UNIVERSIDAD DE INVESTIGACIÓN DE TECNOLOGÍA EXPERIMENTAL YACHAY

Escuela de Ciencias Biologicas e Ingeniería

Antimicrobial activity of soil bacteria against *Escherichia coli, Pseudomonas aeruginosa* and *Staphylococcus aureus*

Trabajo de integración curricular presentado como requisito para la obtención del título de Biólogo

Autor:

García Quishpe Wendy Vanessa

Tutor:

PhD. Zurita Leal Andrea Cristina

Urcuquí, Mayo 2024

AUTORÍA

Yo, **WENDY VANESSA GARCÍA QUISHPE**, con cédula de identidad 1727661389, declaro que las ideas, juicios, valoraciones, interpretaciones, consultas bibliográficas, definiciones y conceptualizaciones expuestas en el presente trabajo; así cómo, los procedimientos y herramientas utilizadas en la investigación, son de absoluta responsabilidad de el/la autor/a del trabajo de integración curricular. Así mismo, me acojo a los reglamentos internos de la Universidad de Investigación de Tecnología Experimental Yachay.

Urcuquí, Mayo 2023.

Wendy Vanessa García Quishpe

CI: 1727661389

AUTORIZACIÓN DE PUBLICACIÓN

Yo, **WENDY VANESSA GARCÍA QUISHPE**, con cédula de identidad 1727661389, cedo a la Universidad de Investigación de Tecnología Experimental Yachay, los derechos de publicación de la presente obra, sin que deba haber un reconocimiento económico por este concepto. Declaro además que el texto del presente trabajo de titulación no podrá ser cedido a ninguna empresa editorial para su publicación u otros fines, sin contar previamente con la autorización escrita de la Universidad.

Asimismo, autorizo a la Universidad que realice la digitalización y publicación de este trabajo de integración curricular en el repositorio virtual, de conformidad a lo dispuesto en el Art. 144 de la Ley Orgánica de Educación

Urcuquí, Mayo 2023.

Wendy Vanessa García Quishpe

CI: 1727661389

Dedication

To the ones who mean the world to me, my parents and my little sister.

Thank you for being the heartbeat of my life.

Wendy Vanessa García Quishpe

Acknowledgment

Firstly, I want to express my gratitude to my parents and family. I deeply appreciate your encouragement in pursuing my dream of becoming a biologist. To Mayra C., my best friend and partner in crime, I'm grateful to life for bringing you into my life. Thank you for the beautiful moments and cherished memories we've created together. To Wen F. and Jerry S., my best friends for life, thank you for sharing this exciting journey with me. Your unconditional friendship has been invaluable. You were there when life was falling apart for me and I couldn't have asked for better people in life. To the breakfast club, especially Naye G. and Gaby N., for the conversations about life, the affection, and for being such good friends. To my biologuitos, I want to express my appreciation for the wonderful moments we shared during classes and field trips. To Steven, for helping me with some details of this thesis, I truly appreciate your time and help. To Paúl M., thank you for being with me in the final stage of this journey. How lovely was to coincide with you in this life, you are everything my soul was searching for.

My sincere appreciation to my ex-tutor Marco Gudiño, who with his wisdom guided me in the realization of this thesis. I appreciate all the advices, time and knowledge he gave me. Also, I am grateful to Dr. Miguel Viñas and collaborators who helped me in the identification of bacterial strains. To my tutor Andrea Zurita, who despite knowing each other for a short time, helped me in the final details of this thesis.

Finally, to my healing artists Porter Robinson, Madeon, Illenium, MGK, Flume, Excision, San Holo and Quevedo, who with their music brightened my life and healed my heart on the days when life was hard. And as the AREA21 song says "We did it".

Wendy Vanessa García Quishpe

Resumen

La aparición de la resistencia bacteriana a diversos fármacos ha hecho que la búsqueda de nuevos compuestos se torne prioritario para el tratamiento de infecciones. Se calcula que hay entre 100 millones a 1.000 millones de tipos diferentes de bacterias con funciones y papeles únicos. En un gramo de suelo existen millones de microorganismos que potencialmente podrían producir metabolitos con actividad antimicrobiana. La mayoría de ellos se encuentra en los 20 cm del horizonte superficial el cual contienen materia orgánica. Estudios recientes se han centrado en las especies bacterianas que liberan compuestos antimicrobianos como posibles fármacos terapéuticos para tratar enfermedades infecciosas. Con este antecedente, el objetivo de este trabajo es aislar y caracterizar las bacterias productoras de antimicrobianos del suelo. Para este estudio se cultivaron y caracterizaron bacterias presentes en el suelo de la Universidad de Investigación de Tecnología Experimental Yachay. Se realizó ensayos antagónicos entre las bacterias aisladas y las especies de bacterias *Escherichia coli*, *Pseudomonas aeruginosa*, y *Staphylococcus aureus*. Los resultados obtenidos demuestran que los metabolitos producidos por las cepas aisladas, UITEY-030 y UITEY-055, inhibieron el crecimiento de las cepas de *Staphylococcus aureus* y *Escherichia coli*, respectivamente.

Palabras Clave:
Bacterias de suelo, resistencia antibiotica, compuestos antimicrobianos, actividad antimicrobiana

Abstract

labelchap:abstract The emergence of bacterial resistance to various drugs has made the search for new compounds a priority for the treatment of infections. It is estimated that there are between 100 million and 1 billion different types of bacteria with unique functions and roles. In one gram of soil there are millions of microorganisms that could produce metabolites with potential antimicrobial activity. Most reside in the top 20 cm of the surface horizon, which contains organic matter. Recent studies have focused on bacterial species that release antimicrobial compounds as possible therapeutic drugs to treat infectious diseases. Therefore, the aim of this work was to isolate and characterize antimicrobial-producing soil bacteria. For this study, bacteria present in the soil of Universidad de Investigación de Tecnología Experimental Yachay, were cultured and characterized. Antagonistic assays were performed between the bacterial isolates and *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The results obtained show that the metabolites produced by the isolated strains UITEY-030 and UITEY-055 inhibited the growth of *Staphylococcus aureus* and *Escherichia coli* strains, respectively.

Keywords:
Soil bacteria, antimicrobial resistance, antimicrobial compounds, antimicrobial activity

Contents

List of Tables

List of Figures

Chapter 1

Introduction

The multidrug-resistant organisms and antimicrobial-resistant (AMR) infections have become a global issue for public health in the era of modern medicine (Danquah et al., 2022; Uddin et al., 2021). For instance, AMR causes 700,000 fatalities annually worldwide with the potential for an increase to 10 million by 2050 (Skarżyńska et al., 2020; Indraningrat et al., 2016). Therefore, it is crucial to quickly find novel antibacterial compounds (Aminov, 2010; Moellering, 2011).

Bioactive chemicals have traditionally been obtained from natural products (Atanasov et al., 2021). Soil bacteria due to their diversity and broad metabolic capacities, have been the subject of in-depth research (Bach et al., 2018). Studies have demonstrated that a large number of these bacteria produce specific compounds with antibiotic activity against a variety of diseases, including drug-resistant strains (Bibi et al., 2017; Waller and Sampson, 2018). One of the most promising resources in the biotechnology field is the ability of soil bacteria to produce antimicrobial substances and bioactive chemicals (Zhang et al., 2021). Since they may be used as an alternative to treat infections caused by drug-resistant microorganisms, these compounds are of significant interest to the scientific community (Elbendary et al., 2018).

Over the years, scientists have isolated and characterized a wide variety of antimicrobial compounds. Some of the most well-known examples include tetracycline, first isolated in 1948, which was extracted from Actinobacteria, a soil phylum bacterium (Grossman, 2016). Since then, many other antibiotics have been purified, including streptomycin and erythromycin, which have been crucial in treating bacterial infections.

The production of antimicrobial compounds is a natural process that has evolved to allow these organisms to compete with other microorganisms in their environment. Different factors, such as changes in temperature, pH, or the presence of other microorganisms, trigger the production of these compounds (Almajano et al., 2007). Recent studies have shown that competition

among soil bacteria is one of the primary mechanisms for producing antimicrobial compounds (Tyc et al., 2014). In fact, the synthesis of inhibitory compounds might play a crucial role in the interspecific competition among bacterial communities (Tyc et al., 2017).

Although the production of antimicrobial substances can inhibit the growth of certain bacterial strains competing for resources (Serwecińska, 2020), these compounds may also have other effects on bacteria such as acting as signaling molecules (Romero et al., 2011) or modifying their gene expression (Mitosch and Bollenbach, 2014).

Screening for these kinds of chemicals could help in the development of more effective drugs against some of the most prevalent pathogenic bacteria. The identification of bacteria that are capable of the synthesis of these compounds requires the collection and isolation of bacteria from the edaphic habitat.

1.1 Problem statement

Global public health is affected by the complicated and multifaceted issue of antibiotic resistance (Prestinaci et al., 2015). The development and spread of drug-resistant microorganisms make it difficult to treat infectious diseases effectively. Although the development of AMR is a natural process, the misuse of antibiotics is the main cause of the public health crisis caused by the unchecked expansion of this phenomenon (Dhingra et al., 2020). Resistance to several medicines including penicillin, began to emerge in bacteria more than fifty years ago. Bacteria such as *S. aureus* are among the ones that develop this resistance (Stapleton and Taylor, 2002). In 2017, the World Health Organization (WHO) released a list of infections for which additional antibiotic research was urgently needed. The ESKAPE pathogens (*E. faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa*, and *Enterobacter* species) were given ”priority status” (Mancuso et al., 2021). By 2050, it is predicted that antibiotic-resistant illnesses could cause up to 10 million fatalities annually, costing the world economy over $100 trillion (Strathdee et al., 2020).

The discovery rate of new antibiotics has declined in recent years (Zada et al., 2021). The difficulty in discovering new compounds with unique structures and mechanisms of action, as well as the high cost and lengthy timetables related to drug discovery (Nwobodo et al., 2022). Soil microbes are a potential source of novel antibiotics (Cycoń et al., 2019). In fact, many antibiotics currently in use were first identified in soil microorganisms. These microorganisms, evolving over millions of years, have generated a diverse variety of secondary metabolites exhibiting po-

tent antibacterial activity (Demoling et al., 2007). Identifying and characterizing novel antibiotic compounds from soil bacteria may help address the issue of AMR. It may be possible to identify new classes of antibiotics with unique structures and modes of action by utilizing the potential of soil microorganisms. These antibiotics can aid in the fight against drug-resistant bacteria and boost the efficiency of existing treatments.

1.2 Hypothesis

Soil bacteria have the potential to be a source of important antimicrobial compounds for the development of new antibiotics to fight against pathogenic bacteria.

1.3 Objectives

1.3.1 General Objective

To assess the antimicrobial activity of soil strains isolated from the campus of the Universidad de Investigación de Tecnología Experimental Yachay (UITEY) against *Escherichia coli* ATCC 25927, as well as from clinical isolated strains: *E. coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

1.3.2 Specific Objectives

- To assess the abundance of soil bacteria through counting the Colony Forming Units (CFU) in different soil samples.
- To screen the antibiotic activity of soil bacteria against *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*.
- To evaluate the antimicrobial effect of dissolved metabolites of the growth of soil bacteria against *Escherichia coli, Pseudomonas aeruginosa*, and *Staphylococcus aureus*.

Chapter 2

Literature Review

2.1 Soil Bacteria Diversity

The world's most varied species are bacteria, and the topsoil, is where soil bacteria have been frequently isolated (Whitman et al., 1998). These microorganisms participate in intricate communities with fungi and archaea to sustain the edaphic life (Deveau et al., 2018). Since microbial communities decompose organic matter and promote the turnover of nutrients to the soil, they are essential to maintain the soil ecosystems. In addition, the diversity of soil bacteria is an intriguing and intricate subject that has drawn a lot of interest from the scientific community. There are millions of bacteria in one gram of soil, which shows how diverse soil microbes are. For instance, a gram of dry soil typically contains 108 to 109 bacterial cells, and molecular analysis have revealed that a single gram of soil includes 4000 to 7000 distinct bacterial genomes (Tate, 2020).

This diversity is influenced by factors such as the kind of soil, pH, moisture, temperature, and the presence of other microorganisms, among other variables that impact the diversity (Luo et al., 2021). The complexity of soil bacteria diversity is evident because there are more bacterial species in one gram of soil than plant species in the Amazon rain forest (Fonseca et al., 2018). Another aspect is the soil management practices, such as tillage, irrigation, and fertilization, which can influence on the physical and chemical properties of the soil and consequently alter the composition and diversity of soil bacteria (Hartman et al., 2008). Changes in the soil community composition can have an impact on the ecosystem's stability and sustainability (Ondreičková et al., 2018).

2.2 Importance of Soil Bacteria

The diversity of soil bacteria is critical for preserving the health of soil ecosystems, as they play a crucial role in their maintenance (Chen et al., 2020). The decomposition of organic materials, nutrient cycling, nitrogen fixation, or the production of antibiotics are only a few of the functions of the different soil genera of soil bacteria (Hemkemeyer et al., 2021). The diversity of soil bacteria makes them a promising source of novel bioactive compounds, including antibiotics (Table 2.1) (Hutchings et al., 2019). In fact, soil bacteria may produce a variety of antimicrobial substances, such as: polyketides, peptides and terpenoids (Srinivasan et al., 2021). These compounds have been successful in the treatment of pathogenic bacteria, including multidrug resistant strains. Among these microorganisms, the genus *Streptomyces* has been considered as a potential source for this type of compounds (Chater, 2016). Some important antibiotics produced by this genus are streptomycin, tetracycline and erythromycin, among others (Schatz et al., 2005; Petkovic et al., 2006; Washington and Wilson, 1985).

2.3 Antibiotic Resistance Soil Bacterial Genes

Antibiotic resistance genes (ARGs) constitute a fascinating and diverse collection of genetic components crucial to the intricate soil ecosystem. These genes can be either intrinsic (originating from the host bacteria) or acquired (via horizontal gene transfer) resistance mechanisms (Hu et al., 2017). They possess a remarkable ability to equip bacteria with defenses against various challenges, ranging from heavy metal contamination to excessive antibiotic and pesticide usage (Hawkins et al., 2018; Sun et al., 2021). Through intricate genetic pathways, these genes empower bacteria to counteract the adverse effects of these compounds, ensuring their survival and enabling adaptation to harsh soil environments.

The pressure of antibiotic selection can prompt resistant bacteria to acquire ARGs from the environmental resistome (Sultan et al., 2018; Wright, 2007). The resistome encompasses all ARGs present in both pathogenic and non-pathogenic bacteria within a microbial community, a concept introduced by D'Costa et al. (2006). Furthermore, the widespread use of antibiotics has given rise to resistant and multi-antibiotic-resistant bacteria (MAR). These MARs contain genes that confer resistance to common antibiotic classes, such as aminoglycosides, amphenicols, β -lactams, sulfonamides, and tetracycline (Forsberg et al., 2012).

Table 2.1: Natural antibiotics produced by bacteria and fungi.

Family	Bacteria Species	Antibiotic Produced
Micromonosporaceae	*Dactylosporangium aurantiacum*	Fidaxomicin
	Micromonospora purpurea	Gentamicin
Paenibacillaceae	*Bacillus brevis*	Gramicidin A
	Bacillus subtilis	Bacitracin A
	Paenibacillus polymyxa	Colistin
Pseudomonadaceae	*Pseudomonas fluorescens*	Mupirocin
Pseudonocardiaceae	*Amycolatopsis orientalis*	Vancomycin
	Saccharopolyspora erythraea	Erythromycin
Streptomycetaceae	*Streptomyces aureofaciens*	Tetracycline
	Streptomyces fradiae	Fosfomycin
	Streptomyces griseus	Streptomycin
	Streptomyces kanamyceticus	Kanamycin A
	Streptomyces orchidaceus	Seromycin
	Streptomyces pristinaespiralis	Pristinamycin
	Streptomyces puniceus	Viomycin
	Streptomyces roseosporus	Daptomycin
	Streptomyces tenebrarius	Tobramycin
	Streptomyces venezuelae	Chloramphenicol

Family	Fungi Species	Antibiotic Produced
Hypocreaceae	*Cephalosporium acremonium*	Cephalothin
Nectriaceae	*Fusidium coccineum*	Fusidic Acid
	Fusarium lateritium	Fusafungine
Trichocomaceae	*Penicillium* sp.	Penicillium

Note. Adapted from "Antibiotics: past, present and future," by Hutchings et al., 2019, *Current Opinion in Microbiology*, 51:72–80 (https://doi.org/10.1016/j.mib.2019.10.008), CC-BY-NC.

Remarkably, bacteria use a wide range of resistance mechanisms, including active drug efflux, inactivating a drug, modifying a drug target, and limiting drug uptake to counteract the toxic impacts of pollutants (Reygaert, 2018). Among the resistance genes found in soil bacteria are those coding for blaTEM, mecA, and vanA, among others (da Rocha et al., 2014; Schwendener and Perreten, 2022).

The astounding adaptability and versatility of bacteria are evident in their ability to withstand drugs, toxins, and assaults from other organisms. To ensure their survival, they must effectively manage these challenges. Understanding the interconnections between resistance genes in soil bacteria holds potential benefits for human health and agricultural practices. By decoding the genetic information within these genes, scientists can develop novel strategies to address antibiotic resistance and advance sustainable soil management.

2.4 The Problem of Antibiotic Resistance

The global health catastrophe of antibiotic resistance seriously threatens worldwide public health (Salam et al., 2023). It is a complicated and multidimensional issue that calls for a multifaceted solution. Infections caused by antibiotic-resistant bacteria, such as respiratory diseases, urinary tract infections, and skin and soft tissue infections, are now commonly seen in healthcare facilities (Lushniak, 2014). Physicians are running out of antibiotic alternatives to treat infectious diseases due to the inefficacy of many previously dispensable antibiotics.

Misuse of antibiotics is the primary driver of antibiotic resistance, as Sir Alexander Fleming also warned that "public demand (for the drug) will initiate an era of abuses." (Lushniak, 2014). In fact, studies have shown that between 30 % and 50 % of the treatment indications, drugs prescription, or duration of therapy have been unnecessary or even incorrectly applied (Fridkin et al., 2014; Ohl and Luther, 2011). In addition, inappropriate antibiotic prescriptions expose specific patients to potential side effects of antibiotic therapy without providing any therapeutic benefit (Lushniak, 2014). Due to this excessive use, rates of antibiotic excretion and environmental release have significantly increased, which has led to an increase in the appearance of antibiotic-resistant bacterial strains (Serwecińska, 2020).

Additionally, the issue of antibiotic resistance affects not only humans but also animals and the environment. Antibiotic resistance is greatly exacerbated by the use of antibiotics in livestock farms as prophylactics, growth promoters and drugs (Mann et al., 2021). At least 30 distinct

types of antibiotics, primarily macrolides, penicillins, and tetracyclines, are often employed in agriculture and cattle production (Laxminarayan and Teillant, 2015). Aquaculture is another area where antibiotics are used indiscriminately, commonly for the growth of fish, shrimp, and shellfish. These practices cause the entrance of enormous doses of antibiotics into the aquatic environment (Cabello, 2006). These chemical compounds may remain for a very long time in this ecosystem favoring the horizontal transference of antimicrobial-resistant genes among sediments and watercourses bacteria (Lushniak, 2014).

Antibiotics are currently scarce, with fewer available to treat infections. Therefore, the human, animal, and environmental health sectors must work together to confront the global health catastrophe brought on by antibiotic resistance. Furthermore, it is critical to understand that infections that are resistant to these compounds are more common and more challenging to treat. Hence, medical professionals should only recommend antibiotics when necessary. Otherwise, these infections will thus increase the risk of mortality and lengthen hospital stays increasing healthcare costs (Lee et al., 2020).

Chapter 3
Methodology

3.1 Study Area

Soil samples were collected from Centro de Desarrollo e Innovación Agroalimentario Yachay (CEDIY) in October 2022. This site is located in Urcuqui, Ecuador (0°24.4430'N and 78°10.2790'W) (Appendix 1, Figure 7.1). The sampling area has low rainfall and high solar radiation. In terms of vegetation, there was low diversity with predominance of Poaceae and Rosaceae plant families. Randomly, two areas of 100 m^2 were chosen for this study. The coordinates of the first area were at 0°24.0100'N and 78°10.6640'W, while the second area is located at 0°23.9960'N and 78°10.6650'W.

3.2 Soil Sampling

Sampling was carried out from the top 20 cm of soil with a metal auger. From each area, three compound samples constituted by 5 sub samples were collected as shown in Figure 3.1. Each compound sample was placed in a plastic bag, transported and stored in the laboratory at 4 °C. Soil temperature was measured in situ using a digital thermometer.
All experiments were carried out in the laboratory of UITEY campus. Soil pH was measured from a suspension of soil in distilled water.

3.3 Bacterial Isolation

Ten grams of soil of each compound sample were diluted in 90 mL of sterile distilled water and serial dilutions were performed (Figure 3.2). One hundred microliters were inoculated on nutrient agar (Sigma Aldrich) and spreaded by using a Digralsky spreader before incubating the plates at 30°C overnight. This process was repeated three times to guarantee the accuracy of

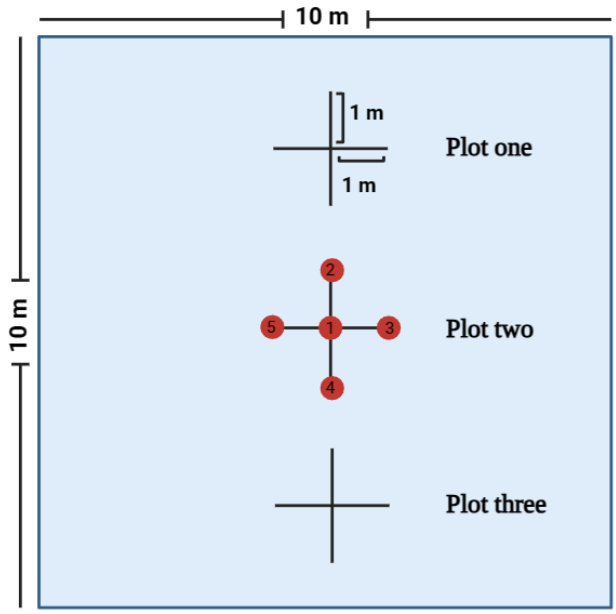

Figure 3.1: Graphic representation of the sampling method. There were three plots in each area constituted by 5 subsamples (represented by red dots).

the results. Colonies counting was performed manually. To isolate pure cultures, each unique colony morphotype was picked up with a bacteriological loop, spread on nutrient agar plate, and incubated as described above.

3.4 Characterization of Strains

The bacterial colonies were characterized regarding their color, size, elevation, shape, and border. Additionally, Gram staining and catalase test were performed in order to differentiate the morphotypes.

The data obtained was then grouped together to create a heat map in which each value of each variable was assigned a range from 0 to 1. For example, the size variable is divided into small, medium, and large, where the value of 0 corresponds to small, 0.5 to medium, and 1 to large. The other variables were categorized in the same way.

3.5 Assessment of the Antimicrobial Effect

The antimicrobial assays were conducted in the following ways: In the first part, the test strains (*E. coli* ATCC 25927 and the clinical isolates of *E. coli, S. aureus*, and *P. aeruginosa*) were cultured in nutrient broth until they reached and absorbance greater than zero. Each test strain was

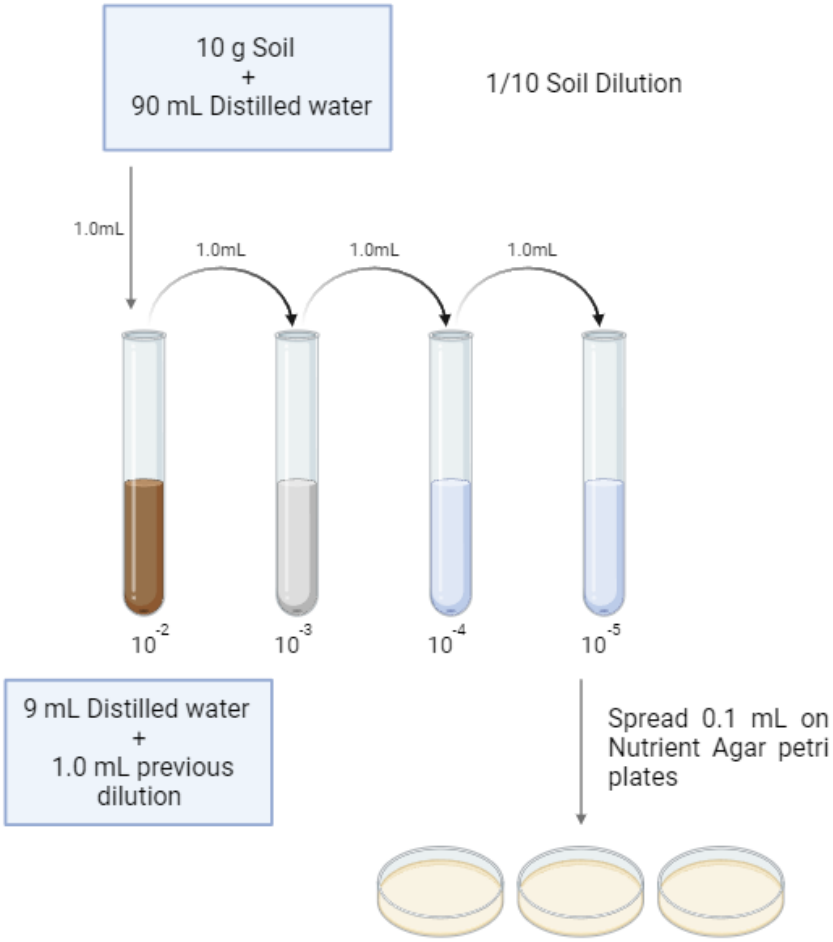

Figure 3.2: Outline of the serial dilution method for determining the number of bacteria per gram of soil.

inoculated on nutrient agar by running the swab over the surface three times, then the soil bacteria were inoculated into the petri dish. Figure 3.3 represents how this methodology was made. The plates were incubated at 30 °C overnight. Positive results were verified by the appearance of inhibition zones.

To assess the second part of the antimicrobial assay of the soluble compounds secreted by the soil bacteria filtrate was prepared using nutrient broth and the soil strains, which were incubated overnight to allow the release of their metabolites into the medium. To standardize the effect of the soluble compounds with the test strains, the absorbance of these strains was about 0.3 in nutrient broth.

Subsequently, 1.5 mL of the filtrate from each soil bacterial strain was taken and mixed with 0.5 mL of the test strains. For the growth control, 1.5 mL of nutrient broth was taken and mixed with 0.5 mL of the test strains (Figure 3.4). The mixture was then incubated overnight and sub-

sequently inoculated on nutrient agar. Finally, the colonies were counted and compared with the control group. For the first control, 1.5 mL of nutrient broth was taken and mixed with 0.5 mL of the four test strains, while for the second control, 2 mL of nutrient broth was taken. Both controls were seeded on nutrient agar for comparison. This whole procedure was repeated in triplicates.

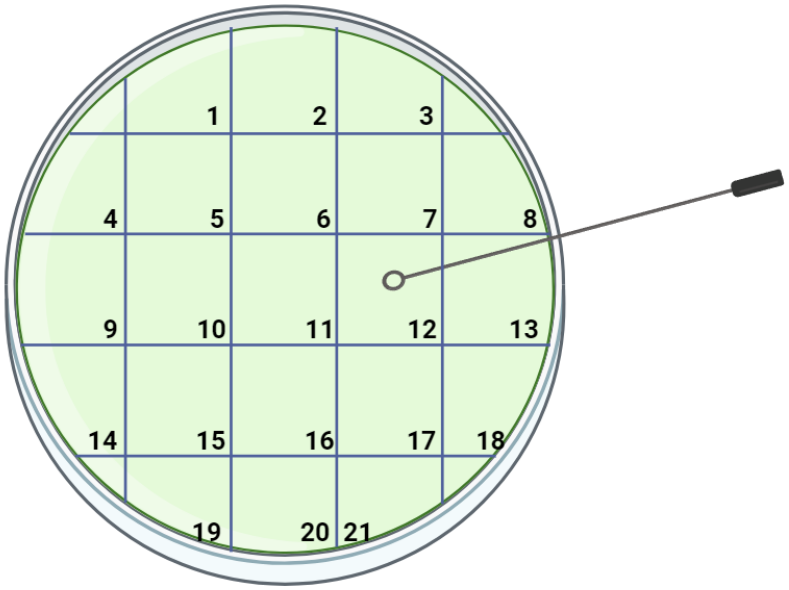

Figure 3.3: Graphic representation of the first part of the antimicrobial assay.

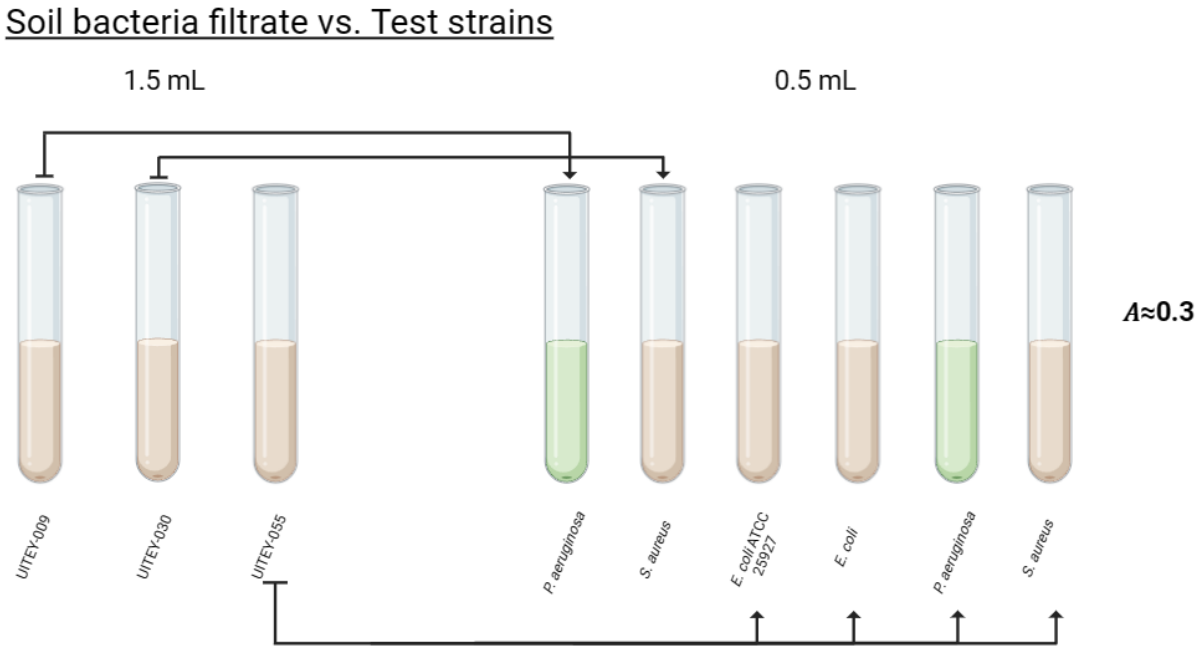

Figure 3.4: Graphic representation of the second part of the antimicrobial assay.

3.6 Statistical Analysis

Data analysis was done using Python program. The variables were examined using the Mann-Whitney U test due to the small number of replicates (Apprendix 3, Table 7.2). It was assumed that the means of the groups that were tested were equivalent to the control mean. $P < 0.05$ was used to define statistical significance. Shapiro-Wilk's test was also used to evaluate the normal distribution (Apprendix 3, Table 7.3).

Chapter 4

Results

4.1 Isolation of Soil Bacteria

The results of the process of bacterial isolation showed interesting patterns in the richness of the soil strains between the first and the second area of the study carried out in Urcuquí, Ecuador. In the first area (P-1), the mean CFU/g values obtained from the 10^{-4} dilution were found to be 16.5×10^6, indicating a moderate bacterial population there. The mean CFU/g number in the second area (P-2), however, was significantly higher at 18.8×10^6, indicating a relatively larger bacterial abundance. The particular CFU/g numbers collected for each sampling site within both locations are presented in Table 4.1 to give a more thorough perspective. At the end of this procedure, a total of seventy-one different bacterial strains were isolated from the composite soil.

In addition, the pH of each of the points in each area was measured and it was found that soil's pH average values varied from 7.52 to 7.86 describing the soil as slightly alkaline.

Table 4.1: CFU/g soil at each point in the two areas of the sampling area. P1-1, P1-2, and P1-3 represent each of the plots from the first area where the samples were collected. P2-1, P2-2, and P2-3 correspond to the second area.

Area	**CFU/g soil**
P1-1	20.1×10^6
P1-2	9.5×10^6
P1-3	19.9×10^6
P2-1	33.6×10^6
P2-2	19.1×10^6
P2-3	3.8×10^6

4.2 Characterization of Soil Bacteria

The bacteria found in both areas were characterized to analyze the microbial diversity in these two regions to and understand their distribution. After the characterization process (Appendix 1, Table 7.1), it was found that there is a main division. Figure 4.1 shows the clustering based on morphological studies, classifying the isolates into several clusters representing phenotypically related bacterial groups.

Within each clade, it is also observed that there are several groups according to the variables they share. However, it was found that there is a clade comprised only of bacteria with irregular colony borders, while another clade is dominated by bacteria with entire colony borders. The same occurs with the shape variable, where one clade was found to predominate circular shaped bacteria compared to the other, where there was a predominance of irregular shaped bacteria.

Although these bacteria were taken from two different areas, these results may suggest that there is no certain type of relationship, as factors such as pH and vegetation do not influence the location of the bacteria, that is, they were not site-specific. That is why, in Figure 4.1, the distribution of bacteria does not follow a specific order since bacteria from the two areas make up one of the two main clades.

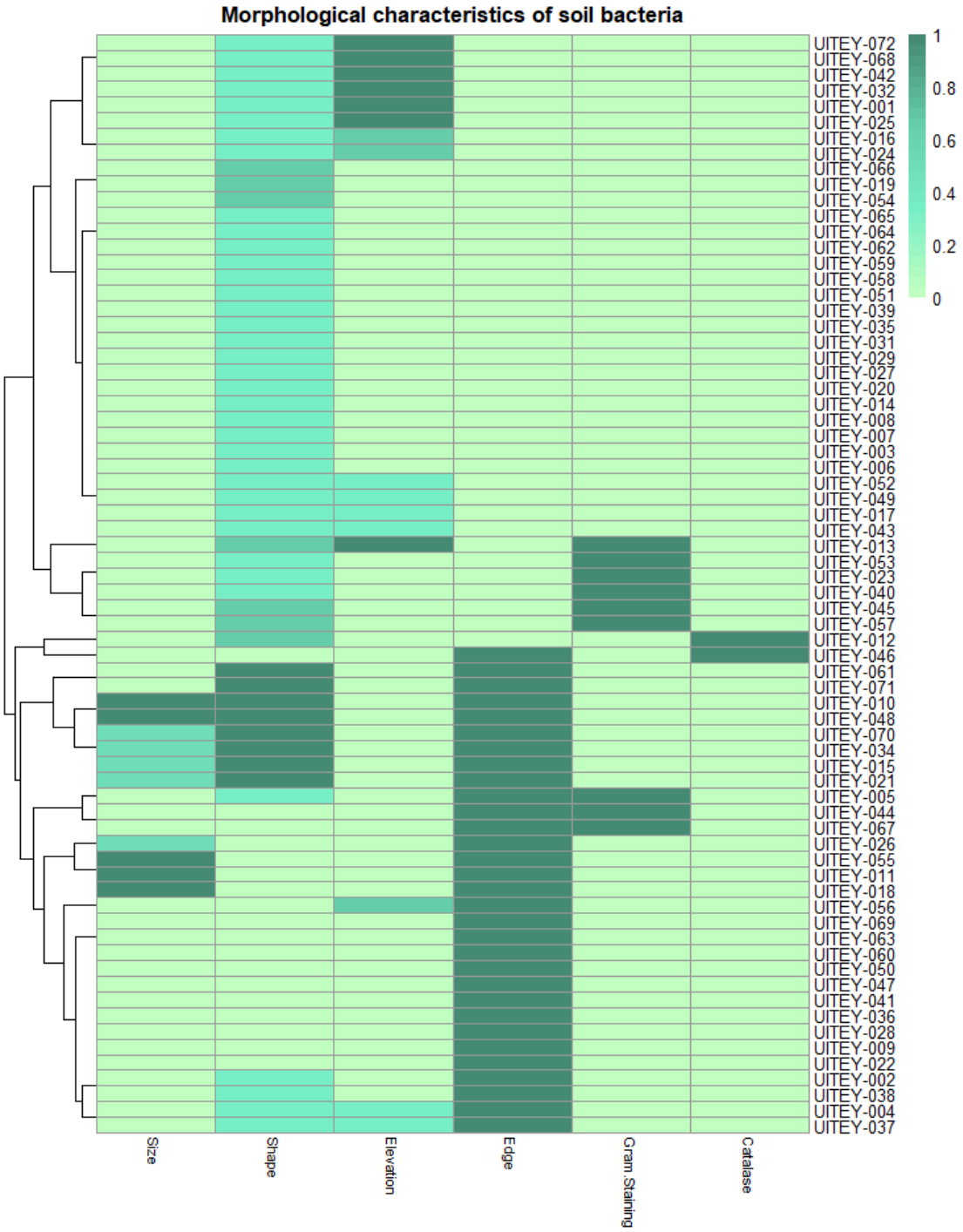

Figure 4.1: A heat map with a dendrogram showing the grouping of 71 soil bacteria according to their morphological characteristics. The color scale represents the value of each of the variables (size, shape, elevation, edge, Gram staining, and catalase type), which were grouped in a range from 0 to 1.

4.3 Assessment of the Antimicrobial Effect

In order to evaluate the potential antibacterial activity of the metabolites synthesized by the soil bacteria strains, *E. coli* ATCC 25927, *E. coli, S. aureus*, and *P. aeruginosa* were exposed against soil bacteria. Results of this first screening indicate that only three (4,22 %) of the 71 strains tested demonstrated antimicrobial activity. The phenotypic identification of the strains was performed in the laboratory of Dr. Miguel Viñas at the University of Barcelona. Corresponding to the strains:

- UITEY-009: *Bacillus cereus*
- UITEY-030: *Bacillus circulans*
- UITEY-055: *Bacillus mycoides*

Regarding the UITEY-009 strain, in the presence of *P. aeruginosa*, it exhibited a slightly inhibitory activity. The number of *P. aeruginosa* colonies did not decrease; however, within the colonies of UITEY-009, there were no *P. aeruginosa* colonies. Meanwhile, the UITEY-030 strain displayed remarkably strong activity when exposed to *S. aureus*, as evidenced by the formation of an inhibition zone around the soil strain on the agar plate. This activity was observed exclusively in this particular soil strain.

Moving forward, the UITEY-055 strain was tested against all the strains under examination. UITEY-055 exhibited no evidence of antimicrobial activity against *E. coli* ATCC 25927, as colonies of *E. coli* ATCC 25927 flourished alongside the soil bacteria. However, when confronted with *E. coli, S. aureus*, and *P. aeruginosa*, UITEY-055 displayed no microbial activity, akin to what was observed with UITEY-009. Within the colonies, there was an absence of the confronted strains. The results of this assay are presented in Figure 4.2.

Based on the previous findings, a subsequent assay was conducted with corresponding controls to validate the results (Figure 4.3). The CFU/mL obtained from the UITEY-009 strain against *P. aeruginosa* exhibited no significant inhibition. The number of colonies in the presence of *P. aeruginosa* remained nearly the same as in the control group ($P>0.5$). This confirmed the absence of inhibitory substances and showed no antimicrobial activity of the soil strain. Conversely, when comparing the UITEY-030 strain with *S. aureus* notable activity was observed; the

count of *S. aureus* colonies significantly diminished when exposed to the metabolites of the soil strain (P>0.5). The control group affirmed the presence of a compound preventing the proliferation of *S. aureus* colonies. Lastly, the UITEY-055 strain demonstrated a discernible effect only in the presence of *E. coli*, as the count of colonies decreased (P>0.5). The disparity between confrontation and control is also visible in Figure 4.3, G and H.

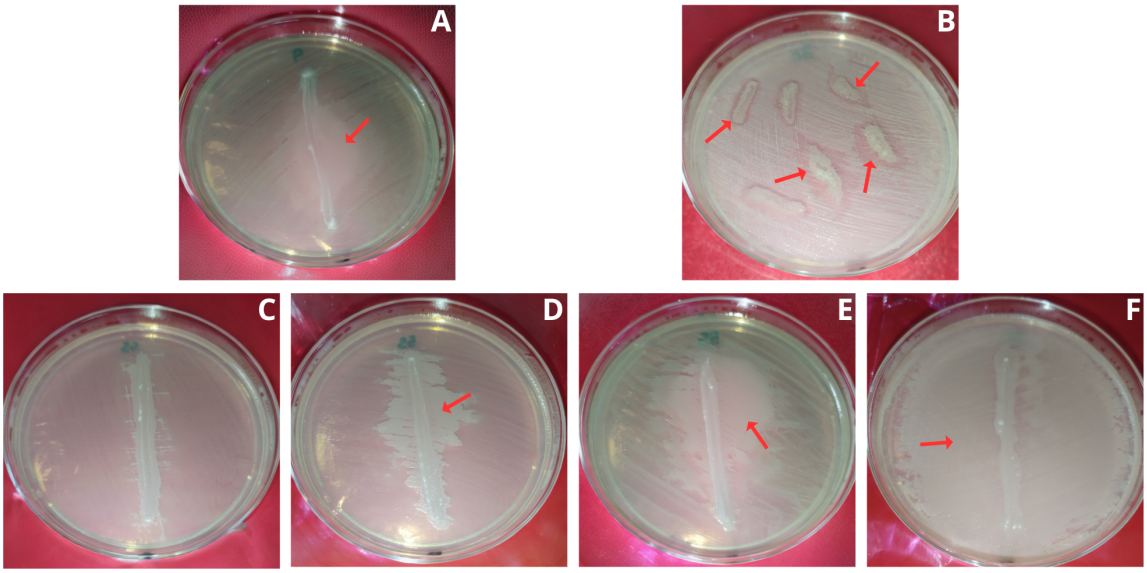

Figure 4.2: Soil isolates showing antimicrobial activity. A represents UITEY-009 strain against *P. aeruginosa*, B corresponds to UITEY-030 strain against *S. aureus*, while C, D, E and F correspond to UITEY-055 strain that was tested against *E. coli* ATCC 25927, *E. coli, S. aureus*, and *P. aeruginosa*, respectively.

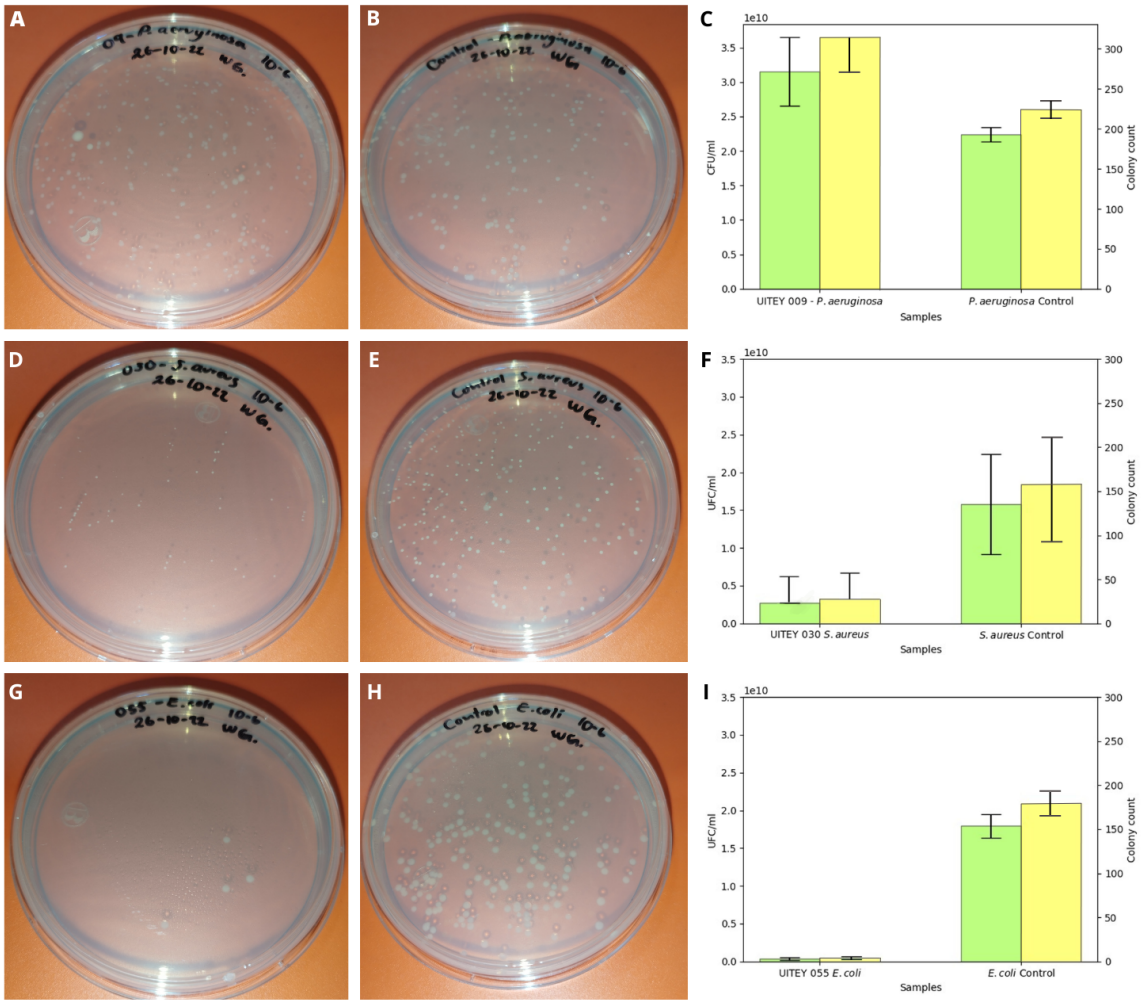

Figure 4.3: Soil strain filtrate inhibition assay. A and B represent the confrontation between the filtrate of UITEY-009 strain and *P. aeruginosa*, where B is the growth control for *P. aeruginosa*. Similarly, D and E represent the confrontation between UITEY-030 filtrate and *S. aureus* along with its control. Finally, G and H refer to strain UITEY-055 filtrate vs. *E. coli*, also with its control. Bar graphs (C, F and I) show CFU/ml (green bar) and the number of colonies (yellow bar) of the three confrontations.

Chapter 5

Discussion

Further research into the discovery and characterization of soil bacterial strains holds significant potential, including the examination of their functional traits, ecological roles, and prospective applications across various sectors. Due to the escalating number of bacteria that have developed antibiotic resistance in recent years, one of the primary research priorities is the identification of new antibiotics (Sitotaw et al., 2022). Consequently, the quest for novel compounds with antimicrobial potential against emerging pathogens is of utmost global urgency (Terreni et al., 2021). Bacteria extracted from soil play a crucial role in this field. However, in recent years, the search for these bacterial strains has expanded to new environments, such as marine ecosystems, mangroves and even forest ecosystems (Stonik et al., 2020; Hamid et al., 2015; Sharma and Thakur, 2020).

Antibiotic resistance by *Staphylococcus aureus* is a significant worldwide public health concern (Rungelrath and DeLeo, 2021). *S. aureus* can cause a wide range of infections, from mild skin issues to severe diseases like pneumonia, sepsis and endocarditis (Ahmad-Mansour et al., 2021; He and Wunderink, 2020; Kwiecinski and Horswill, 2020). *S. aureus* can acquire resistance to several antibiotics in the penicillin family. These include methicillin, which is the most common form of resistance (Larsen et al., 2022). A study conducted in India concludes that methicillin resistance is a public health problem that affects developing countries more than developed countries. The latter have greater ease and access to medicines and thus avoid inappropriate use of antibiotics (Nazar et al., 2019). Similarly, *Pseudomonas aeruginosa*, a bacterium commonly found in the environment, can lead to human infections, particularly in patients with compromised immune systems or within hospital settings (Wu et al., 2015). This bacterium acquires resistance genes through the horizontal transfer of genetic material with other bacteria (Botelho et al., 2019). Likewise, *Escherichia coli*, a bacterium inhabiting the gut of animals and humans,

can induce life-threatening conditions such as sepsis, gastroenteritis, and urinary tract infections (Nielsen et al., 2021). While many strains are indeed harmless, *E. coli* multidrug resistance has emerged as an increasingly concerning issue in both human and veterinary medicine (Poirel et al., 2018). According to a study, multidrug-resistant *Escherichia coli (E. coli)* is alarmingly common in Chinese pig farms. The isolates had diverse resistance genes, virulence factors, and plasmids, and they showed resistance to last-resort drugs. In order to slow the emergence of antimicrobial resistance, the findings highlight the urgent need for robust antimicrobial stewardship and control measures (Peng et al., 2022). Therefore, the purpose of this study was to isolate soil bacteria that have the potential to inhibit the growth of strains such as *E. coli* ATCC 25927, *E. coli, S. aureus*, and *P. aeruginosa*. These soil strains were obtained from Urcuquí, Imbabura.

After performing the antimicrobial assays, it was established that only two bacterial strains (UITEY-030 and UITEY-055) exhibited antimicrobial activity (Figure 4.3); the data indicate that when confronted against *S. aureus* and *E. coli*, respectively, there is a decline in the number of colonies of the test strains. A review article published in 2021 also found that out of 263 bacterial isolates found in soil samples from India, only 3 showed any antibacterial action toward the human pathogens *S. aureus, E. coli, P. aeruginosa*, and *K. pneumoniae* (Prashanthi et al., 2021). This indicates that few bacteria exhibit antimicrobial activity. Furthermore, the antagonistic activity of these strains was shown to be different in the first screening, since in the first assay strain UITEY-030 produced an inhibition zone and strain UITEY-055 produced a kind of growth monopolization; this could be due to the existence of a different secondary metabolite released by each strain when is confronted. This is supported by a study that demonstrated that interspecific interactions have a significant impact on the antimicrobial activity of soil bacteria. The study found that a substantial proportion of bacterial isolates showed antimicrobial activity only in monoculture, while others showed activity only when tested in interactions (Tyc et al., 2014). The specific processes and mechanisms by which these bacteria inhibit the growth of pathogens should be clarified through further study. Understanding the mechanism of action of the secondary metabolites produced by these strains might help to understand how effective they might be against other bacteria.

Regarding the Mann-Whitney U test, the p-value for UITEY-009 strain against *P. aeruginosa* was not statistically significant, indicating that the mean of the confrontation group against the control group is equal. This is corroborated by the number of colonies since a relatively similar

number of colonies is visually observed in the petri dish of both groups (Figure 4.3, A and B). It is important to note that this result was expected since the soil strain does not possess antimicrobial metabolites and therefore both groups are similar. The p-value for UITEY-030 strain against *S. aureus* was also not statistically significant. This was unexpected because in Figure 4.3, both D and F, it is clearly observed that there was a very notable difference in the confrontation and control group. These results might be attributed to the relatively small number of replicates employed in the experiment, which might have reduced the statistical power to identify a significant difference. In the case of UITEY-055 strain against *E. coli*, the p-value was also not statistically significant, however it was observed a clearly difference in the number of colonies in the confrontation respect to the control. Despite the result, this may show that the UITEY-055 strain produces bioactive substances or metabolites to prevent *E. coli* from growing. Therefore, we can infer that UITEY-030 and UITEY-055 strains may have the ability to produce bioactive compounds or metabolites with the capacity to inhibit the growth of pathogenic bacteria. Identifying the specific metabolites produced by these strains and how they interact with the other bacteria to inhibit their growth is work that may take shape in the future. To understand the processes underlying the inhibitory effects, this could entail molecular research and chemical analyses.

In addition, it is interesting to notice that the strains UITEY-030 and UITEY-055 are both members of the genus *Bacillus* sp. This genus is widely recognized for producing antibiotics with antagonistic activity against various bacterial and fungal infections (Sansinenea and Ortiz, 2011). They are also characterized by releasing a variety of secondary metabolites, such as siderophores, antibiotics and antifungals (Sansinenea and Ortiz, 2011). These metabolites can influence the rhizosphere microbiota, creating a hostile environment for pathogens or activating host defensive mechanisms (Velusamy and Gnanamanickam, 2008). In a study, it was also found that bacterial species belonging to this genus demonstrated the ability to inhibit the growth of *E. coli* and *S. aureus*, leading to the determination that the antibiotic produced by the strains is a broad-spectrum antibiotic (Sandhya et al., 2015).

On the other hand, it can be inferred that the bacterial diversity found in the soil demonstrates the complexity of microbial communities and suggests that the distribution of bacteria may be influenced by causes other than environmental ones (Figure 4.1). It is known that agricultural practices, urbanization, and deforestation are anthropogenic processes that can interfere with the bacterial composition of an area. Moreover, factors such as pH, water quantity, and nutrient

availability are affected by these processes. Additionally, the presence of plant species also has an impact on microbial communities. By influencing the quantity and quality of litter, plants can alter soil microbial populations, creating competition for nutrients, particularly carbon compounds (Rousk et al., 2010). This is one of the primary reasons why bacteria found in one area can also be found in another area. There is no specific location because the aforementioned factors have contributed to a relatively homogeneous distribution in both locations.

These findings contribute to a broader understanding of Ecuador's soil microbiota and lay the foundation for future research on the ecological functions and potential applications of these diverse bacterial communities. Understanding bacterial diversity in soil and their ability to inhibit pathogen growth may have applications in agriculture and biotechnology, such as the development of biological control agents for plant diseases (Hayat et al., 2010). Finally, it is important to recognize the limitations of this study because the samples were taken from a specific region of Ecuador. The results obtained are not representative of the entire country's soil bacterial diversity; however, they are still relevant and important.

Chapter 6
Conclusions

In conclusion, the study conducted at the Universidad de Investigación de Tecnología Experimental Yachay campus provides information on the variety of flourishing bacteria in soil ecosystems and their potential as sources of novel antibiotics. The viable bacterial count present in the soil was measured using the UFC of the two study areas, giving a notion of the number of bacteria in 100 m^2. Additionally, 71 soil bacterial strains were distinguished and isolated from both areas, which were used for subsequent antimicrobial activity testing.

After performing antimicrobial assays, the data showed that some of the soil isolates (UITEY-030 and UITEY-055) obtained exhibited a spectrum of antimicrobial effects against pathogenic strains, such as *S. aureus* and *E. coli*, indicating the value of additional research into these bacteria to pinpoint and define the bioactive substances causing their antimicrobial activity. These compounds are effective against many pathogenic bacteria, including multidrug-resistant strains, so it is essential to continue investigating soil bacteria's potential, as well as to identify the mechanisms of action of these compounds and optimize the conditions for their production. Using soil bacteria to produce new antibiotics could be a promising approach to combating antibiotic resistance and developing new treatments for infectious diseases.

The discovery of these soil bacteria underlines the potential of these microbes in the hunt for novel antimicrobial treatments. Future studies could identify and characterize these inhibitory substances, analyze their action methods, and investigate their potential applications in antimicrobial therapy and other relevant disciplines. Additionally, more composite soil samples from other environments with increased vegetation cover are recommended to expand the study and explore a wider range of microbial diversity. To combat bacterial resistance, it is also crucial to look into the mechanisms of action of inhibitory substances and run synergy studies to increase their effects. Overall, the study's findings highlight the importance of continued research into

soil bacteria's potential as a source of new antibiotics and the need to develop effective strategies to combat antibiotic resistance.

Bibliography

Ahmad-Mansour, N., Loubet, P., Pouget, C., Dunyach-Remy, C., Sotto, A., Lavigne, J.-P., and Molle, V. (2021). Staphylococcus aureus toxins: An update on their pathogenic properties and potential treatments. *Toxins*, 13(10):677.

Almajano, M., Carbó, R., Delgado, M., and Gordon, M. (2007). Effect of pH on the antimicrobial activity and oxidative stability of oil-in-water emulsions containing caffeic acid. *Journal of Food Science*, 72(5):C258–C263.

Aminov, R. I. (2010). A brief history of the antibiotic era: Lessons learned and challenges for the future. *Frontiers in Microbiology*, 1.

Atanasov, A. G., , Zotchev, S. B., Dirsch, V. M., and Supuran, C. T. (2021). Natural products in drug discovery: advances and opportunities. *Nature Reviews Drug Discovery*, 20(3):200–216.

Bach, E. M., Williams, R. J., Hargreaves, S. K., Yang, F., and Hofmockel, K. S. (2018). Greatest soil microbial diversity found in micro-habitats. *Soil Biology and Biochemistry*, 118:217–226.

Bibi, F., Faheem, M., Azhar, E., Yasir, M., Alvi, S., Kamal, M., Ullah, I., and Naseer, M. (2017). Bacteria from marine sponges: A source of new drugs. *Current Drug Metabolism*, 18(1):11–15.

Botelho, J., Grosso, F., and Peixe, L. (2019). Antibiotic resistance in pseudomonas aeruginosa – mechanisms, epidemiology and evolution. *Drug Resistance Updates*, 44:100640.

Cabello, F. C. (2006). Heavy use of prophylactic antibiotics in aquaculture: a growing problem for human and animal health and for the environment. *Environmental Microbiology*, 8(7):1137–1144.

Chater, K. F. (2016). Recent advances in understanding streptomyces. *F1000Research*, 5:2795.

Chen, Q.-L., Ding, J., Zhu, Y.-G., He, J.-Z., and Hu, H.-W. (2020). Soil bacterial taxonomic diversity is critical to maintaining the plant productivity. *Environment International*, 140:105766.

Cycoń, M., Mrozik, A., and Piotrowska-Seget, Z. (2019). Antibiotics in the soil environment—degradation and their impact on microbial activity and diversity. *Frontiers in Microbiology*, 10.

da Rocha, C. N., Varela, A. R., Schwartz, T., Nunes, O. C., and Manaia, C. M. (2014). blaTEM and vanA as indicator genes of antibiotic resistance contamination in a hospital–urban wastewater treatment plant system. *Journal of Global Antimicrobial Resistance*, 2(4):309–315.

Danquah, C. A., Minkah, P. A. B., Junior, I. O. D., Amankwah, K. B., and Somuah, S. O. (2022). Antimicrobial compounds from microorganisms. *Antibiotics*, 11(3):285.

D'Costa, V. M., McGrann, K. M., Hughes, D. W., and Wright, G. D. (2006). Sampling the antibiotic resistome. *Science*, 311(5759):374–377.

Demoling, F., Figueroa, D., and Bååth, E. (2007). Comparison of factors limiting bacterial growth in different soils. *Soil Biology and Biochemistry*, 39(10):2485–2495.

Deveau, A., Bonito, G., Uehling, J., Paoletti, M., Becker, M., Bindschedler, S., Hacquard, S., Hervé, V., Labbé, J., Lastovetsky, O. A., Mieszkin, S., Millet, L. J., Vajna, B., Junier, P., Bonfante, P., Krom, B. P., Olsson, S., van Elsas, J. D., and Wick, L. Y. (2018). Bacterial–fungal interactions: ecology, mechanisms and challenges. *FEMS Microbiology Reviews*, 42(3):335–352.

Dhingra, S., Rahman, N. A. A., Peile, E., Rahman, M., Sartelli, M., Hassali, M. A., Islam, T., Islam, S., and Haque, M. (2020). Microbial resistance movements: An overview of global public health threats posed by antimicrobial resistance, and how best to counter. *Frontiers in Public Health*, 8.

Elbendary, A. A., Hessain, A. M., El-Hariri, M. D., Seida, A. A., Moussa, I. M., Mubarak, A. S., Kabli, S. A., Hemeg, H. A., and Jakee, J. K. E. (2018). Isolation of antimicrobial producing actinobacteria from soil samples. *Saudi Journal of Biological Sciences*, 25(1):44–46.

Fonseca, J. P., Hoffmann, L., Cabral, B. C. A., Dias, V. H. G., Miranda, M. R., de Azevedo Martins, A. C., Boschiero, C., Bastos, W. R., and Silva, R. (2018). Contrasting the microbiomes from forest rhizosphere and deeper bulk soil from an amazon rainforest reserve. *Gene*, 642:389–397.

Forsberg, K. J., Reyes, A., Wang, B., Selleck, E. M., Sommer, M. O. A., and Dantas, G. (2012). The shared antibiotic resistome of soil bacteria and human pathogens. *Science*, 337(6098):1107–1111.

Fridkin, S. K., Baggs, J., Fagan, R. P., Magill, S. S., Pollack, L. A., Malpiedi, P., Slayton, R. B., Khader, K., Rubin, M. A., Jones, M. L., Samore, M. H., Dumyati, G. K., Dodds-Ashley, E. S., Meek, J. I., Yousey-Hindes, K., Jernigan, J. A., Shehab, N., Herrera, R., McDonald, C. L., Schneider, A. K., and Srinivasan, A. (2014). Vital signs: Improving antibiotic use among hospitalized patients. *Morbidity and Mortality Weekly Report*, 63:194 – 200.

Grossman, T. H. (2016). Tetracycline antibiotics and resistance. *Cold Spring Harbor Perspectives in Medicine*, 6(4):a025387.

Hamid, A., Ariffin, S., and Mohamad, S. (2015). Identification and optimal growth conditions of actinomycetes isolated from mangrove environment. *Malaysian Journal of Analytical Sciences*, 19:904–914.

Hartman, W. H., Richardson, C. J., Vilgalys, R., and Bruland, G. L. (2008). Environmental and anthropogenic controls over bacterial communities in wetland soils. *Proceedings of the National Academy of Sciences*, 105(46):17842–17847.

Hawkins, N. J., Bass, C., Dixon, A., and Neve, P. (2018). The evolutionary origins of pesticide resistance. *Biological Reviews*, 94(1):135–155.

Hayat, R., Ali, S., Amara, U., Khalid, R., and Ahmed, I. (2010). Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of Microbiology*, 60(4):579–598.

He, H. and Wunderink, R. G. (2020). Staphylococcus aureus pneumonia in the community. *Seminars in Respiratory and Critical Care Medicine*, 41(04):470–479.

Hemkemeyer, M., Schwalb, S. A., Heinze, S., Joergensen, R. G., and Wichern, F. (2021). Functions of elements in soil microorganisms. *Microbiological Research*, 252:126832.

Hu, Y., Gao, G. F., and Zhu, B. (2017). The antibiotic resistome: gene flow in environments, animals and human beings. *Frontiers of Medicine*, 11(2):161–168.

Hutchings, M. I., Truman, A. W., and Wilkinson, B. (2019). Antibiotics: past, present and future. *Current Opinion in Microbiology*, 51:72–80.

Indraningrat, A., Smidt, H., and Sipkema, D. (2016). Bioprospecting sponge-associated microbes for antimicrobial compounds. *Marine Drugs*, 14(5):87.

Kwiecinski, J. M. and Horswill, A. R. (2020). Staphylococcus aureus bloodstream infections: pathogenesis and regulatory mechanisms. *Current Opinion in Microbiology*, 53:51–60.

Larsen, J., Raisen, C. L., Ba, X., Sadgrove, N. J., Padilla-González, G. F., Simmonds, M. S. J., Loncaric, I., Kerschner, H., Apfalter, P., Hartl, R., Deplano, A., Vandendriessche, S., Bolfíková, B. Č., Hulva, P., Arendrup, M. C., Hare, R. K., Barnadas, C., Stegger, M., Sieber, R. N., Skov, R. L., Petersen, A., Angen, Ø., Rasmussen, S. L., Espinosa-Gongora, C., Aarestrup, F. M., Lindholm, L. J., Nykäsenoja, S. M., Laurent, F., Becker, K., Walther, B., Kehrenberg, C., Cuny, C., Layer, F., Werner, G., Witte, W., Stamm, I., Moroni, P., Jørgensen, H. J., de Lencastre, H., Cercenado, E., García-Garrote, F., Börjesson, S., Hæggman, S., Perreten, V., Teale, C. J., Waller, A. S., Pichon, B., Curran, M. D., Ellington, M. J., Welch, J. J., Peacock, S. J., Seilly, D. J., Morgan, F. J. E., Parkhill, J., Hadjirin, N. F., Lindsay, J. A., Holden, M. T. G., Edwards, G. F., Foster, G., Paterson, G. K., Didelot, X., Holmes, M. A., Harrison, E. M., and Larsen, A. R. (2022). Emergence of methicillin resistance predates the clinical use of antibiotics. *Nature*, 602(7895):135–141.

Laxminarayan, R., T. V. B. and Teillant, A. (2015). The economic costs of withdrawing antimicrobial growth promoters from the livestock sector.

Lee, X. J., Stewardson, A. J., Worth, L. J., Graves, N., and Wozniak, T. M. (2020). Attributable length of stay, mortality risk, and costs of bacterial health care–associated infections in australia: A retrospective case-cohort study. *Clinical Infectious Diseases*, 72(10):e506–e514.

Luo, Y., Yuan, H., Zhao, J., Qi, Y., Cao, W.-W., Liu, J.-M., Guo, W., and Bao, Z.-H. (2021). Multiple factors influence bacterial community diversity and composition in soils with rare earth element and heavy metal co-contamination. *Ecotoxicology and Environmental Safety*, 225:112749.

Lushniak, B. D. (2014). Antibiotic resistance: A public health crisis. *Public Health Reports*, 129(4):314–316.

Mancuso, G., Midiri, A., Gerace, E., and Biondo, C. (2021). Bacterial antibiotic resistance: The most critical pathogens. *Pathogens*, 10(10):1310.

Mann, A., Nehra, K., Rana, J., and Dahiya, T. (2021). Antibiotic resistance in agriculture: Perspectives on upcoming strategies to overcome upsurge in resistance. *Current Research in Microbial Sciences*, 2:100030.

Mitosch, K. and Bollenbach, T. (2014). Bacterial responses to antibiotics and their combinations. *Environmental Microbiology Reports*, 6(6):545–557.

Moellering, R. C. (2011). Discovering new antimicrobial agents. *International Journal of Antimicrobial Agents*, 37(1):2–9.

Nazar, A., Imran, Y., Rastogi, V., and Singhal, P. (2019). Prevalence and antibiotic susceptibility pattern of methicillin resistant staphylococcus aureus (MRSA) at a tertiary care hospital from northern india. *International Journal of Current Microbiology and Applied Sciences*, 8(09):2352–2358.

Nielsen, K. L., Stegger, M., Kiil, K., Lilje, B., Ejrnæs, K., Leihof, R. F., Skjøt-Rasmussen, L., Godfrey, P., Monsen, T., Ferry, S., Hammerum, A. M., and Frimodt-Møller, N. (2021). Escherichia coli causing recurrent urinary tract infections: Comparison to non-recurrent isolates and genomic adaptation in recurrent infections. *Microorganisms*, 9(7):1416.

Nwobodo, D. C., Ugwu, M. C., Anie, C. O., Al-Ouqaili, M. T. S., Ikem, J. C., Chigozie, U. V., and Saki, M. (2022). Antibiotic resistance: The challenges and some emerging strategies for tackling a global menace. *Journal of Clinical Laboratory Analysis*, 36(9).

Ohl, C. A. and Luther, V. P. (2011). Antimicrobial stewardship for inpatient facilities. *Journal of Hospital Medicine*, 6(S1).

Ondreičková, K., Piliarová, M., Bušo, R., Hašana, R., Schreiber, Ľ., Gubiš, J., and Kraic, J. (2018). The structure and diversity of bacterial communities in differently managed soils studied by molecular fingerprinting methods. *Sustainability*, 10(4):1095.

Peng, Z., Hu, Z., Li, Z., Zhang, X., Jia, C., Li, T., Dai, M., Tan, C., Xu, Z., Wu, B., Chen, H., and Wang, X. (2022). Antimicrobial resistance and population genomics of multidrug-resistant escherichia coli in pig farms in mainland china. *Nature Communications*, 13(1).

Petkovic, H., Cullum, J., Hranueli, D., Hunter, I. S., Peric-Concha, N., Pigac, J., Thamchaipenet, A., Vujaklija, D., and Long, P. F. (2006). Genetics of streptomyces rimosus the oxytetracycline producer. *Microbiology and Molecular Biology Reviews*, 70(3):704–728.

Poirel, L., Madec, J.-Y., Lupo, A., Schink, A.-K., Kieffer, N., Nordmann, P., and Schwarz, S. (2018). Antimicrobial resistance in iescherichia coli/i. *Microbiology Spectrum*, 6(4).

Prashanthi, R., G.K., S., S., K., and L., M. (2021). Isolation, characterization, and molecular identification of soil bacteria showing antibacterial activity against human pathogenic bacteria. *Journal of Genetic Engineering and Biotechnology*, 19(1).

Prestinaci, F., Pezzotti, P., and Pantosti, A. (2015). Antimicrobial resistance: a global multifaceted phenomenon. *Pathogens and Global Health*, 109(7):309–318.

Reygaert, W. C. (2018). An overview of the antimicrobial resistance mechanisms of bacteria. *AIMS Microbiology*, 4(3):482–501.

Romero, D., Traxler, M. F., López, D., and Kolter, R. (2011). Antibiotics as signal molecules. *Chemical Reviews*, 111(9):5492–5505.

Rousk, J., Bååth, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., Knight, R., and Fierer, N. (2010). Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME Journal*, 4(10):1340–1351.

Rungelrath, V. and DeLeo, F. R. (2021). istaphylococcus aureus/i, antibiotic resistance, and the interaction with human neutrophils. *Antioxidants &amp Redox Signaling*, 34(6):452–470.

Salam, M. A., Al-Amin, M. Y., Salam, M. T., Pawar, J. S., Akhter, N., Rabaan, A. A., and Alqumber, M. A. A. (2023). Antimicrobial resistance: A growing serious threat for global public health. *Healthcare*, 11(13):1946.

Sandhya, M. S., Ramyakrishna, E., Divya, P. S., Kumar, A. P., Rajkumar, K., Yazein, E., and Burgula, S. (2015). Isolation of antibiotic producing bacteria from soil.

Sansinenea, E. and Ortiz, A. (2011). Secondary metabolites of soil bacillus spp. *Biotechnology Letters*, 33(8):1523–1538.

Schatz, A., Bugie, E., Waksman, S. A., Hanssen, A. D., Patel, R., and Osmon, D. R. (2005). The classic: Streptomycin, a substance exhibiting antibiotic activity against gram-positive and gram-negative bacteria. *Clinical Orthopaedics and Related Research*, &NA(437):3–6.

Schwendener, S. and Perreten, V. (2022). The bla and mec families of -lactam resistance genes in the genera macrococcus, mammaliicoccus and staphylococcus: an in-depth analysis with emphasis on macrococcus. *Journal of Antimicrobial Chemotherapy*, 77(7):1796–1827.

Serwecińska, L. (2020). Antimicrobials and antibiotic-resistant bacteria: A risk to the environment and to public health. *Water*, 12(12):3313.

Sharma, P. and Thakur, D. (2020). Antimicrobial biosynthetic potential and diversity of culturable soil actinobacteria from forest ecosystems of northeast india. *Scientific Reports*, 10(1).

Sitotaw, B., Ayalew, F., Girma, A., Mekonnen, A. B., Jardan, Y. A. B., Nafidi, H.-A., and Bourhia, M. (2022). Isolation and identification of promising antibiotic-producing bacteria. *Open Chemistry*, 20(1):1283–1291.

Skarżyńska, M., Leekitcharoenphon, P., Hendriksen, R. S., Aarestrup, F. M., and Wasyl, D. (2020). A metagenomic glimpse into the gut of wild and domestic animals: Quantification of antimicrobial resistance and more. *PLOS ONE*, 15(12):e0242987.

Srinivasan, R., Kannappan, A., Shi, C., and Lin, X. (2021). Marine bacterial secondary metabolites: A treasure house for structurally unique and effective antimicrobial compounds. *Marine Drugs*, 19(10):530.

Stapleton, P. D. and Taylor, P. W. (2002). Methicillin resistance in staphylococcus aureus: Mechanisms and modulation. *Science Progress*, 85(1):57–72.

Stonik, V. A., Makarieva, T. N., and Shubina, L. K. (2020). Antibiotics from marine bacteria. *Biochemistry (Moscow)*, 85(11):1362–1373.

Strathdee, S. A., Davies, S. C., and Marcelin, J. R. (2020). Confronting antimicrobial resistance beyond the COVID-19 pandemic and the 2020 US election. *The Lancet*, 396(10257):1050–1053.

Sultan, I., Rahman, S., Jan, A. T., Siddiqui, M. T., Mondal, A. H., and Haq, Q. M. R. (2018). Antibiotics, resistome and resistance mechanisms: A bacterial perspective. *Frontiers in Microbiology*, 9.

Sun, F., Xu, Z., and Fan, L. (2021). Response of heavy metal and antibiotic resistance genes and related microorganisms to different heavy metals in activated sludge. *Journal of Environmental Management*, 300:113754.

Tate, R. L. (2020). *Soil Microbiology*. Wiley.

Terreni, M., Taccani, M., and Pregnolato, M. (2021). New antibiotics for multidrug-resistant bacterial strains: Latest research developments and future perspectives. *Molecules*, 26(9):2671.

Tyc, O., de Jager, V. C., van den Berg, M., Gerards, S., Janssens, T. K., Zaagman, N., Kai, M., Svatos, A., Zweers, H., Hordijk, C., Besselink, H., de Boer, W., and Garbeva, P. (2017). Exploring bacterial interspecific interactions for discovery of novel antimicrobial compounds. *Microbial Biotechnology*, 10(4):910–925.

Tyc, O., van den Berg, M., Gerards, S., van Veen, J. A., Raaijmakers, J. M., de Boer, W., and Garbeva, P. (2014). Impact of interspecific interactions on antimicrobial activity among soil bacteria. *Frontiers in Microbiology*, 5.

Uddin, T. M., Chakraborty, A. J., Khusro, A., Zidan, B. R. M., Mitra, S., Emran, T. B., Dhama, K., Ripon, M. K. H., Gajdács, M., Sahibzada, M. U. K., Hossain, M. J., and Koirala, N. (2021). Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and

future prospects. *Journal of Infection and Public Health*, 14(12):1750–1766. Special Issue on Antimicrobial Resistance.

Velusamy, P. and Gnanamanickam, S. S. (2008). The effect of bacterial secondary metabolites on bacterial and fungal pathogens of rice. In *Secondary Metabolites in Soil Ecology*, pages 93–106. Springer Berlin Heidelberg.

Waller, D. G. and Sampson, A. P. (2018). Chemotherapy of infections. In *Medical Pharmacology and Therapeutics*, pages 581–629. Elsevier.

Washington, J. A. and Wilson, W. R. (1985). Erythromycin: A microbial and clinical perspective after 30 years of clinical use (second of two parts). *Mayo Clinic Proceedings*, 60(4):271–278.

Whitman, W. B., Coleman, D. C., and Wiebe, W. J. (1998). Prokaryotes: The unseen majority. *Proceedings of the National Academy of Sciences*, 95(12):6578–6583.

Wright, G. D. (2007). The antibiotic resistome: the nexus of chemical and genetic diversity. *Nature Reviews Microbiology*, 5(3):175–186.

Wu, W., Jin, Y., Bai, F., and Jin, S. (2015). Pseudomonas aeruginosa. In *Molecular Medical Microbiology*, pages 753–767. Elsevier.

Zada, S., Sajjad, W., Rafiq, M., Ali, S., Hu, Z., Wang, H., and Cai, R. (2021). Cave microbes as a potential source of drugs development in the modern era. *Microbial Ecology*, 84(3):676–687.

Zhang, Q.-Y., Yan, Z.-B., Meng, Y.-M., Hong, X.-Y., Shao, G., Ma, J.-J., Cheng, X.-R., Liu, J., Kang, J., and Fu, C.-Y. (2021). Antimicrobial peptides: mechanism of action, activity and clinical potential. *Military Medical Research*, 8(1).

Appendices

Chapter 7

Appendix

7.1 Appendix 1.

Figure 7.1: Study area for soil sampling. The two plots are covered with Rosaceae and Poaceae families.

Table 7.1: Morphological characterization of the 71 soil bacterial colonies obtained after isolation.

Code	Size (mm)	Color	Shape	Elevation	Margin	Gram Stain	Catalase
UITEY-001	3	White	Round	Convex	Entire	+	+
UITEY-002	4	White	Round	Flat	Ondulate	+	+
UITEY-003	4	White	Round	Flat	Entire	+	+
UITEY-004	3	White	Round	Raised	Ondulate	+	+
UITEY-005	2.5	Orange	Round	Flat	Ondulate	-	+
UITEY-006	4	White	Round	Flat	Entire	+	+

UITEY-007	3.8	White	Round	Flat	Entire	+	+
UITEY-008	2	White	Round	Flat	Entire	+	+
UITEY-009	11	White	Irregular	Flat	Ondulate	+	+
UITEY-010	45	White	Filamentous	Flat	Ondulate	+	+
UITEY-011	40	White	Irregular	Flat	Ondulate	+	+
UITEY-012	1	White	Punctiform	Flat	Entire	+	-
UITEY-013	1	White	Punctiform	Convex	Entire	-	+
UITEY-014	3	White	Round	Flat	Entire	+	+
UITEY-015	23	White	Filamentous	Flat	Ondulate	+	+
UITEY-016	1	White	Round	Flat	Entire	+	+
UITEY-017	3	White	Round	Raised	Entire	+	+
UITEY-018	50	White	Irregular	Flat	Ondulate	+	+
UITEY-019	1	White	Punctiform	Flat	Entire	+	+
UITEY-020	2	White	Round	Flat	Entire	+	+
UITEY-021	25	White	Filamentous	Flat	Ondulate	+	+
UITEY-022	6	White	Irregular	Flat	Ondulate	+	+
UITEY-023	2	White	Round	Flat	Entire	-	+
UITEY-024	2	White	Round	Flat	Entire	+	+
UITEY-025	3	White	Round	Convex	Entire	+	+
UITEY-026	21	White	Irregular	Flat	Ondulate	+	+
UITEY-027	3	White	Round	Flat	Entire	+	+
UITEY-028	2	White	Irregular	Flat	Ondulate	+	+
UITEY-029	2.5	White	Round	Flat	Entire	+	+
UITEY-030	4	White	Round	Raised	Ondulate	+	+
UITEY-031	1	Beige	Round	Flat	Entire	+	+
UITEY-032	2	White	Round	Convex	Entire	+	+
UITEY-034	25	White	Filamentous	Flat	Ondulate	+	+
UITEY-035	2	White	Round	Flat	Entire	+	+
UITEY-036	1	White	Irregular	Flat	Ondulate	+	+

UITEY-037	3	White	Round	Raised	Ondulate	+	+
UITEY-038	3	Beige	Round	Flat	Ondulate	+	+
UITEY-039	1	White	Round	Flat	Entire	+	+
UITEY-040	4	White	Round	Flat	Entire	-	+
UITEY-041	6	White	Irregular	Flat	Ondulate	+	+
UITEY-042	2	White	Round	Convex	Entire	+	+
UITEY-043	1.5	White	Round	Raised	Entire	+	+
UITEY-044	1	White	Irregular	Flat	Ondulate	-	+
UITEY-045	1	White	Punctiform	Flat	Entire	-	+
UITEY-046	7	White	Irregular	Flat	Ondulate	+	-
UITEY-047	5	White	Irregular	Flat	Ondulate	+	+
UITEY-048	55	White	Filamentous	Flat	Ondulate	+	+
UITEY-049	3	White	Round	Flat	Entire	+	+
UITEY-050	4	White	Irregular	Flat	Ondulate	+	+
UITEY-051	3	Beige	Round	Flat	Entire	+	+
UITEY-052	3	Beige	Round	Raised	Entire	+	+
UITEY-053	4	White	Round	Flat	Entire	-	+
UITEY-054	1	Beige	Punctiform	Flat	Entire	+	+
UITEY-055	55	White	Irregular	Flat	Ondulate	+	+
UITEY-056	2	White	Irregular	Raised	Ondulate	+	+
UITEY-057	1	White	Punctiform	Flat	Entire	-	+
UITEY-058	2	White	Round	Flat	Entire	+	+
UITEY-059	3	White	Round	Flat	Entire	+	+
UITEY-060	3	Beige	Irregular	Flat	Ondulate	+	+
UITEY-061	17	White	Filamentous	Flat	Ondulate	+	+
UITEY-062	4	Beige	Round	Flat	Entire	+	+
UITEY-063	7	White	Irregular	Flat	Ondulate	+	+
UITEY-064	2.5	Beige	Round	Flat	Entire	+	+
UITEY-065	3	White	Round	Flat	Entire	+	+

UITEY-066	1	White	Punctiform	Flat	Entire	+	+
UITEY-067	5	White	Irregular	Flat	Ondulate	-	+
UITEY-068	1.5	White	Round	Convex	Entire	+	+
UITEY-069	15	White	Irregular	Flat	Ondulate	+	+
UITEY-070	25	White	Filamentous	Flat	Ondulate	+	+
UITEY-071	11	White	Filamentous	Flat	Ondulate	+	+
UITEY-072	1	White	Round	Convex	Entire	+	+

Table 7.2: Mann-Whitney U results

Confrontation strains	**Test Statistic (U)**	**P-value**
UITEY-009 vs. *P. aeruginosa*	9.0	0.1
UITEY-030 vs. *S. aureus*	0.0	0.1
UITEY-055 vs. *E. coli*	0.0	0.1

Table 7.3: Shapiro-Wild results

		Test Statistic (w)	**P-value**
Confrontation strains	UITEY-009 vs. *P. aeruginosa*	0.891	0.359
	UITEY-030 vs. *S. aureus*	0.772	0.051
	UITEY-055 vs. *E. coli*	0.923	0.463
Control strains	*P. aeruginosa*	0.986	0.780
	S. aureus	0.956	0.596
	E. coli	0.793	0.100