolated *Actinobacteria* from mangrove soil in the Andhra Pradesh region, India. Among their isolates, strains GN1 and GN2 exhibited significant antimicrobial activity against pathogenic bacteria, including E. coli, *Staphylococcus aureus*, *Bacillussubtilis*, and *Pseudomonas aeruginosa*. Similarly, **Dholakiya *et al*., (2017)** isolated *Actinobacteria* from the Gulf of Khambhat in Gujarat, India, and their screening revealed considerable inhibitory effects on both Gram-negative and Gram-positive bacteria, such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas* sp.,

Bacillussubtilis, and *Staphylococcus aureus*.

In another study by **Aouar (2012),** six *Actinobacteria* strains isolated from rhizosphere soil displayed notable antibacterial activity against *Escherichiacoli*, *Staphylococcus aureus*, and *Bacillussubtilis*. However, **Benamira &Abriche (2018)** reported contrasting results, where their isolated strain coded as "J" exhibited antimicrobial activity against *Bacillussubtilis* and *Staphylococcus aureus* but did not affect *Escherichia coli*.

Comparing our findings with these studies reveals the variable antimicrobial activity of different *Actinobacteria* strains against various pathogens. Our results align with previous research in demonstrating significant activity against Gram-positive and Gram-negative bacteria, particularly *Staphylococcus aureus* and *Escherichia coli*.

The investigation of EPS production in the isolated *Actinobacteria* strains H3, H8, and H13 revealed promising results. EPS production is a crucial trait exhibited by many bacteria, including *Actinobacteria*, and has significant potential for various biological and industrial applications.

The recorded EPS yield of approximately 0.202% for all three strains indicates the capacity of these *Actinobacteria* to produce substantial amounts of EPS under the given growth conditions. This finding highlights their suitability as potential sources of valuable extracellular polysaccharides.

EPS production is influenced by various factors, including media composition, growth conditions, and genetic factors. Future research should focus on optimizing EPS production conditions to enhance the yield even further. Additionally, understanding the structure and composition of the produced EPS will provide valuable insights into its properties and potential applications.

In comparison to other studies, such as the research by **Elnahas *et al*., (2017)** and **Urrutia *et al*., (2021)**, which investigated EPS production in different *Streptomyces* strains, our results demonstrate the production of EPS in *Actinobacteria* and open new avenues for exploring their potential bioactivities.

EPS has shown promising biological activities in other studies, such as antioxidant properties and inhibition of cancer cell migration and proliferation. Therefore, it would be interesting to explore the functional properties of EPS from our *Actinobacteria* strains and assess their potential as prebiotics or food additives.

Furthermore, safety evaluations are crucial when considering potential therapeutic applications of EPS. It is essential to investigate whether EPS poses any risks, such as septicemia, to ensure its safe use in medical and biotechnological applications.

Conclusion

Conclusion

This study focused on the isolation and characterization of *Actinobacteria* strains from saline soils, with a specific emphasis on their exopolysaccharide (EPS) production and antimicrobial activity. The research successfully isolated and purified 20 *Actinobacteria* strains from the soil samples collected in 'sabkha' area in Mosran, Djelfa. The morphological and cultural characteristics of these strains were analyzed using various culture media, including ISP1, ISP2, ISP3, and ISP4.

The investigation into EPS production revealed that three selected strains (H3, H8, and H13) exhibited notable EPS production, with a yield of approximately 2.02 g/L .EPS production is a crucial trait exhibited by bacteria, including *Actinobacteria*, and the findings contribute to our understanding of the biological activities and potential applications of these microorganisms in biotechnology.

Moreover, the antimicrobial activity of the isolated *Actinobacteria* strains was evaluated against pathogenic bacteria (*Staphylococcus aureus* and *Escherichia coli*) and non-pathogenic microorganisms (*Penicillium sp.* And *Saccharomyces cerevisiae*). The results demonstrated varying degrees of antimicrobial activity, with some strains exhibiting broad-spectrum activity and inhibiting the growth of multiple pathogens. These findings highlight the potential of *Actinobacteria* as a source of novel antimicrobial agents, contributing to the ongoing search for effective treatments against pathogenic bacteria and fungi, including yeast.

The study also explored the tolerance of the isolated *Actinobacteria* to salinity, pH, and temperature. The *Atinobacteria* strains displayed adaptability to high salt concentrations and exhibited diverse responses to different pH levels and temperature conditions. These findings enhance our understanding of the ecological adaptations and survival mechanisms of *Actinobacteria* in extreme environments.

The isolation and characterization of *Actinobacteria* from saline soils provide valuable insights into their diversity and adaptability in extreme environments. The observed morphological, physiological, and biochemical characteristics confirmed the identification of the isolated strains as *Actinobacteria*. The variation in cultural characteristics, EPS production, and antimicrobial activity highlighted the metabolic versatility and potential biotechnological applications of *Actinobacteria*.

This study contributes to the growing body of research on *Actinobacteria* derived from extreme environments and their potential for biotechnological applications. The findings expand our knowledge of these microorganisms and their bioactive compounds, opening avenues for further exploration in fields such as biotechnology, agriculture, and pharmaceutical sciences.

In conclusion, the study demonstrates the significance of studying *Actinobacteria* from saline soils, highlighting their unique ecological niche and untapped microbial diversity. The findings provide valuable insights into their potential as sources of novel bioactive compounds and antimicrobial agents. Further research in this field holds promise for the development of new therapeutic approaches and biotechnological advancement.

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Appendix

Appendix 01 Culture media used.

1. Media and chemicals

Phisiological water

NaCl 0.9% : 9 g
Distilled water : 1 L
Autoclave at 121°C for 15-20 minutes.

Soyabean Casein Digest Agar

Casein enzymatic hydrolysate : 15.0 g
Soyatone (soya peptone) : 5.0 g
Sodium chloride : 5.0 g
Agar : 15.0 g
Distilled water : 1 L
40 grams of SCDA media powder / 1L Distilled water
PH : 7.3±0.2 at 25 °C
Autoclave at 121°C for 15-20 minutes.

Starch casein agar with 10% NaCl and without NaCl

Starch casein medium soluble starch : 10 g
Casein : 1 g
Dipotassium hydrogen phosphate K_2HPO_4 : 0.5 g
Magnesium sulphate $MgSO_4$: 0.5 g
0.5 mL of antifungal solution which constitutes (Amphotericin B : 10g, Amphotericin B titrating : 1000µg/mg)
Distilled water : 1000 ml
PH : 7.2±0.2 at 25 °C
Autoclave at 121°C for 15-20 minutes.

International Streptomyces Project medium-1 (ISP-1)

Tryptone : 5 g (Peptone meat was used instead)
Yeast extract : 3 g
Agar : 18 g
Distilled water : 1 L
PH : between 7 and 7.4.
Autoclave at 121°C for 15-20 minutes.

International Streptomyces Project medium-2 (ISP-2)

Yeast extract : 4 g
Malt extract : 10 g
D-glucose : 4 g
Agar : 18 g
Distilled water : 1 L
PH : 7.2
Autoclave at 121°C for 15-20 minutes.

International Streptomyces Project medium-3 (ISP-3)

Oat meal : 20 g (Ammonium sulfate was used instead)
Trace salt solution : 1 ml
Distilled water : 1000 ml
Agar : 18 g
PH : between 7 and 7.4.

Trace salt solution

$CuSO_4$.5H2O 0.64 g didn't exist, so it wasn't used
$FeSO_4.7H_2O$: 0.11 g
$MnCl_2.4H_2O$: 0.79 g
$ZnSO_4.7H_2O$: 0.15 g
Distilled water : 100 ml
PH : between 7 and 7.4.
Autoclave at 121°C for 15-20 minutes.

International Streptomyces Project medium-4

Solution1 :

Soluble starch 10g in cold distilled water (500 ml)

Solution 2 :

Dipotassium hydrogen phosphate : 1.0 g
Magnesium sulphate heptahydrate : 1.0 g
Sodium chloride : 1.0 g
Ammonium sulphate : 2.0 g
Calcium carbonate : 2.0 g
Distilled water 500 ml Trace salt solution 1 ml Mix both Solution 1 and Solution 2
Agar : 18 g
PH : between 7 and 7.4
Autoclave at 121°C for 15-20 minutes.

Mueller Hinton Agar (MHA)

Beef extract : 2.0 g
Acid hydrolysate of casein : 17.5 g
Starch : 1.5 g
Agar : 17.0 g
Final pH : 7.3 ± 0.1 at 25ºC
Suspend : 38 g of the medium in 1000ml of distilled water.
Heat with frequent agitation and boil for one minute to completely dissolve the medium.
Autoclave at 121°C for 15 minutes. Cool to room temperature.
Pour cooled Mueller Hinton Agar into sterile petri dishes on a level, horizontal surface to give uniform depth.
Allow to cool to room temperature.
Store the plates at 2-8 ºC.

Appendix 02 strains used.

2. Cultural caracteristics

Table 8 : Cultural caracteristics of *Actinobacteria* strains in different ISP media.

Strains	Media	Mycelium color	
		MS	MA
H3	ISP2	Beige	White
	ISP3	Beige	Light orange
	ISP4	Beige	Yellow
H4	ISP 2,4	Beige	White
	ISP 3	Beige	Light orange
H5	ISP 2	Marron and beige	White and grey
	ISP 3	Grey	White
	ISP 4	Beige	Green in center, light grey in edge
H6	ISP 2.4	Beige	White
H7	ISP 4	Beige	white
H8	ISP 2.4	Beige	white
H9	ISP 2.3.4	Beige	White
H10	ISP 2.3.4	Beige	White
H11	ISP 2.3.4	Beige	White
H12	ISP 2	Beige	White
	ISP 3	Beige	Beige
	ISP 4	Beige	Orange
H13	ISP 2	Beige	White
	ISP 3	Beige	Beige
	ISP 4	Beige	Orange
H14	ISP 2	Beige	White
	ISP 3	Beige	Beige
	ISP 4	Beige	Orange
H17	ISP 2	Beige	White
	ISP 3.4	Beige	Beige
H18	ISP 2	Orange	White
	ISP 4	Beige	White
H19	ISP 2	yellow in the edge, orange in center	White
	ISP 4	Beige	White
H21	ISP 2	Marron in the edge, beige in center	White
H22	ISP 2	Beige	White
H23	ISP 2.4	Beige	White
H24	ISP 2	Beige	White
	ISP 4	Beige	Beige
H25	ISP 2.4	Beige	White

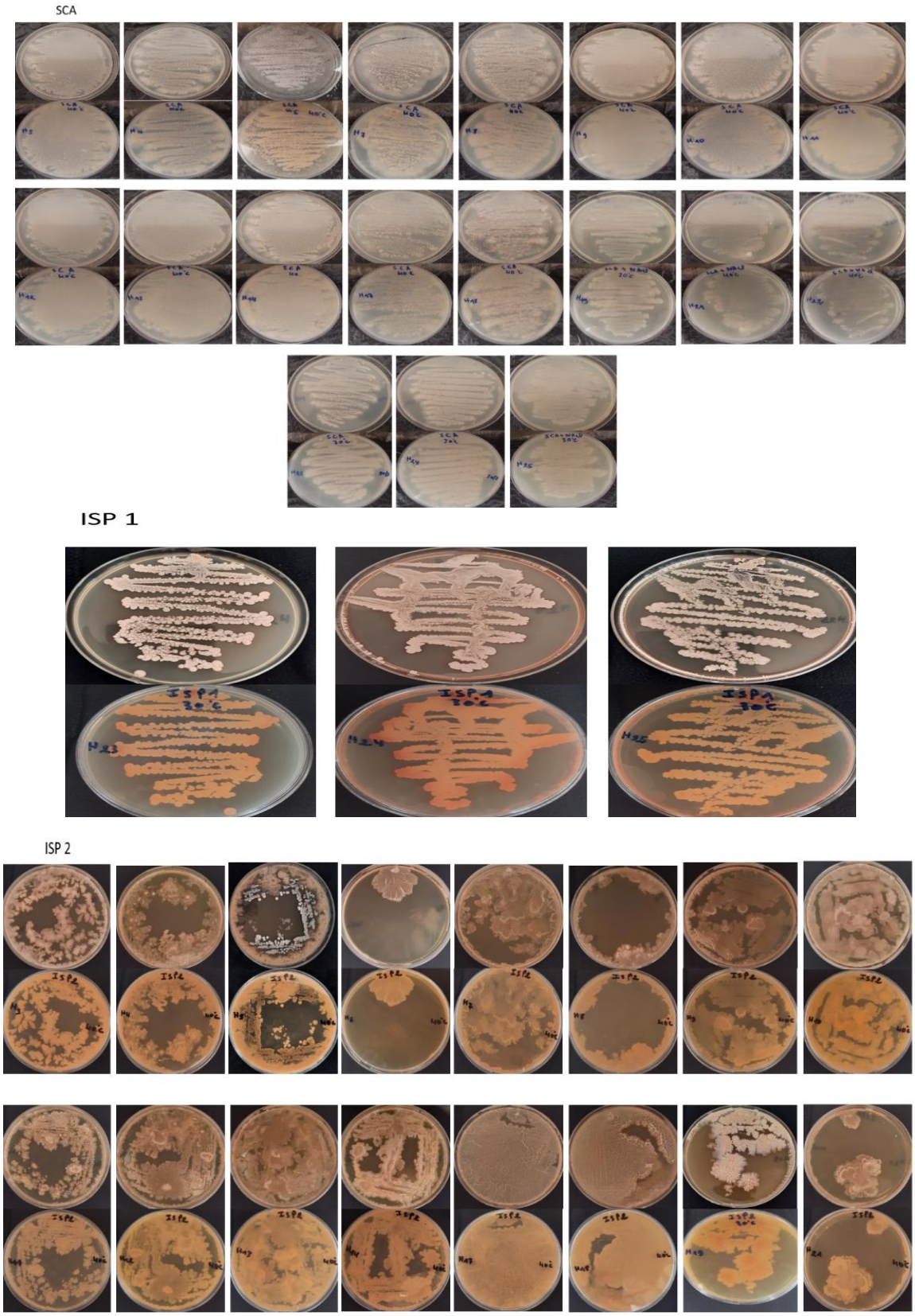

Fig 14 : Morphological Variations in *Actinobacteria* : SCA, ISP 2, ISP 1.

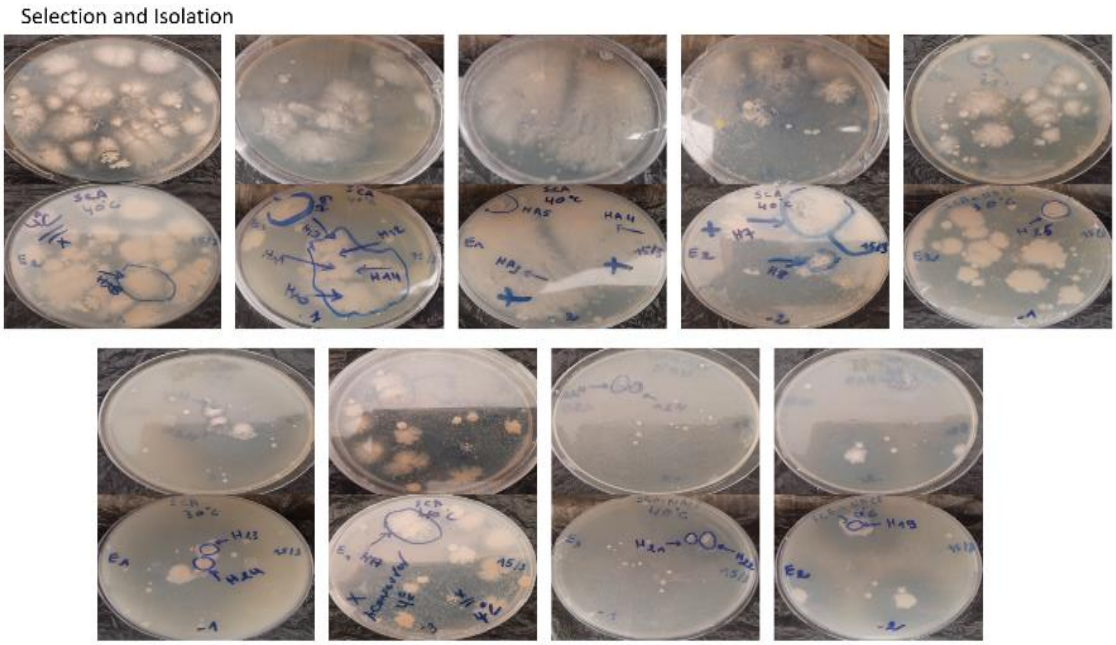

Fig 15 : *Actinobacteria* Selection and Isolation.