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ANTIMICROBIAL AND ENZYMATIC ACTIVITY OF SOIL BACTERIA ISOLATED FROM THE NILGIRIS AND ERODE DISTRICTS, TAMIL NADU, INDIA AND ITS PLANT GROWTH PROMOTING PROPERTIES

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Soil has generally been utilized to track down new anti-infection makers, at present a significant number of the 'old' anti-toxins are currently being controlled in the lab and synthetic changed to frame new variants of more established anti-microbials.In this study, soil samples were collected from a variety of locations, including agricultural and forest areas, and they were analyzed for antibiotic production. After the primary screening, the bacterial isolates were characterized, their antimicrobial activity against *E. coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumonia, and Pseudomonas aeruginosa* was checked, and their biochemical tests were done, and Bacillus sp. was found to be the bacterial isolate. Proteins known as soil enzymes are responsible for catalyzing specific substrate-dependent biochemical reactions in the soil that are essential for microbial life. The activity of amylolysis and proteolysis was examined. Different plants with particular growth patterns were found to have an activity that promoted plant growth. According to the findings of this study, the pharmaceutical industry may be able to harness antibiotic-producing bacteria strains for therapeutic purposes. This work may be used to further control microbial strains and provide potential information on the production of antibiotics. In addition, the ingredient that encourages plant growth can be identified and utilized extensively in agricultural industries.

Keywords: Soil sample; antibiotic producing bacteria; antimicrobial activity; enzymatic activity; plant growth promotion.

1. INTRODUCTION

Soil is a mixture of organic matter, minerals, and living organisms Forest oil is one of the most important soils which is considered not too cold and covers about 40% of the country. Agricultural soil is the soil that is fertile and rich widely and used for agricultural purposes Between 1945 and 1978, the genus greatest was the source of over 55% of the antibiotics found, totaling over 5,000 compounds. Microorganisms from the genera *Penicillium, Streptomyces, Cephalosporium, Micomonospora*, and *Bacillus* comprise the relatively small group of those currently in use [1].

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The ability of bacteria to produce various secondary metabolites—basically known as antibiotics—allows them to survive off of other microbes. Human diseases have been treated with antibiotics for ages [2].

Forthebreakdownoforganicmatterandtherecyclingofol dplantmaterial, soil microorganisms (bacteria and fungi) are crucial. In order for the soil to continue to sustain both agricultural production and the supply of other ecosystem services, certain soil bacteria and fungus create relationships with plant roots that reflect the soil's ability to respond to agricultural intervention [3].

Aside from infections, microscopic organisms and Achaea are the littlest animals tracked down in the soil. They are the t common microorganisms in the dirt and have different basic capabilities, including fixing nitrogen. Some bacteria are capable of forming colonies of soil minerals, which can have an impact on how these minerals age and degrade. The general composition of the soil can have an impact on the number of bacteria that grow there. The presence of more minerals in a region may result in a larger number of bacteria. Additionally, these bacteria will produce aggregates, which will improve the overall health of the soil [4].

This study is evaluated the Antimicrobial and enzymatic activity of soil bacteria and its growth-promoting properties for plants.

2. MATERIALS AND METHODS FOR SAMPLE COLLECTION

The Nilgiris Western Ghats forest in Tamil Nadu served as the location for the collection of the soil sample. The field of soil that was collected from the Erode district in Tamil Nadu is located at Lat11.433936°N and Long77.327768°E.

The sites were digged into 5-15 cm and approximately 5g of soil was collected in sterile containers and transported into the laboratory [5].

2.1 Collection of Test Strains

On nutrient broth medium, four bacterial strains *Staphylococcus aureus, Klebsiella pneumonia, Escherichia coli, and Pseudomonas aeruginosa* were isolated, and biochemical tests were carried out to confirm these strains' identities. These bacteria were selected because these are the bacteria usually considered to be potential opportunistic pathogens commonly associated with diseases [6].

2.2 Crowded Plate Technique

Each soil was weighed in 10 milliliters of sterile distilled water to obtain a 1:10 dilution and thoroughly mixed with vigorous shaking after being sieved to remove fine soil particles. The supernatant was used in subsequent dilutions after the sediment had been allowed to settle. A 1:10 dilution of 1 ml of stock culture was transferred into 9 ml of sterile distilled water in another test tube to create 1:100 dilutions. Until 1:100,000 dilutions were achieved, this transfer process from the previous tube continued. Each dilution's soil inoculums were inoculated separately into petri dishes using 0.1 milliliters of nutrient agar media with a pH of 7 to 7.2. The dishes were inverted for two days at room temperature. The colonies that produced the zone of clearance were sub cultured in nutrient agar, and the pure cultures of those colonies were kept at 4°C until they were used again [7].

The secondary screening was used to check the isolated cultures' ability to kill the test strains of bacteria. After being grown overnight in nutrient broth, the colonies were further screened for their ability to inhibit pathogenic bacteria like *E. coli, P. aeruginosa, S. aureus, and K. pneumonia* [8]. An antimicrobial assay was carried out on Mueller Hinton agar by autoclaving 3.8 grams of the powdered medium in 1000 milliliters of distilled water. The spread plate method was used to inoculate each of these plates with 0.1 milliliters of the pathogenic culture. The colonies that were isolated were cultured overnight in a centrifuge, and the supernatant was absorbed onto the discs. After being plated on these plates, these discs were incubated for an entire night. Biochemical tests were used to identify the antibiotic producers from the isolates that produced the best inhibition zones [9].

2.3 Microscopic Examination

Gram staining: One loop of culture was spread on a clean glass slide, the smear was heat-fixed, the smear was flooded with crystal violet, and the smear was flooded with Grams of iodine. After waiting one minute, the smear was washed, and decolorizing agents (90 percent alcohol) were added and washed.

After that, the counter-stain safranin was added and washed for twenty seconds. Under a microscope, the slide was examined, and the results were recorded [10].

Endospore staining: A single loop containing culture was spread on a clean glass slide, allowed to air dry, and then heat fixed. Covered in absorbent paper, the

smear is placed in a water bath, sprayed with malachite green, and allowed to steam for five minutes. Add safranin for one minute after the slide has been rinsed with water. Again, this water was air-dried and examined under a 100x microscope [11].

2.4 Biochemical Test

2.4.1 Indole, methyl red, voges proskauer, citrate utilisation, catalase and oxidase test

A starch hydrolysis test was carried out in order to assess the bacterial isolates' capacity for amylase. Starch hydrolysis agar, the selective medium, was made and sterilized for 15 minutes at 121°C and 15 lb pressure. The media was carefully poured in Petri plates in a laminar flow after being sterilized. In laminar flow, the media was allowed to solidify at room temperature. The bacterial disengages were moved Petri plates with the assistance of a cleaned vaccination circle. For 24 hours, the petri plates were kept at 37°C. The bacterial colonies were directly coated with an iodine solution. In the presence of a dark blue background, it is possible to observe hydrolysis zones [12].

2.4.2 Amylolytic activity

A starch hydrolysis test was carried out in order to assess the bacterial isolates' capacity for amylase. Starch hydrolysis agar, the selective medium, was made and sterilized for 15 minutes at 121°C and 15 lb pressure. The Petri was carefully poured into Petri plates in a laminar flow following sterilization. In laminar flow, the media was allowed to solidify at room temperature. Using a sterilization loop, the bacteria Perlites were transferred to Petri plates. For 24 hours, the Petri plates were kept at 37°C. The bacterial colonies were directly coated with the iodide solution. In the presence of a dark blue background, it is possible to observe hydrolysis zones [13].

2.4.3 Proteolytic activity

The capacity for proteolysis of isolated bacteria was tested qualitatively on skim milk media, indicating that the microbes are able to integrate protein which is shown by clear zones around the colon [14].

2.4.4 Plant growth-promoting activity

The isolated colony which showed the highest zone of inhibition was taken and their plant growth-promoting activity was checked by soaking the seeds (*Vigna radiate*, *Pennisetum glaucum*, *Macrotylam Uniflorum*, and *Amaranthuscruentus)* in the culture for about 30 minutes and planting it in the soil along with control and is kept for germination for 10 days. The growth of the plant was noted for each day and sprinkled with water daily for their germination to take place [15].

3. RESULTS AND DISCUSSION

3.1 Utilizing the Crowded Plate Method, Bacteria from the Soil were Isolated

Seclusion of microorganisms from the soil by jam-packed plate technique Plates was noticed for the presence of any settlement with a reasonable zone around it. Plates with subcultures and 400 colonies were chosen because they showed colonies that were well-defined but in a crowd. Dilutions of 1:100 and 1:1000 revealed distinct zones of inhibition around various kinds of colonies [16].

3.2 Sub Culturing the Colonies onto Nutrient Broth that has a Clear Zone

FA1, FA2, FA3, FA4, FA5, FA6, FB1, FB2, FC1, FC2, FC3, FC4, FC5, and **FD1** were the subcultured colonies on, nutrient broth. The colonies on agricultural soil nutrient agar plates were referred to as Aa1, Aa4, Ab2, Ab4, Ac1, Ac4, and Ad2 [17].

3.3 Identify Bacterial Strains

Biochemical tests on each microorganism confirmed the bacterial strains *E. coli, P. aeruginosa, S. aureus,* and *K. pneumonia* that were collected [18].

3.4 Secondary Screening

Zones of inhibition were used to measure the antibiotics' effectiveness against a variety of gram-positive and gram-negative microbial strains. By performing a disc diffusion assay against the test strains of *E. coli, K. pneumonia, S. aureus,* and *P. aeruginosa*, the antibiotic efficacy of the bacterial isolates was evaluated. The forest soil's inhibition zone was measured. Against *E. coli, K. pneumoniae, S. aureus,* and *P. aeruginosa*, the isolates **FA5, FB1, FC3, and FC4** demonstrated a significant zone of inhibition. Antimicrobial activity against *E. coli, K. pneumonia, S. aureus,* and *P. aeruginosa* was lowest in the isolates **FA2, FA3, FA6, FB1, FC1, FC2, and FC5**. Separates **FA1, FA4 and FD1** showed no zone of a hindrance. Isolates **FA5** produced effective antimicrobials among the isolated colonies, and the agricultural soil's inhibition zone was measured. When tested against *K. pneumonia, S. aureus,* and *P.*

aeruginosa, isolates **Aa1 and Ac4** demonstrated a significant zone of inhibition. A prominent zone of inhibition against *E. coli* was observed with **Aa4 and Ac4**. In order to confirm the identity of this strain, additional biochemical tests were carried out in colonies colony [19].

Table 1. Biochemical tests to confirm the bacterial strains' identities

Biochemical tests	*E. coli*	*K. pneumonia*	*P. aeruginosa*	*S. aureus*
Gram staining	-	-	-	+
Indole Test	+	-	-	-
Methyl red Test	+	-	-	+
Voges-Proskauer Test	-	+	-	+
Citrate utilization Test	-	+	+	-

Table 2. Antimicrobial activity of soil-dwelling microbes from agriculture and forests against the pathogenic test strains (an mm-wide inhibition zone was observed)

Baterial isolated (forest soil)	*Escherichia coli*	*Klebsiella pneumonia*	*Pseudomonas aeruginosa.*	*Staphylococcus aureus*
FA1	-	-	-	-
FA2	15mm	-	-	-
FA3	-	18mm	17mm	12mm
FA4	-	-	-	-
FA5	22mm	15mm	18mm	-
FA6	-	8mm	-	-
FB1	20mm	-	8mm	17mm
FB2	-	14mm	-	-
FC1	15mm	-	-	-
FC2	-	-	15mm	-
FC3	10mm	14mm	-	17mm
FC4	18mm	-	16mm	-
FC5	13mm	-	-	-
FD1	-	-	-	-
Control	-	-	-	-

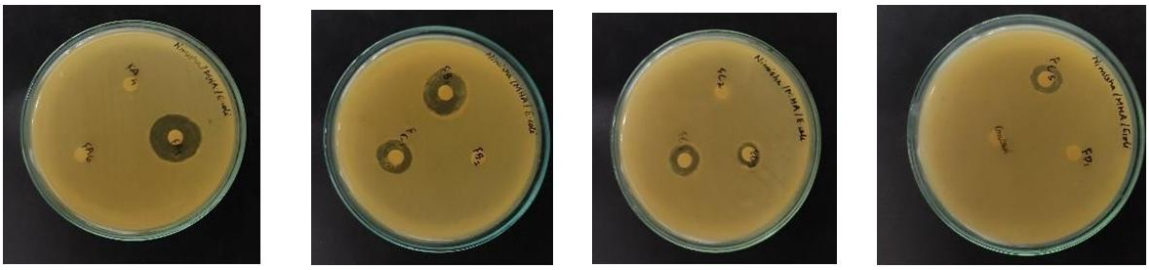

Fig. 1. Isolated bacteria against test strain *E. coil*

Fig. 2. Isolated bacteria against test strain *S. aureus*

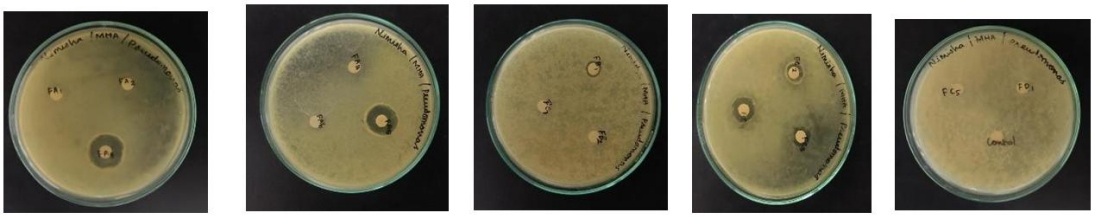

Fig. 3. Isolated bacteria against test strain *P. aeruginosa*

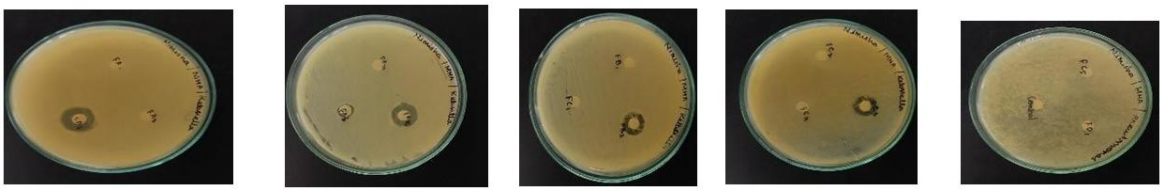

Fig. 4. Isolated bacteria against test strain *K. pneumoniae*

Table 3. Biochemical tests for colony-producing effective antimicrobial activity

Bacterialisolates (agricultural soil)	*Escherichia coli*	*Klebsiella pneumonia*	*Staphylocous aureus*	*Pseudomonas aeruginosa.*
Aa1	-	15mm	8mm	9mm
Aa4	12mm	-	-	-
Ab2	-	-	-	-
Ab4	-	-	-	-
Ac1	-	-	-	-
Ac4	14mm	11mm	10mm	12mm
Ad1	-	-	-	-
Ad2	-	-	-	-

Fig. 5. Isolated bacteria against test strain *E. coil*

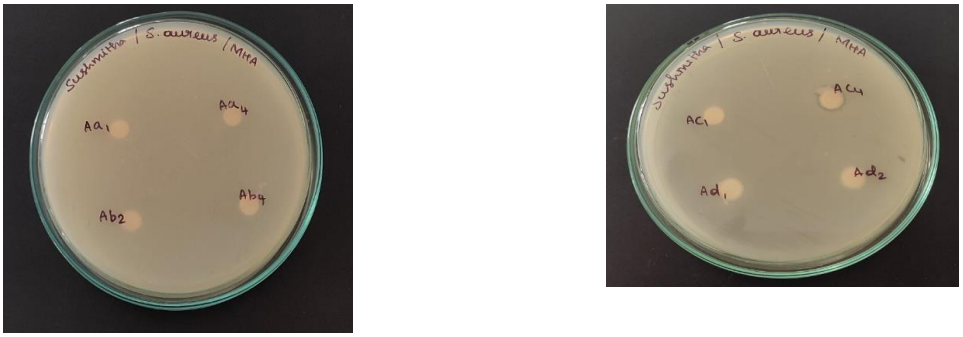

Fig. 6. Isolated bacteria against test strain *S. aureus*

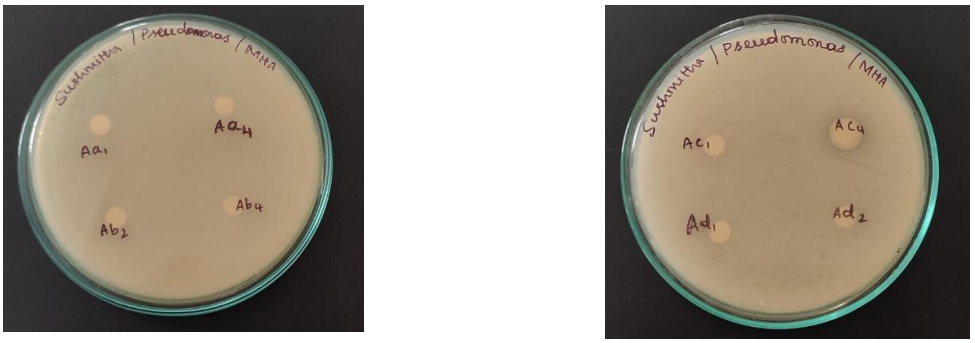

Fig. 7. Isolated bacteria against test strain *P. aeruginosa*

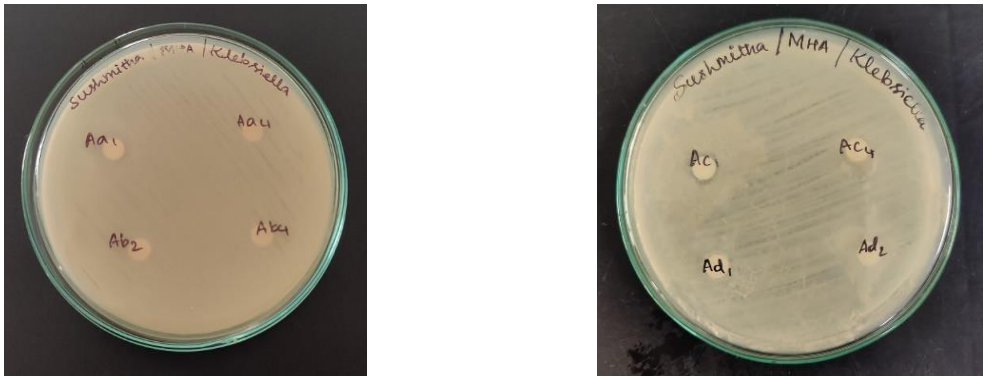

Fig. 8. Isolated bacteria against test strain *K. pneumoniae*

Chart 1. Bacterial isolates

Tests/colony	FA5	Ac4
Gram staining	+	+
Endospore staining	+	+
Motility Test	+	+
Indole Test	-	-
Methyl red Test	+	+
Voges Proskauer Test	-	-
Citrate utilization Test	+	+
Catalase test	+	+
Oxidase test	+	+

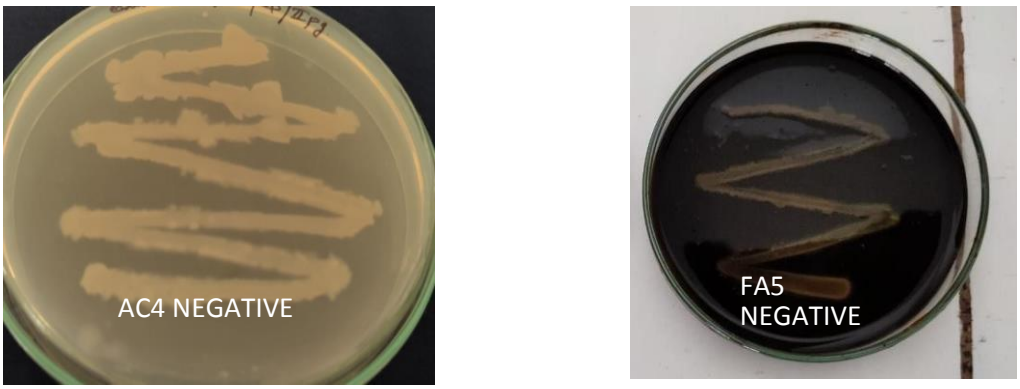

Fig. 9. Amylolytic activity

Fig. 10. Proteolytic activity

Table 4. Plant growth promoting activity (Shoot and root measured in cm)

Seed		Withculture	Without culture
Vignaradiate	SHOOT	20cm	17cm
	ROOT	12cm	6cm
Pennisetumglacum	SHOOT	26cm	15cm
	ROOT	11cm	5cm
Macrotylama	SHOOT	14cm	13cm
Uniflorum	ROOT	5cm	4cm
Amaranthus	SHOOT	5.7cm	2.5cm
cruentus	ROOT	1cm	0.5cm

Fig. 11. Culture of *Vigna radiate*

Fig. 12. Culture of *Pennisetum glacum*

Fig. 13. Culture of *Macrotylama uniflorum*

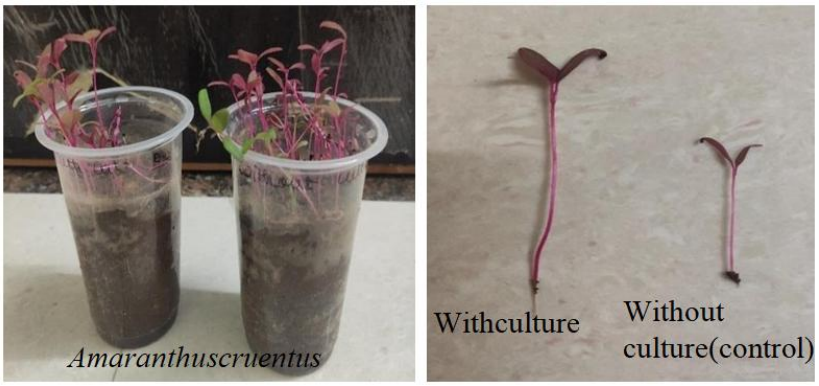

Fig. 14. Culture of *Amaranthus cruentus*

3.5 Characterization of Microbial Isolates

The bacterial isolates **(FA5 and Ac4)** with the greatest antimicrobial activity were selected, and their identities were determined by a variety of morphological and biochemical tests. Isolate colonies on nutrient agar were creamy, raised, and opaque. Both of the colonies belonged to the genus *Bacillus sp*., according to biochemical tests [20].

3.6 Amylolytic Activity

The isolates **FA5 and Ac4** showed prominent growth on starch hydrolyzing agar but no zone of inhibition was observed after the addition of iodine [21].

3.7 Proteolytic Activity

The isolate **FA5 and Ac4** showed prominent growth on skim milk agar by the formation of zone of clearance after incubation [22].

3.8 Effect on Plant Growth Promoting Activity

There was prominent growth observed in which the seeds were soaked in bacterial culture broth **(FA5 and Ac4)** than the one which was soaked in water and planted [23].

4. CONCLUSION

The purpose of this investigation was to assess the antimicrobial, amylolytic, and proteolytic capabilities of soil bacteria as well as their impact on the activity that promotes plant growth. The soil sample was collected from the forest of The Nilgiris which was sieved to extract fine soil particles were serially diluted to see the antibiotic-producing bacteria on nutrient agar which was subculture in nutrient broth then, their antimicrobial activity was checked on MHA by well diffusion method which was being tested against the test strains *Escherichia coli, Klebsiella pneumonia, Staphylococcus aureus,* and *Pseudomonas aeruginosa*.

The most prominent zone forming isolate **FA5** was collected and sub cultured onto nutrient agar. Microscopic examinations like gram staining and endospore staining was done and was observed to be gram positive and spore forming bacteria.

Biochemical tests were conducted it was observed to be indole negative, MR positive, VP negative and

citrate negative. Further amylolytic and proteolytic activity was noted in which starch was not hydrolyzed and protein were formed. As per the results the bacterial isolates showed similarity with *Bacillus sp*. [24].

The bacterial isolate was further used to check for their effect on the plant growth promoting activity. Both the plants showed prominent growth in which the seed were soaked in culture broth [25].

Several distinct bacterial isolates, including those from *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, were found to produce a distinct zone of inhibition. The findings clearly revealed antibiotic-producing microorganisms in soil samples and call for additional research into their characterization and molecular methods of identification.

It is hereby noted that more antibiotic bacteria can be isolated which can be used against most of the opportunistic pathogens and can be used as bio fertilizers that promote plant growth by providing them sufficient nutrients which will be useful for farmers in the near future [26,27].

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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