

Isolation and Characterization of Soil Microorganisms with Potential Antimicrobial Activity

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Abstract—Soil is a very rich ecosystem and a famous source of microorganisms that produce antimicrobials. Due to the growing antimicrobial resistance rate, the search of the soil-based microbes as the source of new bioactive compounds has become interesting again. The objective of this study was to isolate, screen and characterize soil microorganisms that had antimicrobial activity against some pathogenic bacteria. The serial dilution and culture-based methods were used to process soil samples. Results were 28 microbial isolates that were screened using the cross-streak technique in the determination of antimicrobial activity. The agar well diffusion assay was also used to test potent isolates. Morphological, biochemical and molecular characterization, which included 16S rRNA gene sequencing, were done. The initial screening indicated that 32.1% of the isolates were antimicrobial active with bigger inhibition levels in Gram-positive bacteria. The production of extracellular antimicrobial metabolites was confirmed by secondary screening, with some exhibiting a broad-spectrum activity. *Bacillus subtilis*, *Bacillus licheniformis* and *Streptomyces* spp. were identified as the potent isolates by molecular identification. The microorganisms that obtain their location on soil are still promising sources of antimicrobial agents, and additional research is justified to define the bioactive compounds.

Index Terms—Soil microorganisms; Antimicrobial activity; Actinomycetes; *Bacillus*; *Streptomyces*; Antibiotic resistance

I. INTRODUCTION

One of the most complicated and biologically active ecosystems in the Earth is soil, which is a reservoir of huge microbial diversity. It contains a broad diversity of micro-organisms, bacteria, actinomycetes, fungi,

algae and protozoa which all play important ecological roles in nutrient recycling, organic matter, soil structure and in inhibiting plant and human pathogens [1, 2]. Of special scientific and clinical significance among these varied microbial communities, are those microorganisms in soil that can generate antimicrobial compounds. These microbes produce bioactive secondary metabolites with the help of which they can survive in competitive soil conditions by suppressing the growth of other microbes [3].

The contribution of soil microorganisms in the identification of antibiotics has played a central role in the modern-day medicine. The discovery of antibiotics in the golden era of the mid-20th century was mainly through soil-derived microbial communities, especially the actinomycetes of genus *Streptomyces* [4]. Some of the life-saving drugs like streptomycin, chloramphenicol, tetracycline and erythromycin were discovered in the soil microorganisms and transformed the treatment of bacterial infections [5]. These natural products played an important role in decreasing the deaths associated with infectious diseases as well as the development of surgery and contemporary approaches to therapies.

However, the extensive and frequent use of antibiotics in agriculture, animal care, and human healthcare has led to the rapid development of antimicrobial resistance (AMR) [6]. By importing multidrug-resistant bacteria, including methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococci, and extended-spectrum 2-lactamase-producing bacteria, it has also been seriously impairing the effectiveness of currently available

antimicrobial therapies [7]. The problem of antimicrobial resistance is currently considered a worldwide public health crisis, as it endangers the healthcare systems and raises morbidity, mortality, and economic costs at the global level [8]. There is, therefore, an urgent call to find new antimicrobial agents that have new mechanisms of action.

Soil still forms a potential and under-explored new source of antimicrobial compounds. The high heterogeneity of soil habitats such as agricultural fields, forest ecosystems, rhizosphere, wetlands and contaminated habitats presents unique selective forces leading to microbial adaptation and metabolic diversity [9]. To counter this, soil microorganisms secrete a diverse range of secondary metabolites with antibacterial, antifungal, antiviral and antiparasitic properties with many having distinct chemical structures not present in synthetic collections [10]. Isolation of soil microorganisms entails a series of systematic sampling which is then followed by selective culturing methods that are meant to recover the diverse microbial populations. The dominant microbial cultures can be cultivated and purified using serial dilution and plating on general or selective media [11]. The various primary screening procedures that include cross-streak assays, agar well diffusion, and disc diffusion are regularly utilized in measuring the activity of antimicrobials as well as on pre-selected pathogenic microorganisms [12]. Isolates showing good inhibitory potentials are taken through an additional assessment.

Profiling of antimicrobial producing soil microorganisms is necessary to identify and determine their functionality correctly. Preliminary results are obtained through morphological and biochemical studies, and molecular determination using 16S rRNA gene sequencing can be used to identify the taxonomic position of the organism and phylogenetic analysis [13,14]. In addition to medical applicability, these microorganisms can also be used in agriculture as biological control agents of plant pathogenesis, eliminating the use of chemical pesticides and promoting environmental-friendly practices [15]. In this regard, the discrimination and identification of microorganisms in soil that may have an antimicrobial property is a most sensitive subject of study, not only to lead to the discovery of new drugs, but also to

explore the ecology of soil microorganisms in the age of antimicrobial resistance.

Aim

The aim of the present study is to isolate, screen, and characterize soil microorganisms with potential antimicrobial activity against selected pathogenic microorganisms, with a view to identifying promising microbial isolates capable of producing bioactive antimicrobial compounds.

Objectives

1. To isolate and obtain pure cultures of microorganisms from selected soil samples.
2. To screen the isolated soil microorganisms for antimicrobial activity against selected pathogenic microorganisms.
3. To characterize the potent antimicrobial-producing isolates using morphological, biochemical, and molecular techniques.

II. REVIEW OF LITERATURE

Sadiqi et al., (2022) Collected "10 soil samples from green land, agricultural, and marshy soil locations in Khyber Pakhtunkhwa, Pakistan. Following cultivation on the specified conditions, the bacterial isolates were identified using phenotypic, biochemical, and phylogenetic analyses. Our phylogenetic analysis identified three bacterial isolates, A6S7, A1S6, and A1S10, exhibiting 99% nucleotide sequence similarity with *Brevibacillus formosus*, *Bacillus subtilis*, and *Paenibacillus dendritiformis*. The crude extract was derived from bacterial isolates to evaluate its antibacterial efficacy against various multidrug-resistant strains (MDRS), including *Acinetobacter baumannii* (ATCC 19606), Methicillin-resistant *Staphylococcus aureus* (MRSA) (BAA-1683), *Klebsiella pneumoniae* (ATCC 13883), *Pseudomonas aeruginosa* (BAA-2108), *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), and *Salmonella typhi* (ATCC 14028). The investigation demonstrated that all bacterial extracts exhibit action against both Gram-negative and Gram-positive bacteria at a dosage of 5 mg/mL, effectively inhibiting the growth of *E. coli* in comparison to the positive control, ciprofloxacin" [16]. Fernández-Fernández et al., (2022) evaluated the service-learning initiative "Micro Mundo," which is a part of the worldwide

Citizen Science initiative "Tiny Earth," is highlighted. The study was aimed at testing the antimicrobial properties of soil-derived bacterial isolates, determining the diversity of the selected isolates with antimicrobial-producing capacity, and measuring some antibiotic resistance pattern of the isolates. Also, the Micro Mundo project was utilized to illustrate the high level of interconnectedness between scientific investigation and education that proves the usefulness of service-learning models in raising awareness of antimicrobial resistance (AMR) and enhancing the discovery of innovative antimicrobial solutions. It was found that 2,600 bacterial isolates were received based on 130 soil samples, and active participation in the antimicrobial screening process was done by 16 teachers, 25 university students, and 300 secondary school students. On *Escherichia coli* and *Staphylococcus epidermidis* as indicators, 132 isolates (out of 1000 or so screened) were found to be potential producers of antimicrobials. Taxonomic identification established that *Bacillus* was the most common genus in the selected isolates followed by *Pseudomonas*, *Paenibacillus* and *Serratia* [17].

Prashanthi et al., (2021) assessed the culturing of the soil bacteria with antibacterial activity of various locations in Bangalore, India. The fact that bacteria can produce antibiotics is arguably a defensive mechanism because the compounds are produced as secondary metabolites used to suppress competition amongst other microorganisms in their habitat. The antagonistic action of individual bacterial isolates against human pathogenic bacteria, i.e. *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* were tested using both primary and secondary screening assays. Findings of both screening methods always showed that the isolates PR1, PR2, and PR3 had a high ability to act as an antibacterial agent against all the pathogens used in the tests. In addition to this, the impact of various treatment procedures such as filter sterilization, autoclaving and proteinase K treatment on the activity of culture filtrates was investigated, which showed filter sterilization as the best procedure in preserving the antibacterial activity. Moreover, different carbon and nitrogen sources were also evaluated, and it was shown that growth of antibacterial productions depended on nutrient

availability with each isolate having different carbon and nitrogen preferences [18].

Sapkota et al., (2020) intended to separate, characterize, and test potential actinomycetes that produce antibiotics from Nepalese soils at different elevations. Eleven soil samples collected from various parts of Nepal, at elevations between 1500 and 4380 meters, produced 41 isolates of actinomycete. To determine which isolates were present, scientists used morphological analysis, various sugar consumption evaluations, protein utilization assessments, and hydrolysis testing. They were also classified according to their temperature and pH levels. The first test to see how well antimicrobials worked was done using "*Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 700603), and *Pseudomonas aeruginosa* (ATCC 27853)". Seventy percent of the isolates were *Streptomyces* species, nine percent were *Nocardia* species, and five percent were *Micromonospora* species. Four out of five actinomycete isolates (43.34%) showed strong antibiotic activity in the first test; 46.34% of these were effective against Gram-positive bacteria and 12.19 % against Gram-negative bacteria [19].

Jan et al., (2020) comprehend the distribution and quantity of native microorganisms (IMOs) modified based on humus collected in the forest adjacent to the arable land, and microbial communities of soil microorganisms. The microorganisms in the soil derived in humus and the man-made artificial growth of microbial-based soil additions were described using the molecular and biochemical methods. There were almost 100 times more bacteria (2×10^6 – 13×10^6 CFU/g sample) than fungi (2×10^4 – 8×10^4 CFU/g sample). According to the 16S rDNA and ITS sequence analysis, most of the bacterial and fungal communities in IMOs and humus were composed of *Trichoderma* and *Aspergillus* species, respectively, and *Bacillus* and *Pseudomonas*, respectively. *Sclerotinia sclerotiorum* and *Fusarium oxysporum*, two soil-borne pathogenic fungi, were strongly inhibited by several of the bacterial isolates produced in the humus and the IMOs. Also, the bacteria exhibited phosphate solubilizing activity and siderophore synthesis activity, both of which are required qualities to be able to biologically control

plant pathogenic fungus. The implications of these findings are that IMO, and humus could be useful to support sustainable agriculture [20].

III. METHODOLOGY

The current research was intended as laboratory-based cross-sectional experimental research to isolate, screen and characterize microorganisms in the soil that have an antimicrobial activity about specific pathogenic microorganisms.

3.1 Study Setting

This was done in a Microbiology lab under aseptic conditions. The soil samples have been taken at the desired points in the environment, and the rest of the microbiological processes, screening tests, and the characterization investigations were conducted within the laboratory environment.

3.2 Study Population

The population under examination included:

1. Microorganisms in soil that have been distilled in environmental soil samples.
2. "Pathogenic bacterial strains (Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae) used as test organisms for antimicrobial activity".

3.3 Sampling Technique

The purposive sampling method was adopted in the collection and selective isolation of soil samples and morphologically different microbial isolates. A depth of 5-10 cm was sampled in soil samples which had been collected aseptically following the removal of surface debris.

❖ Inclusion Criteria

- Soil samples collected from natural, non-contaminated environments.
- Microbial isolates showing visible and distinct growth on culture media.
- Isolates exhibiting antimicrobial activity during screening.

❖ Exclusion Criteria

- Contaminated or mixed cultures.
- Isolates showing no antimicrobial activity.

IV. DATA COLLECTION TOOLS AND TECHNIQUES

4.1 Isolation of Soil Microorganisms

The spread plate and serial dilution techniques were used to isolate microorganisms. Soil sample weighing 1 gram was dissolved in 9 milliliters of sterile distilled water and diluted in increments of 10^{-6} . Isolation of bacteria and actinomycetes Nutrient Agar (NA) and actinomycetes isolation agar (AIA) were each given aliquots of thixotropic dilutions (0.1 mL). For bacteria, the incubation time was one to two days at 37°C , whereas for actinomycetes, it was five to seven days at 28°C . Seeking of pure cultures through sub-culture of distinct colonies based on morphology was repeated and agar slants of pure colonies were preserved at 4°C to be further analyzed.

4.2 Primary Screening for Antimicrobial Activity

The main method for screening the isolated microbes was the cross-streak technique. Soil samples were spread out in a single line on a Mueller-Hinton Agar (MHA) plate and then cultured at 37°C for 24 hours to allow metabolite development. Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae were among the test pathogenic organisms that underwent streaking following incubation, with each streak running perpendicular to the previous one. Evidence of antibacterial action was observed by observing decreased development of the pathogen near the producer strain after incubating plates at 37°C for 24 hours.

4.3 Secondary Screening by Agar Well Diffusion Method

The initial screening with the agar well diffusion test was used to screen the isolated which showed good antimicrobial activity in the primary screening. Broth cultures were centrifuged at 10,000 rpm at 10 minutes to get cell-free culture supernatants. MHA plates with seeded test pathogens (0.5 McFarland standard) had a well of 6 mm diameter punched into the plates. We put 100 μL of culture filtrate in each well and incubated the plates at 37°C in 24 hours. The zones of inhibition were recorded in millimeters and compared with the positive controls which were standard antibiotics.

4.4 Morphological and Biochemical Characterization

Strong antimicrobial producing isolates were identified using colony morphology, pigmentation, Gram staining, and cellular features. Biochemical

characterization involved standard biochemical tests, which included catalase test, oxidase test, production of indole, methyl red test, Vibes Proskauer test, use of citrate, starch hydrolysis test and sugar fermentation test using usual microbiological procedures of initial identification.

4.5 Molecular Identification

The amplicon was amplified to "the 16S rRNA gene using universal primers, the PCR results were sequenced, and BLAST analysis was performed to compare the obtained sequences with reference sequences found in the NCBI GenBank database to identify phylogenetic affiliation" The chosen isolates that were found to be potent were further molecularly identified by 16S rRNA gene sequencing. The genomic DNA was purified using a typical bacteria DNA isolation kit. The taxonomic identity was verified by the construction of a phylogenetic tree with the help of the relevant bioinformatics tools.

V. DATA COLLECTION PROCEDURE

The laboratory conditions of conducting all experiments were aseptic. Isolation, screening and characterization of the isolates was done systematically, and all assays were conducted in triplicate to verify the reproducibility and accuracy of the results.

5.1 Statistical Analysis

The findings were presented as mean \pm standard deviation, and each experiment was carried out in triplicate. Zone of inhibition data were analyzed using descriptive statistical methods. Comparative analysis among isolates was conducted to identify the most potent antimicrobial producers.

5.2 Results

5.2.1 Isolation of Soil Microorganisms

Soil samples were sampled by a serial dilution and spread plate followed by recovery of 28 morphologically different microbial isolates. These comprised 21 bacterial isolates, collected on Nutrient Agar (NA) and 7 actinomycetes, collected on the Actinomycetes Isolation Agar (AIA). The isolates were heterogeneous in nature; they had diversity in terms of colony morphology, pigmentation, margin, elevation, and texture.

Table 1. Distribution of Soil Microbial Isolates

Type of Isolate	Culture Medium	Number of Isolates	Percentage (%)
Bacteria	Nutrient Agar (NA)	21	75.0
Actinomycetes	Actinomycetes Isolation Agar (AIA)	7	25.0
Total		28	100

Interpretation:

As indicated in Table 1, bacterial isolates were the largest proportion of the sampled soil microorganisms with 75 percent, (21 out of 28 isolates) and actinomycetes, which represent 25 percent (7 out of 28 isolates). Such preponderance of the bacteria suggests that the soil samples contained high population of heterotrophic bacteria that could easily adapt to nutrient agar environment. Nevertheless, the high percentage of actinomycetes points at the existence of ecologically and pharmacologically significant microorganisms, as the latter is also famous at producing bioactive secondary metabolites, such as antibiotics. The distribution in general indicates a well-differentiated array of microbial soil fauna with a high potential of antimicrobial screening.

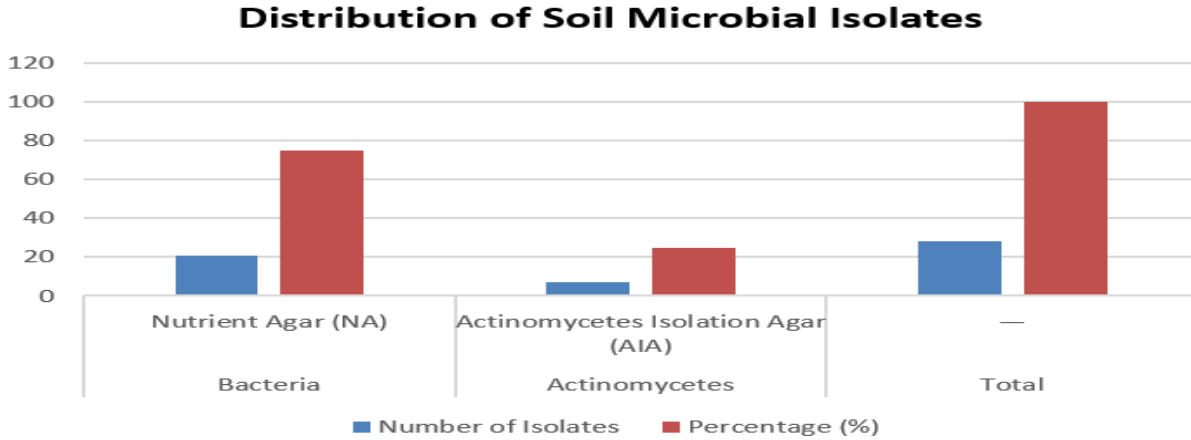


Figure 1: Distribution of Soil Microbial Isolates

5.2.2 Primary Screening for Antimicrobial Activity

Primary screening was done for all the isolates with the cross-streak method against some select pathogenic organisms. Nine isolates (32.1%) out of 28 isolates showed visible inhibition of at least one of the test pathogens. The Gram-positive *Staphylococcus aureus* displayed a higher antimicrobial activity than Gram-negative bacteria.

Interpretation:

Table 2 shows that more soil isolates were able to show an antimicrobial activity against *Staphylococcus aureus* (28.6) than Gram-negative test organisms. It was noticed that moderate activity was observed against *Escherichia coli* (21.4%), with fewer isolates being active against *Pseudomonas aeruginosa* (14.3) and *Klebsiella pneumoniae* (10.7%). This pattern indicates that the isolates of antimicrobial compounds formed by the soil isolates were stronger against Gram-positive bacteria, probably because of the structural variations in bacteria cell wall. In general, the results indicate selective and inconsistent antimicrobial activity with the isolates, which indicates the potential of soil microorganisms as sources of antibacterial agents, especially against Gram-positive pathogens.

Table 2. Primary Screening of Soil Isolates for Antimicrobial Activity

Test Pathogen	Number of Active Isolates	Percentage of Active Isolates (%)
<i>Staphylococcus aureus</i>	8	28.6
<i>Escherichia coli</i>	6	21.4
<i>Pseudomonas aeruginosa</i>	4	14.3
<i>Klebsiella pneumoniae</i>	3	10.7

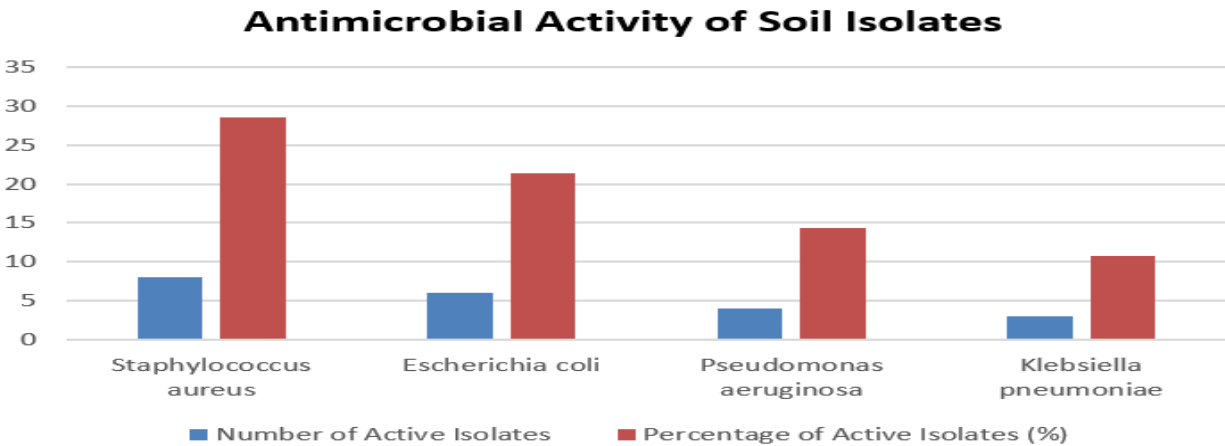


Figure 2: Antimicrobial Activity of Soil Isolates

5.2.3 Secondary Screening by Agar Well Diffusion Method

The 9 strong isolates that were identified during the first screening were subsequently tested by the agar well diffusion assay. Cell-free culture supernatants

developed clear areas of inhibition, and this confirmed the production of extracellular antimicrobial metabolites. The zone diameters were different among isolates and pathogens.

Table 3. Zone of Inhibition (mm) Produced by Potent Isolates (Mean ± SD)

Isolate Code	S. aureus	E. coli	P. aeruginosa	K. pneumoniae
SI-3	18.4 ± 0.6	15.2 ± 0.5	12.1 ± 0.4	11.3 ± 0.3
SI-7	20.1 ± 0.7	16.8 ± 0.6	13.4 ± 0.5	12.6 ± 0.4
SI-11	17.3 ± 0.5	14.0 ± 0.4	11.2 ± 0.3	10.8 ± 0.3
SI-15	21.6 ± 0.8	18.2 ± 0.6	14.5 ± 0.6	13.1 ± 0.5
SI-19	19.0 ± 0.6	16.0 ± 0.5	12.9 ± 0.4	11.9 ± 0.4

Interpretation: As shown in table 3, all the chosen potent soil isolates had quantifiable zones of inhibition against the test pathogens indicating their extracellular antimicrobial action. The most active one was SI-15 which had the highest antimicrobial efficacy as it had the greatest zones of inhibition against *Staphylococcus aureus* (21.6 ± 0.8 mm) and *Escherichia coli* (18.2 ± 0.6 mm) and showed a broad spectrum of action. Overall, the area of inhibition was always greater towards *S. aureus* than Gram-negative ones, which indicates a higher susceptibility of Gram-positive organisms. Comparatively lesser zones were viewed against *Pseudomonas aeruginosa* and *Klebsiella*

pneumoniae indicating inherent resistance related to Gram-negative cell wall structures. The values of the standard deviation are low, therefore, showing that there was good reproducibility of the antimicrobial activity of triplicate experiments.

5.2.4 Morphological and Biochemical Characterization

Morphological appearance showed that many strong isolates were Gram-positive and rod-shaped, and some were filamentous which is characteristic of actinomycetes. Isolates exhibited different metabolic profiles by biochemical tests.

Table 4. Morphological and Biochemical Characteristics of Potent Isolates

Isolate	Gram Reaction	Morphology	Catalase	Oxidase	Starch Hydrolysis	Citrate
SI-3	Positive	Rod	+	-	+	+
SI-7	Positive	Rod	+	+	+	-
SI-11	Positive	Filamentous	+	-	+	+
SI-15	Positive	Rod	+	+	+	+
SI-19	Positive	Rod	+	-	-	+

(+ = Positive, - = Negative)

Interpretation: Table 4 indicates that all strong antimicrobial producing isolates were Gram-positive, majorly rods with one filamentous isolate (SI-11), indicating the existence of actinomycete-like organisms. The presence of catalase in all the isolates shows that they are aerobic and can counter oxidative stress. Diversity in the isolates is noted by differences in oxidase activity, starch hydrolysis, and usage of citrate. Most of these isolates have a positive starch hydrolysis implying the synthesis of extracellular enzymes a phenomenon that is commonly linked with the synthesis of secondary metabolites. Such

morphological and biochemical phenotypes confirm the taxonomic diversity of the isolates and give initial evidence of their possible belonging to the well-known antimicrobial producing genera like *Bacillus* and *Streptomyces*.

5.2.5 Molecular Identification

Three isolates were identified and subjected to molecular identification through 16S rRNA gene sequencing as per the antimicrobial potency. Analysis of the sequence showed that it was highly similar to the known antimicrobial-producing genera.

Table 5. Molecular Identification of Selected Potent Isolates

Isolate Code	Closest Identified Species	Sequence Similarity (%)	GenBank Accession
SI-7	Bacillus subtilis	99.2	Submitted
SI-11	Streptomyces sp.	98.7	Submitted
SI-15	Bacillus licheniformis	99.0	Submitted

Interpretation:

The molecular identity of the strongest antimicrobial-producing isolates according to the 16S rRNA gene sequencing is supported in Table 5. Isolate SI-7 was highly comparable (99.2%) to Bacillus subtilis whereas its counterpart SI-15 was found to be highly comparable (99.0 %) with Bacillus licheniformis, which are among the well-known producers of antimicrobial peptide and secondary metabolites. Isolate SI-11 showed close relationship (98.7 %) to Streptomyces species, a genus that has been recognized to have made great contributions to the discovery of antibiotics. The high values of sequence similarity confirm the taxonomic determination of these isolates and their potential to serve as good sources of bioactive antimicrobial compounds.

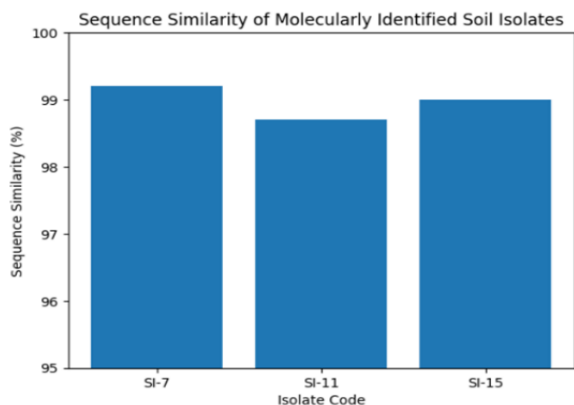


Figure 3: Sequence Similarity of Potent Isolates

VI. DISCUSSION

The current paper confirms that soil is an effective source of antimicrobial-producing microorganisms by supporting the importance of the resource in the current investigation towards finding new agents to counter the threat of antimicrobial resistance. The microbial isolates recovered were 28 morphologically different, with bacteria (75%) and actinomycete (25%) having a significant percentage. This is in line with previous research by Jan et al. (2020) and Prashanthi et al. (2021) [20], who found the preponderance of

bacteria with their fast development and abilities to adjust to general-purpose culture media.

Primary screening showed that 32.1 % of the isolates showed antimicrobial activity on at least one pathogen in the test. The strongest inhibitory effect was recorded about Staphylococcus aureus as compared to Gram-negative organisms. On the same note, Fernández-Fernández et al. (2022) [17] reported similar observations and attribute this trend to the structural simplicity of the Gram-positive cell wall which enables penetration by antimicrobial compounds more easily. The relatively low susceptibility of Escherichia coli, Pseudomonas aeruginosa, and Klebsiella pneumoniae in the current study correlates with the previous data provided by Sadiqi et al. (2022) [16] and indicates the inherent mechanism of resistance of the Gram-negative bacteria, such as the outer membrane barrier and the efflux system.

The antimicrobial metabolites were found to be extracellular, and this was proved by secondary screening using the agar well diffusion method. SI-15 presented the best antimicrobial potential because it had the largest spectrum and best inhibition zones of all the potent isolates. Similar increases in antimicrobial activity in secondary screening have been found by Prashanthi et al. (2021), [18] suggesting that critical roles in antimicrobial action are played by metabolite concentration and diffusion. The standard deviation values are also low in triplicate experiments, and this further indicates the reproducibility and reliability of antimicrobial activity observed.

Morphological and biochemical characterization showed that all the potent isolates were Gram-positive with most of them being rod-shaped and one filamentous isolate suggesting actinomycetes. Molecular identification was done by sequencing the 16S rRNA gene, which revealed essential isolates to be Bacillus subtilis, Bacillus licheniformis, and Streptomyces spp. They are well-documented genera producing various antimicrobial compounds, and so

do Sapkota et al. (2020) [19]. In general, the results of the present research are well aligned to the literature and support the further possibility of using soil microorganisms as sources of viable antimicrobial agents and justify the further purification and characterization of identified bioactive compounds.

VII. CONCLUSION

The current study affirms that the soil can be a powerful and viable source of antimicrobial producing microorganisms that have a high potential of being used in therapeutics. The systematic isolation and screening found that a significant percentage of soil-based isolates had antimicrobial activity against some of the human pathogens with greater inhibitory effect of Gram-positive bacteria in Gram-negative organisms. The extracellular character and reproducibility of the antimicrobial metabolites synthesized by the potent isolates were determined through secondary screening. Morphological, biochemical and molecular characterization of the most successful identified strains as *Bacillus subtilis*, *Bacillus licheniformis* and *Streptomyces* spp. are genera that are well known to produce a wide range of bioactive compounds. These results suggest the overall applicability of the soil microorganisms in antimicrobial discovery and the need to further purify, characterize, and evaluate the identified bioactive compounds in a mechanistic way to address the increasing problem of antimicrobial resistance.

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