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ORIGINAL ARTICLE **OPEN ACCESS**

Isolation and Identification of Antibiotic-Producing Bacteria for the Control of Plant Pathogens

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ABSTRACT
Natural isolates of bacteria are powerful producers of novel bioactive antimicrobial chemicals that may be used in the development of biopesticides and the management of plant diseases. One of the sites in Hyderabad city that is permanently polluted is the waste disposal facilities. Considering the significance of these places, no rigorous study has been done on the diversity and strength of the natural bacteria there. To isolate and identify antibacterial strains that can control plant diseases, the abovementioned locations were chosen. The biochemical characteristics of four strains grown in isolation were studied. Antifungal activity and MIC analysis were performed to demonstrate the isolates' inhibition towards fungal strains, which allowed for the determination of the antibiotic potency of the isolates. The four isolates were undergone biochemical tests and found their basic characteristics. The antifungal effects of the bacterial lysates were tested on Plant pathogenic isolates. The results represented that the bacterial Isolates 1 and 3 showed significant antifungal activity (44 and 42% respectively) against Fusarium oxysporum, and isolates 1 & 4 were shown 44 and 38% against Phytophthora infestans. The MIC of the isolate 1 and 3 strains against fusarium was found 66% active against Plant pathogenic fungi, ranging from 25 to 100µL. Antibiotic-resistant microorganisms were widespread in Hyderabad's urban environment. If the isolates can be properly identified according to the strain level and the metabolites can be fully characterized, we can gain valuable knowledge into the ability for antimicrobial synthesis at the city dump site. This study highlights the antimicrobial potential of bacterial isolates as antifungal agents, which can be used for the control of plant pathogens.
Keywords: *Isolation; Identification; Antibiotic-producing bacteria; Control; Plant pathogens*

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INTRODUCTION

The microbiome plays a crucial role in plant health and vitality. Antibiotics made by bacteria that live in plants are useful in preventing off-plant diseases [17]. The world's population is growing quickly, and so are temperatures, water supplies, and other environmental stresses. This means that we need to immediately begin rethinking our agricultural production system. Plant production and health, as well as plant survivability in the face of biotic and abiotic stressors, are directly influenced by the plant microbiome, or the population of microorganisms present in and on the plant [22]. In addition, it is the foundation for the eco-friendly, sensible adjustments that modern agriculture needs to make. The complicated conversations between plant-associated microbes and between microbes and their plant hosts must be better understood before the biostimulant, biofertilizer, and biopesticide potential of plant-associated microorganisms can be fully realized [2, 5, 16]. Recent research published in Microbial Biotechnology addresses some of these issues by revealing substantial variations in the microbial formulation and diversity between healthy and diseased plants. These findings lend support to the hypothesis that certain native microbial members may aid the plant pathogenic organisms during the infection process [27].

Plant systemic tolerance initiation, lytic enzyme development, nutrition and space competitors, and the manufacturing of pathogen-inhibiting volatiles and antibiotics all contribute to the biocontrol capability of the plant microbiome [22]. The search for microorganisms that promote plant growth by mitigating the impacts of abiotic stress, increasing mineral nutrient uptake from soils, and acting as biocontrol agents has been stoked by environmental issues [10]. Estimates from the last few years show that just 3% of the genetic potential of bacteria for synthesizing bioactive components has been experimentally examined. The development of new antibiotics is urgently required to tackle bacterial strains that have become

resistant, and plant-associated biocontrol agents offer a viable source [9]. For example, in ecological niches where the targeted plant pathogen is present, as well as in soils that are inhibitory [14] might be a more practical reaction. As an example of the latter, a study analyzing the bacterial microbiome of perithecia (sexual fruiting bodies) of *Fusarium graminearum* a. fungus listed in the top ten fungal diseases of significance in phytopathology was recently published in Nature Microbiology [7].

Current research is directed toward discovering combinations of antimicrobials that are effective against a wide variety of plant pathogens and have distinct modes of action. This will increase the efficacy of microbial inocula in agriculture and decrease the incidence of tolerance [5]. Consortia of at least six bacterial types are effective for the biocontrol of agricultural diseases, according to a recent study published in Environmental Microbiology [29]. Most people think about them when discussing contagious diseases. The alarming increase in antimicrobial resistance [25, 26] has stoked a resurgence of intrigue in the discovery of new ecological niches and novel methodologies for isolating bacteria and their metabolic products that may one day be used as antimicrobial drugs [1, 3, 12, 28, 24, 25].

We found that there was a significant presence of antibiotic-producing bacteria in soil samples we collected from the research area's several garbage dumps. Bacteria were grown from the obtained materials. Antimicrobial activity and minimum inhibitory concentrations against plant pathogens were analyzed in the isolated cultures, and biochemical assays were performed on the cultivated organisms. Our results shed light on the wide variety of antimicrobial isolating organisms found in the soils of the research area. This study will assist determine whether there are any strains of bacteria that produce antibiotics that might be used by the pharma industry to create new, strong cultures that generate new antibiotics.

MATERIAL AND METHODS

Sample collection:

Soil samples were collected from the industry dumping sites in and around the different sites of Hyderabad. Only a few hours after being collected, these samples were transported to the laboratory for further testing.

Methodology for Serial Dilution:

Before testing, the samples were diluted with a prescribed volume of distilled water (usually 9 ml) and 1 ml of stock. Each Petri plate (10^{-2},10^{-3},10^{-9}) and 9ml saline water-filled test tube (10^{-9}) were marked with marking pencils to differentiate them from the others. A milliliter of the sample was combined with nine milliliters of sterile water to produce ten milliliters of the finished product (10^{-1}). A uniform suspension of bacteria was created by aggressively shaking the dilution. A 1:100(10^{-2}) dilution was made using an aseptic pipette, and a sterile suspension was transferred to the second test tube. When 1ml of suspension was pipetted into the third test tube from the 1:100 dilution tubes, the result was a dilution of 1:1000. Pipetting 1 ml suspension into the final remaining test tube resulted in the completion of a sequence of 10^{-1} to 10^{-9} dilutions in this manner. We used a pour plate approach to deposit 0.5 percent of each dilution onto nutritional agar medium and incubate the plates at 37 degrees Celsius.

Sub Culturing methodology:

Culturing In Petri Dishes:

The nutrient agar for the organisms that grow was made following the formulation and autoclaved at 121°C and 15 lbs of pressure. After being disinfected, the media was put onto Petri dishes and given time to set. The sample is divided into 0.1ml aliquots and placed in the appropriate Petri plates for spreading. All of the plates were incubated at 37°C for 24 hours, during which time the plates were checked for the emergence of various colonies. Depending on the various colony morphologies, isolated bacterial colonies were selected and streaking on nutritional agar slants. For additional research, these pure cultures were employed.

MORPHOLOGICAL CHARACTERIZATION:

Gram's Staining.

A bacterial culture was used to create a smear for this experiment. After adding the dye and coloring the smear with 1-2 drops of crystal violet, the smear was allowed to sit for one minute before being removed. Water was used to remove the crystal violet stain from the carpet. Grams' stain was applied to the smear and allowed to sit for one minute before being removed. After that, thoroughly clean the area with water. The surface was then cleaned with a 70% ethanol wash to remove any remaining soap residue. Following that, a thorough water rinse was used to remove any remaining residue. It was observed under a microscope for the first time.

Capsule staining

A single loop of Indian ink was attached to the end of a microscope slide and inspected under a microscope. The sterile saline solution was added in one continuous loop to the ink. A negligible amount

of Indian ink has been polluted by foreign microorganisms. It was necessary to take a second slide, this time holding it at a 45-degree angle, to ensure that the ends of both slides came into touch. Following that, the higher slide was dragged down until it came into contact with the lower slide on the other side. When the top slide was pushed backward without being elevated, the stain spread further. To hasten the drying process, the slide was set outside to dry naturally. For a total of three minutes, the smear was saturated with Ethylene blue. Following that, the slide was gently cleaned with water to eliminate any remaining ethylene blue residue. It was placed outside in the sunshine to dry. A microscope was used to examine the specimen.

Spore Staining:

A thick smear was created using a bacterial culture and then stained. The application of heat to the smear. To prevent the water bath from drying out, it was required to cover the smear with tissue paper and expose it to steam for 3 to 4 minutes. Following that, the smear was stained with the malachite green reagent. The slide was withdrawn from the water bath and the tissue was carefully removed from the slide before adding the malachite green. Once the slide was completed, it was cleaned with diss. water. Two minutes of sliding time was required following the addition of six drops of safranin. The slide was then rinsed thoroughly with water and left to dry naturally. It was observed for the first time under a microscope.

BIOCHEMICAL TESTS:

CARBOHYDRATES FERMENTATION

Materials: Materials: Culture, Durham's tubes and sterile fermentation tubes, glucose, sucrose, and lactose broths, as well as an inoculating loop.

Method: The process involved transferring broth into fermentation tubes and inserting Durham's tubes into the fermenting tubes such that the broth also entered the Durham's tubes. 15 minutes at 12°C in an autoclave It was chilled at room temperature after autoclaving. All six inoculated and three non - inoculated tubes were maintained at 35 °C for 24 to 48 hours after the culture was inoculated.

CATALASE ACTIVITY

Materials: Culture, Hydrogen peroxide (3%) (make HI-MEDIA), Four sterile glass slides, Inoculating loop.

Method: A loop full of bacterial culture was placed on the glass slide under sterile conditions. To this inoculated glass slide of bacterial culture, three-four drops of hydrogen peroxide were allowed to flow on the bacterial culture. The formation of gas bubbles was observed.

HYDROGEN SULPHIDE PRODUCTION TEST

Materials: Culture. TSI (Triple Sugar Iron) agar tubes. Inoculating loop. Four sterile test tubes. Cotton plugs

Method: 100 milliliters of distilled water was used to dissolve 6.4 grams of TSI medium (make HI-MEDIA). 15 minutes at 121 °C in an autoclave. To create slants, it was autoclaved, cooled to 50°C, and then poured into four sterile tubes. The tubes were then closed with cotton and let to harden. Three tubes were given a culture inoculation while the fourth tube served as the control. For 48 hours, the tubes were incubated at 36 °C.

INDOLE PRODUCTION TEST

Materials: Tryptone broth, Sterile test tubes Culture, Kovac's reagent, Inoculating needle.

Method: The procedure involved pouring the cooled broth into four clean test tubes. A culture was put into two of the tubes, and a third was preserved as a control. Following the 48 hours of incubation at 35 degrees Celsius, the tubes were discarded. 48 hours later, 1 ml of Kovac's reagent was added to all of the tubes, including the control. After 10–15-minute sessions, the tubes were gently stirred. The test tubes were left upright so the reagent could rise to the surface.

METHYL-RED AND VOGES-PROSKAUER TESTS

Materials: Culture, Sterile test tube, Methyl red pH indicator (make HI-MEDIA), V-P reagent I (naphthol solution) MR VP broth, (make HI-MEDIA), V-P reagent II (40% Potassium hydroxide) (make HI-MEDIA), Inoculating loop.

Method: Following sterilizing, the liquid was chilled to 50 degrees Celsius before being placed into four clean test tubes. A culture was inoculated into three test tubes, and a fourth tube was left uninoculated as a control. The incubation period for all four tubes was 48 hours at 35°C. Two test tubes had five drops of methyl red indicator put in them.

CITRATE UTILIZATION TEST

Materials: Simmon citrate agar media (make HI-MEDIA), Four sterile test tubes, Inoculating loop, Culture,

Method: Media consisting of 3.4 grams of Simmon's citrate agar (pH 6.9) was weighed, diluted in 100 milliliters (ml) of distilled water, and then autoclaved at 15 pounds of pressure for 15 minutes. When the medium had cooled, it was placed into four clean test tubes and angled so that it could harden. The

culture was inoculated onto three Simmon's citrate agar slants using the stab-and-streak method. The uninoculated fourth tube served as a control. For 48 hours, all the test tubes were kept at 37 degrees Celsius.

UREASE TEST:

Materials: Sterile test tubes Urea agar medium (make HI-MEDIA), Urea solution 20 % in 100 ml, Culture, Inoculating loop.

Method: Autoclave at 121°C for 15 minutes, cool to 50°C, and then use 100 ml of distilled water to dissolve 2.8 grams of urea agar medium. Include 0.1 g of glucose and 3.0 ml of a 0.02% solution of phenol red. Pour into the liquid base, steam for an hour, and then cool to 50 degrees Celsius. One hundred milliliters of urea in water (20%). Filtered and then added aseptic conditions to the starter medium to ensure sterility. The solution was evenly dispersed across four clean test tubes, and the medium was allowed to settle while tilted at an angle. Three of the test tubes were inoculated with Culture after solidification, while a fourth served as a control that had not been inoculated. After exposing the slants at 37 degrees Celsius for 24 to 48 hours, they were considered ready for use.

STARCH HYDROLYSIS

Materials: Starch agar medium, Culture, Sterile Petri plate, Iodine indicator, Inoculating loop.

Method: Dissolved the aforesaid ingredients in 100 ml of distilled water in a conical flask, autoclaved the mixture for 15 minutes at 1210 C, and let it cool to 500 C. After thoroughly combining the solution, it was placed into a petri dish and left to solidify. The culture was then plated onto the plate when it had solidified. For 24 hours, the plate was kept in a 37°C incubator. Drop the iodine solution onto the plates and let it sit there for 30 seconds. The iodine solution was very strong, so I poured it off. Look for the sterile area that surrounds the bacterial growth line.

Anti-fungal activity

The dual culture technique identified anti-fungal activity. PDA media was used to cultivate *Fusarium oxysporum*, *Phytophthora infestans* and *sclerotium rolfsii*, all of which are soil-borne plant pathogens. After 96 hours of growth, a block of agar (five millimeters in diameter) was taken from a fungal culture and placed on top of a fresh agar medium in the middle of a Petri dish. Each bacterial culture was streaked with a loopful onto the edge of a Petri dish with a diameter of 90 mm, and the plates were incubated at 302°C for 24 hours. After 5 days of incubation, the inhibition zone between the two cultures was calculated. As a comparison, plates treated with the same fungus but without the bacteria served as controls. Percent inhibition over control and reduction in radial growth was assessed in three independent replicates for each.

Determination of minimum inhibitory concentration (MIC)

The dilution method was used to test the plant extracts for antibacterial activity, and the MICs were calculated (Magiatis et al., 2002). To combat bacteria and other microorganisms, the aforementioned strains were chosen. The antifungal efficacy of plant extracts was evaluated after they were diluted in various solvents and evaluated at varying doses. An agar plug containing a fungal culture was inserted in the middle of the plate to determine whether or not the plug possessed antifungal properties. Then, 2 centimetres out from the centre where the fungus was inserted, wells were drilled (using a sterilized agar borer), and various aliquots of the sample were added to each well before being incubated at 30.2 degrees Celsius for 24 to 96 hours. A value for the percentage of inhibition (I%) was determined.

RESULTS AND DISCUSSION:

An investigation was carried out in this study to determine the presence of antibiotic-producing bacteria in the soil samples collected from two different sites from the industry dumping sites in and around the different sites of Hyderabad. The experimental result obtained in this study were presented in a tabular form showing the microscopic characteristics of the bacterial isolates. A total of four species of bacteria were isolated. The macroscopic characteristics based on size, shape, colony color, margin, texture, surface, and opacity were analyzed for the isolated bacterial cultures.

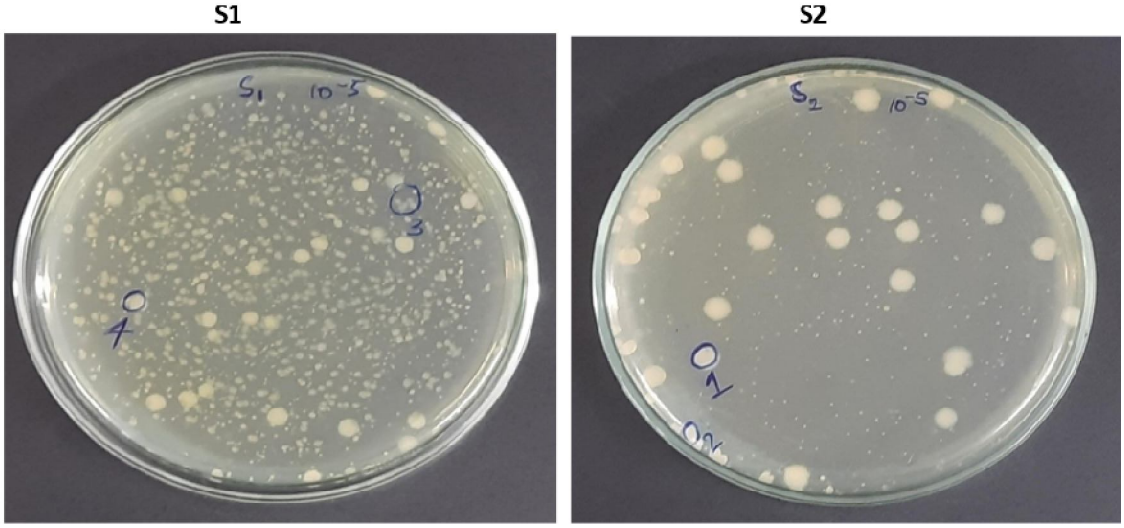


Figure 1: Spread plates of the serially diluted samples

Inoculums were diluted serially, then disseminated sterilely, and incubated at 37 °C for 24 hours on a plate of nutritional agar. One day later, colonies of the expanding bacteria were taken for observation. It was discovered that their morphology was small, smooth, and had delineated edges. Tables and figures below detail the Gram nature and biochemical features of the bacteria isolated.

Table 1: Morphological tests of bacteriological isolates

	I-1	I-2	I-3	I-4
Size	Large	Medium	Medium	Large
Shape	Circular	Irregular	Irregular	Circular
Color	Milky white	White	Pale yellow	Creamy white
Margin	Curate	Entire	Entire	Curate
Texture	Viscous dry	Smooth	Smooth moist	Thick, viscous
Surface	Flat	Flat	Flat	Flat
Opacity	Opaque	Opaque	Translucent	Opaque

Isolation of bacteria:

Once a potentially useful strain was identified by its distinctive colony morphology, pure cultures were obtained by streaking the colonies on nutrient agar slants.

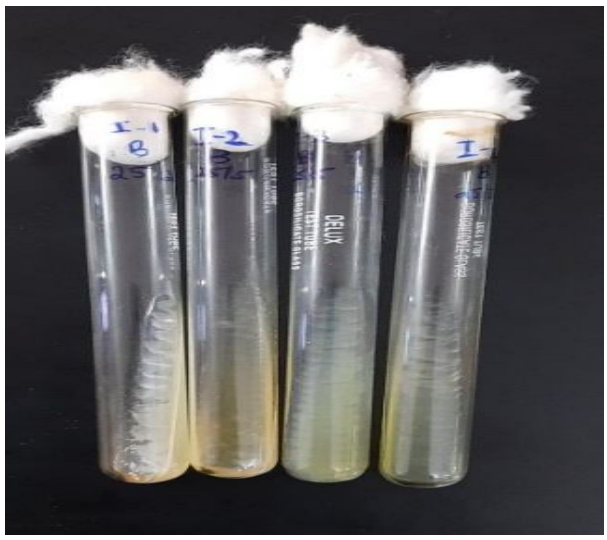


Figure 2: Subcultured isolates

Morphological tests:

Gram's staining was done to identify the shape, size, and structure which differentiates whether the bacteria are Gram (+ve) or Gram (–ve) based on the cell wall composition.

Figure 3: different staining procedures of the bacterial isolates.

GRAM'S STAINING: After the process of staining, purple-colored cocci bacteria were observed in all isolate 3 and purple-colored rod-shaped bacteria was observed in isolate 1,2,4. From the observation, all 4 isolates were identified as gram-positive bacteria. Bacteria

SPORE STAINING: After the process of staining, bacterial cells are observed to be pink-colored cells in all 4 isolates, no pink color was observed, which cleared that all 4 Isolates are non-sporulated.

CAPSULE STAINING: After the process of staining bacterial cells all the isolates are observed purple. No purple color was observed from all 4 Isolates, which declares all isolates are identified as non-capsulated.

BIOCHEMICAL TESTS:

Table 2: Biochemical tests against bacterial isolates.

	I-1	I-2	I-3	I-4
INDOLE	+VE	+VE	-VE	-VE
METHYL RED	+VE	+VE	+VE	-VE
VOGES-PROSKAUER	-VE	+VE	+VE	+VE
CITRATE	+VE	+VE	+VE	+VE
SUGAR	(No Evolution) -VE	(Evolution) -VE	(No Evolution)-VE	(Evolution) -VE
UREASE	+VE	+VE	+VE	+VE
CATALASE	-VE	-VE	-VE	-VE
STARCH	+VE	-VE	-VE	-VE
H2S	-VE	-VE	-VE	-VE

Indole production test:

The indole test measures the microorganism's capacity to convert the amino acid tryptophan into the indole compound. The presence of indole is denoted by the development of red coloration in the media. Isolates 3, 4, and the control group did not show the same development of cherry (deep red) color in the top layers of the tube as found in isolates 1, and 2. Isolate 3,4 shows a negative result for the Indole test because the top layer does not produce the characteristic cherry red color; in contrast, isolates 1 and 2 show a positive result.

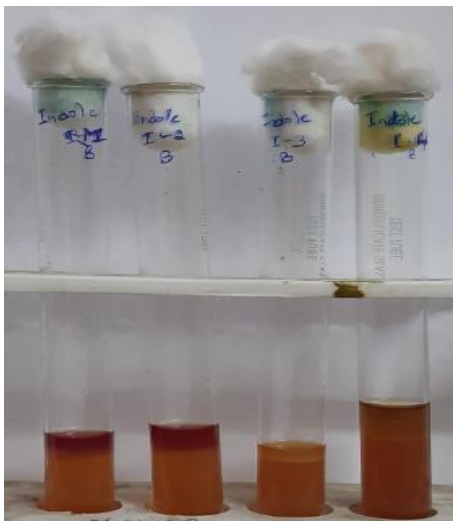


Figure 4: Indole production test

Methyl-red and Voges Proskauer tests:

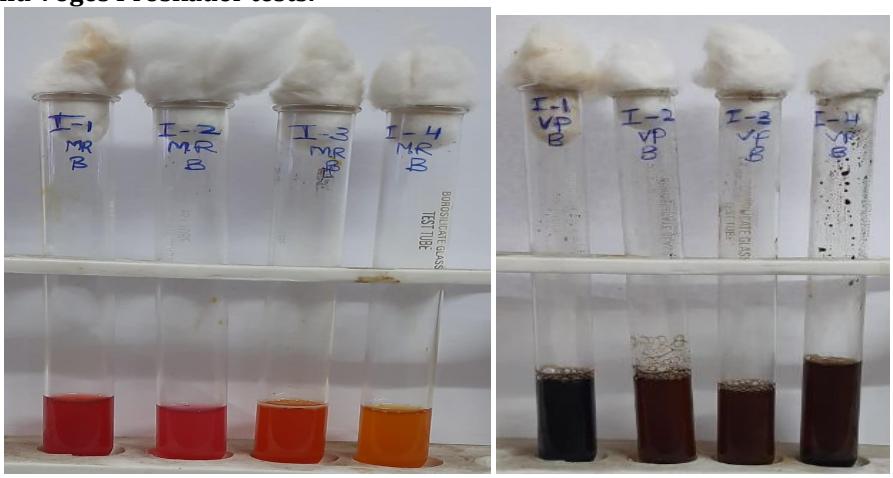


Figure 5: Methyl Red test and Voges Proskaeur test

After inoculating MR media with bacterial isolates and incubating the mixture at 35 degrees Celsius for 48 hours, we added a few drops of a methyl red solution to the medium to indicate the occurrence of mixed acid fermentation metabolites in glucose media and observed the resulting red hue. Isolate 1, 2, and 3 tubes became crimson once methyl red was introduced to them.

Bacterial isolates were inoculated into VP medium and then incubated at 35°C for 2 days with the addition of alpha-naphthol and potassium hydroxide to conduct the VP test. Turning glucose into acetone results in a favorable color change to red. Isolate 2,3,4 became crimson when V-P reagents I; II were introduced to the test tubes. Two, three, or four of the isolates tested positive for VP because they turned red in the V-P test.

Citrate utilization test:

Figure 6: Citrate test

The capability of bacteria to turn citrate into oxaloacetate is further processed into pyruvate and CO_2. After 48 hours of incubation, where the transition from green to vivid blue hue shows a rise in the pH level (above 7.6) of the media. It was noted that there is variation in the medium color for all the isolates. The test shows positive for all 4 isolates. If bacteria cannot utilize citrate, bacterial development would be absent.

Urease test:

20% aqueous solution of urea that has been purified through filtration and added aseptically to the base medium. Four sterile test tubes were filled with the solution after it had been well mixed, and the medium was left to solidify in a slanting position to create slopes. Three test tubes were inoculated with Culture after the solidification process, while a fourth tube served as a comparison control without inoculation. The slants were incubated at 37 °C for 24 to 48 hours.

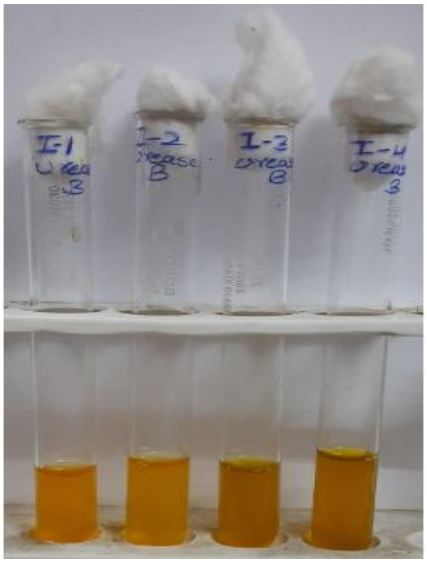


Figure 7: urease test

After incubation and the addition of methyl red, the yellow color was observed in the medium for all 4 Isolates. All 4 bacterial isolates are positive towards the urease test.

Catalase production test:

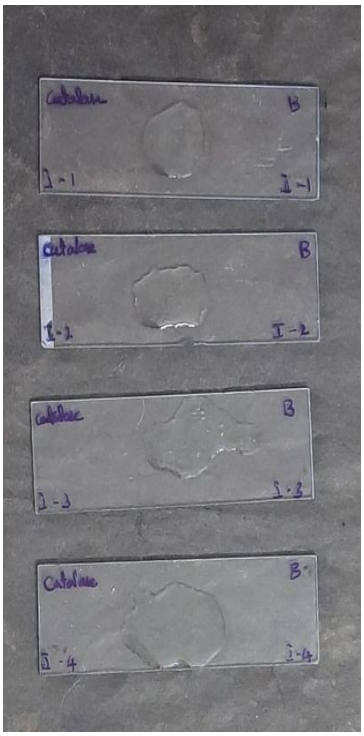


Figure 8: Catalase test

After placing a culture sample on the slide, hydrogen peroxide was applied drop by drop. All 4 isolates tested negative for catalase in samples that formed bubbles, indicating positive outcomes.

Carbohydrate fermentation test:

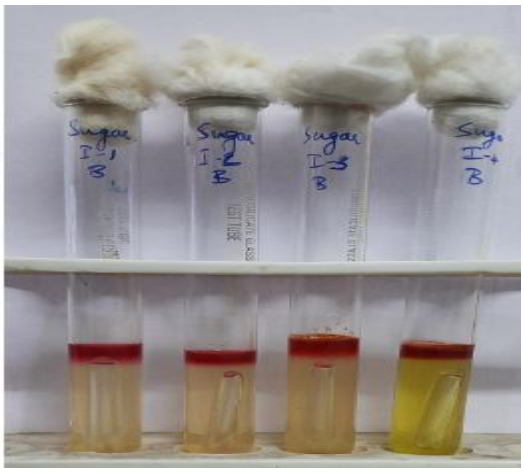


Figure 9: Carbohydrate fermentation test

Autoclaving the carbohydrate broth at 12loC for 15 minutes sterilized the fermentation tubes and Durham's tubes. It was allowed to cool to ambient temperature after being placed in an autoclave. After inoculating the culture, all six inoculation tubes and three control tubes were cultured at 35oC for 24-48 hours. No one of the four isolated strains exhibits a yellowing of the medium after being exposed to methyl red indicator. Therefore, the carbohydrate fermentation test is negative for 4 of the isolates.

H_2S Production test:

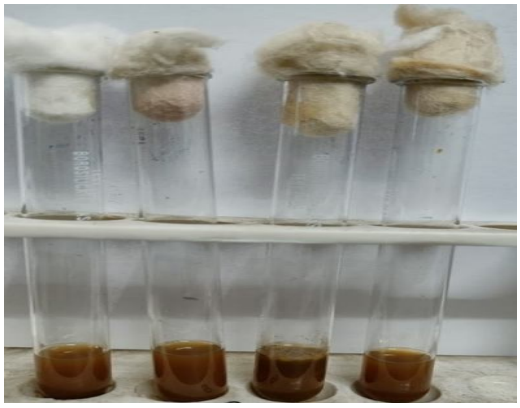


Figure 10: H 2 S test

TSI media was prepared, sterilized, and made 4 slants. Three tubes were inoculated with culture, keeping one tube un-inoculated Comparative to the control. The tubes were incubated at 36^{o}C for 48 hours. After incubation test solution color was not turned black in all 4 isolates.

Amylase test/starch hydrolysis test:

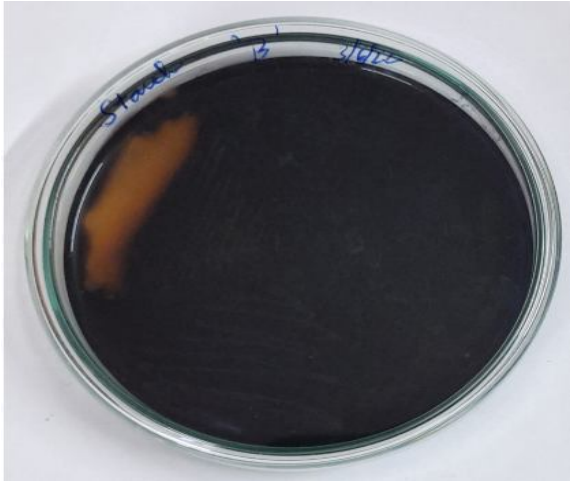


Figure 11: Amylase/ starch hydrolysis test

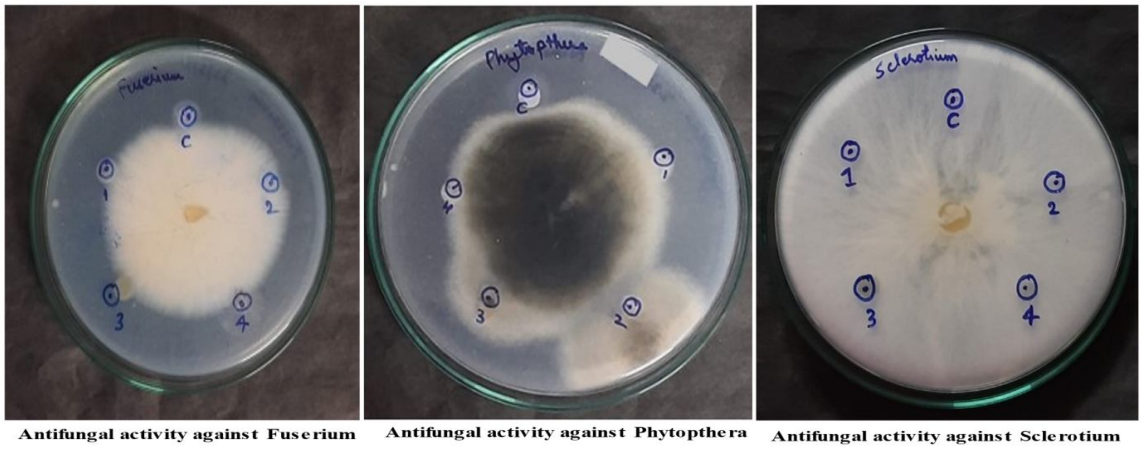
Starch agar medium was prepared, sterilized, and poured into a Petri plate and the medium was allowed to Solidify. After solidification, the plate was inoculated with Culture. The plate was incubated for 24 hours at 37°C. By adding iodine solution to a starch agar plate, the formation of golden yellow color was observed in isolate 1.

ANTIFUNGAL ACTIVITY:

Four of the isolates tested positive for antifungal activity against common plant diseases that reduce harvest yields. This data is summarised in the table below. Further, all the strains showed at least one inhibitory activity against the following three pathogens as measured by the growth inhibition zone through the replacement culture in an antibacterial medium: Fusarium oxysporum, Phytophthora infestans, and Sclerotium.

Table 3: Antifungal activities of different bacterial isolates.

	CONTROL	**1**	**2**	**3**	**4**
Fusarium oxysporum	52%	44%	38%	42%	32%
Phytophthora infestans	46%	44%	0%	26%	38%
sclerotium rolfsii	0%	0%	0%	0%	0%



Antifungal activity against Fuserium Antifungal activity against Phytopthera Antifungal activity against Sclerotium

Figure 12: Antifungal activity against *Fusarium oxysporum, Phytophthora infestans*, and *sclerotium rolfsii*

Out of four isolates in this study on three different fungal strains, two have produced antifungal activities. The 1 & 3 bacterial isolates were shown significant antifungal activity (44 & 42%) among the four isolates on *Fusarium oxysporum* fungal strain. Whereas the 1 & 4 of the bacterial isolates were shown 44 and 38% of antifungal activity against the Phytophthora infestans fungal strain. According to Ortz-Castro, R. et al. [13], it can be inferred that the availability of bacteria encourages plant growth by taking part in plant defense-related processes and suppressing mycelium growth and spore germination. For dangerous plant pathogenic bacteria, the use of microbiologic isolates strains as biological control agents have been documented [19]. Significant emphasis has been paid to the study of finding novel secondary metabolites with a range of biological activities in varied contexts [30]. The use of biological activities in agriculture has nevertheless received relatively little study attention [30]. The five selected isolates from this study's groundbreaking research on the utilization of bacteria in crop growth demonstrated great antifungal activity and can thus be employed as biological control agents to inhibit dangerous infections in crops. Four effective isolates with antifungal properties were found in this study. This improves our expertise in how soil remediation is regulated and how this bacterium influences plant development. Understanding the activity and evolution of microbial communities is crucial for process optimization since microorganisms play a significant role in both composting and promoting plant growth [20]. This is because changing the type and quantity of input materials can change the microbial population and the existence of numerous bacteria can either positively or negatively affects composting [8].

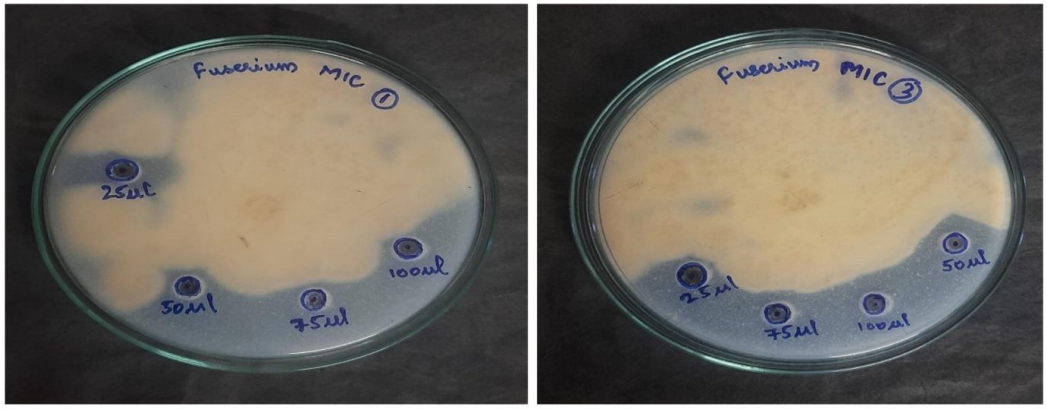
ANTIFUNGAL Minimum Inhibition Concentrations (MIC values)

Table 4: Minimum inhibition concentrations of the bacterial isolates on plant pathogens.

	Phytophthora infestans		***fusarium oxysporum***	
SAMPLE	**1**	**4**	**1**	**3**
25µL	52%	56%	56%	48%
50µL	56%	58%	58%	54%
75µL	60%	62%	62%	58%
100µL	64%	64%	64%	66%

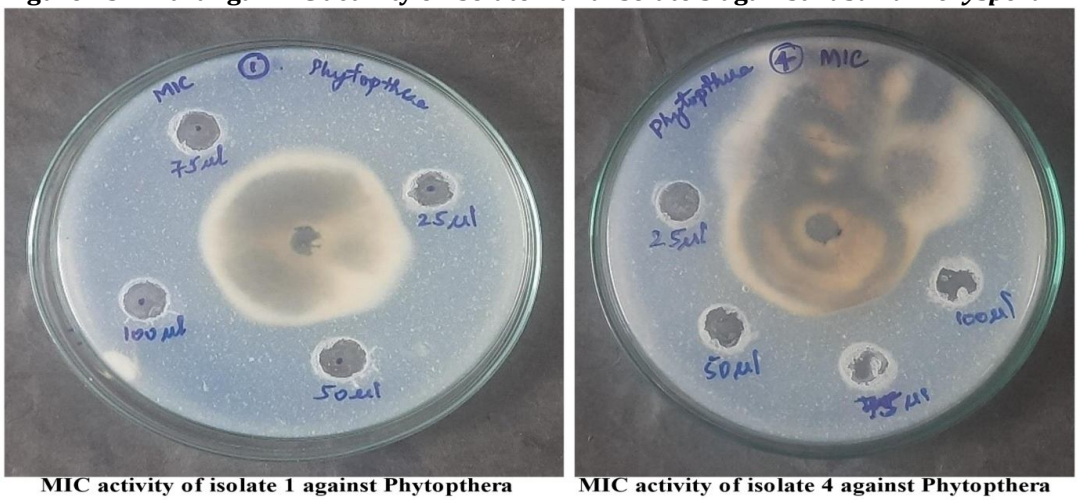
Furthermore, plant pathogens like *Fusarium oxysporum* and *Phytophthora infestans* were used to test the antifungal activity of isolated strains. Only two of the four fungal strains tested in this investigation were effective at producing inhibitory zones against pathogenic microorganisms that had been identified. The maximum inhibition zone produced by bacterial isolates was 66%, and it was effective against *Fusarium oxysporum*. Zones of inhibition against the fungus *Phytophthora infestans* were quite good for the same isolated bacteria.

The minimum inhibitory concentration (MIC) values (Table 4) show that all the *Fusarium oxysporum* and *Phytophthora infestans* fungal strains tested show significant antimicrobial activity against the four bacterial strains used in the study, with the highest values for isolate 3 of *Fusarium oxysporum* strain 66% of inhibition zone with 100µL sample, and the lowest values for isolate 1 of *Phytophthora infestans* showing 52% inhibition zone with 25µL.



MIC activity of isolate 1 agains Fuserium MIC activity of isolate 3 agains Fuserium

Figure 13: Antifungal MIC activity of isolate 1 and isolate 3 against *Fusarium oxysporum*



MIC activity of isolate 1 against Phytopthera MIC activity of isolate 4 against Phytopthera

Figure 14: Antifungal MIC activity of isolate 1 and isolate 4 against *Phytophthora infestans*

The capacity of relatively halophilic bacteria to combat plant diseases like Grey Mould tomato plants caused by *B. cinerea* was first reported by Sadfi-Zouaoui *et al*., [18]. Hydrolytic enzymes such as proteases, chitinases, amylases, laminarinases, lipases, and cellulases are the main target of halotolorants, as demonstrated by this team. In another study, it was reported that the antibacterial and antifungal capabilities of halophiles may occur owing to their numerous plasmids S. Todkar *et al*., [21] *C. albicans* is a

human pathogenic fungus, yet the D6A strain demonstrated an inhibition zone of 39 mm against it. In this regard, the antifungal activity of a slightly halophilic strain of actinomycete HB-11 was observed against diverse fungal species.

CONCLUSIONS

Antibiotics produced by the isolated bacteria were shown to be effective against plant pathogenic fungi in the current investigation. There was promising evidence that some isolates could generate new antibacterial chemicals. The purpose of this study was to identify and isolate microorganisms capable of creating effective antibiotics from the area's waste dump. Isolates from these regions performed well in antifungal experiments, suggesting they could be a rich source of novel antibacterial and antifungal drugs. To determine the mechanism of action of antimicrobial substances, additional analytical purification of extracts is required.

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