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Biochemical Metabolite Analysis: GC Nitrogen and Carbon for Antibacterial Activity and Molecular Identification of Soil-Producing Antibiotic Bacteria

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Abstract: In order to combat infections and cure illnesses, fungi and bacteria produce antibiotics. The need for new antibiotic discoveries is a result of antibiotic resistance. Since pathogenic bacteria are common in soil, many techniques have been employed to create strong natural antibiotics against them. Bacteria that produce antibiotics were isolated from soil in this investigation. Taken from the Bhopal region. Of the five isolates, two were able to inhibit Klebsiella pneumoniae and Escherichia coli growth. Although the strains differed in culture, morphological and biochemical tests recognized them as Pseudomonas. Pseudomonas fluorescence and aeruginosa were determined to be the amplified 16S rRNA PCR products after sequencing revealed 98% and 97% similarity scores, respectively. Based on this research, it is possible that Pseudomonas species generate antibiotics that suppress a variety of microorganisms and boost their growth. Regarding their ability to inhibit bacteria, the isolates favored distinct sources of carbon and nitrogen. The culture filtrates that were used were sterile, filter-sterilized, and proteinase K-treated; the sterilized filtrates exhibited greater antibacterial activity. 18.5 And 15.5 mm inhibition zones were shown for streptomycin and culture filtrates. All isolates' antibacterial activity against human pathogens is reported for the first time in this study. Culture filtrate antibacterial activity was significantly decreased by proteinase K. It appears from these results that these three bacteria may be isolates with antibacterial properties. More than 5 mg/mL ciprofloxacin, all bacterial extracts inhibited E. coli growth. As per the study, these species possess the ability to generate antimicrobial compounds that combat microbial infections and strains that are resistant to drugs. Propanoic acid, oxalic acid, phenol, and hexadecanoic acid were among the antimicrobial substances identified by GC-MS in bacterial extracts.

Keywords: Antibiotic, soil, Bacteria, Pseudomonas, Metabolites Phylogenetic.

1. Introduction

The majority of bioactive microorganisms are found in the top few inches of agricultural soils [1]. Microorganisms are supported by oceanic crust rocks, frigid temperatures, hot springs, and deep depths [2–5]. Soil microorganism activity and diversity are influenced by both biotic and abiotic factors. Microbes are influenced by a variety of factors, including temperature, humidity, pH, chemical makeup, nutrients, and vegetation. Soil health is affected by seasonal changes. Lastly, organic wastes from agriculture enrich soil microbes [6]. Nutrient cycling is carried out by *Clostridium, Caulobacter, Frankia Nitrobacter, Nitrosomonas, Pseudomonas, Rhizobium* and *Thiobacillus* [7].

Researchers in the fields of human health, agriculture, and animal husbandry have discovered, characterized, and used numerous bacteria that generate metabolites, enzymes, antibiotics, and unique compounds in recent years [8, 9]. Secondary metabolites are produced by microbes with structures. Specific secondary metabolites eliminate infections. Germs are killed by antibiotics [10]. Antibiotics for humans are mostly bacterial. Most of the 500 antibiotics that are discovered each year are produced by soil bacteria [11, 12]. Antibiotics, which are low-molecular-mass secondary

metabolism products (< 1500 kDa), are produced by a small group of microorganisms during the late growth phase (idiophase) [10]. Antibiotics that fight germs are produced by bacteria [13]. *Bacillus, Cephalosporium, Micomonospora, Penicillium and Streptomyces* are the sources of the majority of antibiotics [14]. Gram-positive bacteria are killed by the bacteriocins found in Polypeptide Bacillus [15, 16].

Using 12•9 × 109 units (10•7 per person) in 2010, India was the country that used the most antibiotics [17]. As many antibiotics lose their effectiveness and pathogenic bacteria develop resistance, there is an increasing demand for bacterial antibiotics worldwide [18–19]. Researchers in medicine and pharmaceuticals were concerned about drug resistance in bacteria. Alternative antimicrobial agents are required for dairy, food preservation, and clinics [20–21]. When antibiotics are used inappropriately, pathogenic bacteria become less effective and resistant to drugs. Antibiotic-resistant pathogens pose a challenge to contemporary medicine. The prevention of infectious diseases is jeopardized by antibiotic drug resistance [22]. Bacteria alter genes and remove medication efficacy in order to survive in the hostile environment [23].

New antibiotics or antimicrobials are needed because antibiotic-resistant bacteria pose a threat to public health [24]. Many studies on the synthesis of novel antibiotics from a variety of plants and microorganisms have been conducted in recent decades [25–28]. Soil texture, flora, moisture content, and nutrient availability all have an impact on these microbial species and populations [29]. Antibiotics are made by bacteria and are used by them in defense. Bacteria are protected and signaled by antibiotics [30–31].

The development of antibiotics depends on natural products [32]. We desperately need novel antimicrobials that can either kill or control a wide variety of microorganisms. Antibiotics are used in modern medicine. New antibiotics are needed because common antibiotics become less effective against certain pathogenic strains [33–34]. Microorganisms that produce secondary metabolites have distinct structures and roles. Antibiotic-like bioactive metabolites are produced by certain soil microflora [29]. Novel antimicrobials were used in other significant studies on soil bacterial isolation [35–36].

Multidrug-resistant bacterial pathogens proved difficult for public health officials to contain, increasing their danger. Bacterial pathogen resistance frequently leads to secondary infections in life-threatening conditions such as cancer, surgery, transplantation, etc. and impacts current medication treatment [37, 38]. The rapid development of MDR strains makes drug treatment difficult [39–40]. Microbes create antibiotics to protect their home. Antibiotics are used to treat illnesses in humans [41]. Medicine was revolutionized by antibiotics [42]. Drug-resistant pathogens, however, are the result of abuse or overuse [43, 44]. Growing antibiotic resistance poses a threat to modern medicine [45]. Thus, it is essential to discover novel antibiotics that eradicate resistant bacteria. Drug resistance has numerous causes, including misuse of antibiotics and careless disposal. Antibacterial products are used therapeutically in agriculture, human medicine, and animals to aid in the survival and migration of resistant bacteria [466]. Soil resistance bacteria are spread by the improper use of animal wastes as fertilizers. Drug-resistant bacteria are spread by consuming animal products [47]. New antibiotics that are safe, efficient, and effective are required to fight drug-resistant pathogens.

The most promising new sources of antibiotics are microbial secondary metabolites [48, 49]. Numerous bacteria in soil produce metabolites that kill coexisting microorganisms, thereby acting as antibiotics [50]. Temperature, pH, moisture content, and nutrients all affect soil antibiotic diversity [44]. As was previously mentioned, Pseudomonas species, which produce antibiotics, are primarily found in soil. They can adapt to any habitat because of their capacity to produce spores [51]. To ascertain the diverse physiology of Pseudomonas species, intricate biochemical tests are required. Promising results are obtained from phylogenetic analysis of bacteria using 16S rRNA and gyrase B sequence analysis [51]. We employed molecular techniques to separate and identify antibiotic-producing bacteria from agricultural soil in Bhopal, Madhya Pradesh, India that exhibited antibacterial activity.

2. Materials and Methods

Collection of samples

Samples of soil were taken from waste dumps in the Indian city of Bhopal. Following the removal of all surface debris, the area was excavated to a depth of 4-5 cm, and using a sterile spatula and a plastic bag, about 10-15 g of soil were

collected. To gather soil samples, five distinct locations within the disposal area were utilized. The soil sample-containing bag was clearly labeled, brought into the laboratory, and kept at 4 0C until additional examination.

Soil bacteria isolation and maintenance

The well-known "Crowded plate" method was used to isolate soil bacteria after the recommended serial dilution procedure. To obtain a 1:10 dilution, one gram of each soil sample was weighed and then soaked in ten milliliters of sterile distilled water. It was given a good shake and then allowed to settle for sediment. After being collected, the supernatant was serially diluted. Using a glass rod, 100 µl of each dilution was aseptically spread out on nutrient agar plates that had been labeled. The agar plates were incubated at 37 oC for 24 to 48 hours after a few seconds. To obtain pure colonies, the recognizable colonies were chosen for streaking on an agar plate individually. For later research, pure culture was kept at 4 °C.

The isolated bacteria's physiological characteristics

Under a microscope, the morphology of each bacterial isolate colony on agar plates was examined. Following a 24-hour incubation period at 37ºC, the color, shape, appearance, colony diameter, and transparency of each individual colony were assessed. Thermo Fisher Scientific, Massachusetts, USA) gram staining method was utilized to differentiate between gram-positive and gram-negative bacteria.

Secondary screening for bacteria that produce antibiotics

The antibacterial activity of pure colonies derived from various bacterial isolates was evaluated against two gram-negative bacteria, namely Escherichia coli and Klebsiella pneumoniae. Gram negative bacteria were chosen because they have a history of causing serious infections in humans and because they contain endotoxin. Bacteria that produce antibiotics were subjected to secondary screening using the agar diffusion method. Using the spread plate technique, 100 µl of the pathogenic culture was inoculated into each plate. The separated colonies' overnight culture was centrifuged, and the 250µl supernatant was absorbed on the discs for later use. After being put on the agar plates, these discs were left in the incubator for the entire night. A blank disc was employed as a control group. Each inhibited zone's diameter was measured in order to gauge how strongly the test pathogens were antagonistic.

Biochemical examination of the separated microorganisms

In order to assess the chemical nature of the bacterial isolates, biochemical characterization was performed. Following standard protocols, we performed tests for oxidase, catalase, Voges-Proskauer, methyl red, indole production, starch hydrolysis, citrate utilization, carbohydrate fermentation, and growth on MacConkey agar. (Fig. 1)

Mass spectrum analysis of metabolites using gas chromatography

GC-MS analysis of bacterial secondary metabolites was performed on Thermo Scientific GC Focus Series DSQ. A constant flow rate of 1 mL per minute with an infection volume of 1 µL was used with helium gas as the carrier gas. The injector and hot oven were maintained at 250 °C and 110 °C, respectively, and increased by 10 °C per minute to 200 °C, then 5 °C per minute to 280 °C, closing after 9 min at 280 °C. Different compounds' GC column peaks were eluted and their retention time recorded. After matching data with compound mass spectra, the database searched for similar compounds with the same molecular mass and retention time. The current research compared the bioactivities of bacterial extracts and their components to previously studied natural compounds.

Sources of nitrogen and carbon and their impact on antibacterial activity

It was investigated how various carbon and nitrogen sources affected the antibacterial activity of the culture filtrates. Each 250-ml Erlenmeyer flask was filled with 50 milliliters of the synthetic medium that had been modified with different sources of nitrogen (0.3%) and carbon (1%) before being sterilized. The synthetic medium contained the following ingredients: 10 g of sucrose, 1.2 g of K_2HPO_4, 0.8 g of KH_2PO_4, 0.2 g of $MgSO_4$ $7H_2O$, 0.3 g of NH_4NO_3, 1000 ml of water, and pH 6.8–7.00. Carbon sources included arabinose, fructose, galactose, glucose, lactose, maltose, mannitol, and sucrose. Nitrogen sources included casein, NH_4Cl, NH_4NO_3, $NaNO_3$, $NH_4H_2PO_4$, KNO_3, $(NH_4)_2SO_4$, and urea. Following PR1, PR2, and PR3 inoculation, the flasks were shaken and incubated at 30 °C for 48 hours. After 48 hours, the liquid cultures of PR1, PR2, and PR3 were placed in a centrifuge and spun at 5000 × g. Filters used in cell-free culture were gathered and kept at 4 °C.

Genomic DNA extraction from specific bacteria

To get rid of the supernatant for the cell pellet, the bacterial broth was centrifuged. After being cleaned with 0.9% saline, the pellet was suspended in the digestion buffer. Following the manufacturer's instructions, an automated DNA extractor (Invent Technologies Ltd., Dhaka, India) was used to extract genomic DNA. Using a spectrophotometer set to measure wavelengths between 260 and 280 nm, the concentration of isolated DNA was determined. Prior to usage, the genomic DNA's purity was examined. 16S rRNA amplification and sequencing for molecular identification of bacteria GoTaq® Green Master Mix (Promega Corporation, Wisconsin, USA) was used to amp up the 16S rRNA gene fragments in accordance with the manufacturer's instructions. The forward (5'-AGA GTT TGA TCM TGG CTC AG-3') and reverse (5'-CGG TTA CCT TGT TAC GAC TT-3') primers were made from universal primers. To sum up, the PCR began with an initial denaturation that lasted three minutes at 95 °C. 32 cycles of optimum denaturation were carried out, with 30 s per cycle at 95 °C. After 30 seconds of annealing at 55 °C, there was a 1-minute extension at 72 °C. The last step of the PCR was extension for five minutes at 72 °C. In the electrophoresis tank, the amplified PCR products were run on a 1% agarose gel. Ethidium bromide was used to stain the gel, and an AlphaImager transilluminator (Alpha Innotech, California, USA) was used to scan the gel. The amplified gene segments came from Invent Technologies Ltd. in Dhaka, India, and were purified and sequenced. Using the sequence alignment editor BioEdit 7.2, 16S rRNA gene sequences were aligned and then exported into BLAST to find matches with preexisting reference sequences.

3. Results and Discussion

Separate isolates were filtered out using the crowded plate method.

Following the crowded plate technique, soil samples were cultured on media and colonies with distinct morphology were discovered. Plates with densely populated but clearly defined colonies were chosen for screening. At dilution 104, five distinct colonies displayed characteristics that could be identified. In order to preserve their pure cultures for later use, these five antagonistic bacterial colonies (PI-1, PI-2, PI-3, PI-4, and PI-5) were isolated.

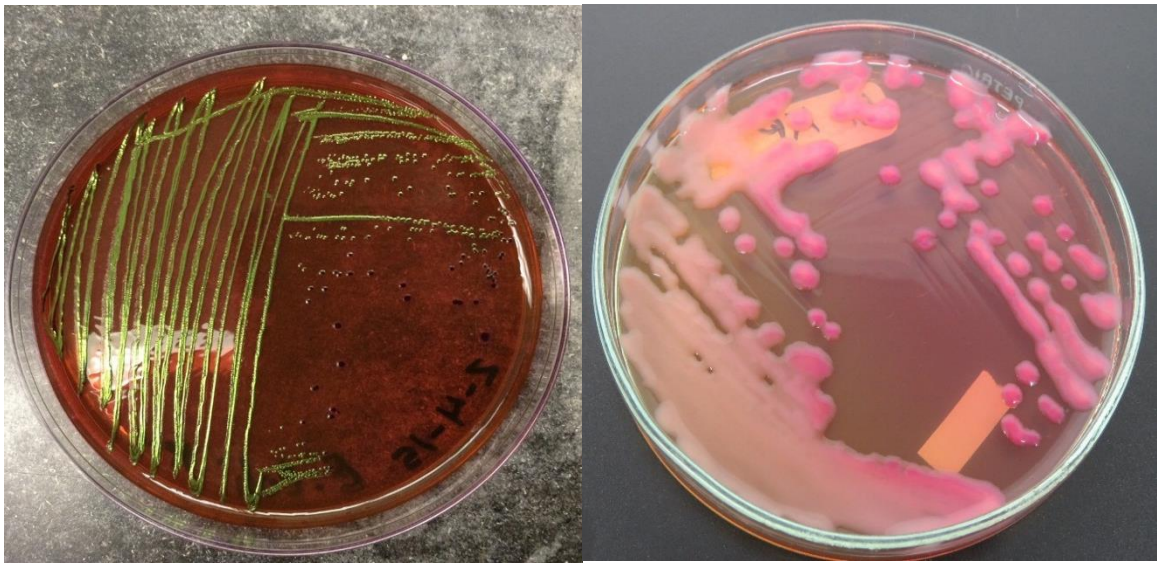

Figure 1. Isolates of E.coli and K. Pneumonia

Table 1. Antagonistic activity of bacterial isolates against test pathogens.

Test Pathogen	Isolates	Zone of inhibition (mm)
Escherichia coli	PI-1	none
	PI-2	14.9
	PI-3	08.99
	PI-4	05.26
	PI-5	none
Klebsiella pneumonia	PI-1	07.00
	PI-2	04.75
	pI-3	12.7
	PI-4	07.35
	PI-5	none

Table 2. Colony morphology and gram staining of bacterial isolates.

Metabolites of various isolates

The antibiotic efficacy of the five bacterial isolates was assessed using the disc diffusion assay against the pathogenic test strains of Klebsiella pneumoniae and Escherichia coli. (Fig.1) The outcome demonstrated that every isolate had antibacterial activity, with the exception of PI-5. While PI-1, PI-2, PI-3, and PI-4 demonstrated zones of inhibition against K. pneumoniae, PI-2, PI-3, and PI-4 demonstrated prominent zones of inhibition against E. coli (Table 1). For additional examination, the top two isolates (PI-2 and PI-3) were chosen.

Characteristics	PI-2	PI-3
Shape of colony	Round	Circular
Surface texture	Flat	Flat
Edge	Partial	Partial
Elevation	Elevated	Umbonate
Pigmentation	Creamy white	Creamy white
Growth in broth	Pellicle	Pellicle
Gram staining	Positive (+)	Positive (+)

Table 3. Biochemical test results for the identification of bacterial isolates.

Biochemical test	PI-2	PI-3
Oxidase Test	+	+
Catalase Test	+	+
Voges-Proskauer Test	+	-
Methyl Red Test	-	+
Indole Test	-	-
Starch Hydrolysis Test	+	+
Citrate Utilization Test	-	-

Mentioned in Table 3, a number of biochemical tests were carried out. According to the results above, PI-2 and PI-3 are members of the gram-positive Pseudomonas species. (Fig.2)

Characterization of PI-2 and PI-3 morphologically

The standard technique for analyzing the morphology of bacterial colonies on an agar plate is used to describe the growth of bacteria. PI-2 and PI-3 were found to be smooth-surfaced, circular-shaped colonies under a microscope. Table 2 displays the specific features of their colony morphology. Since both isolates were growing at the broth's surface, the growth in the broth was pellicle-like. They were aerobic, based on this result. Additionally, the gram staining method was used to determine the characteristics of their cell walls. The findings indicated that both isolates

had maintained their purple hue, identifying them as gram-positive bacteria. PI-2 and PI-3's microscopic observations revealed that they had bacilli as their natural shape (Figure 1). To verify the identity of the isolated strains, which are

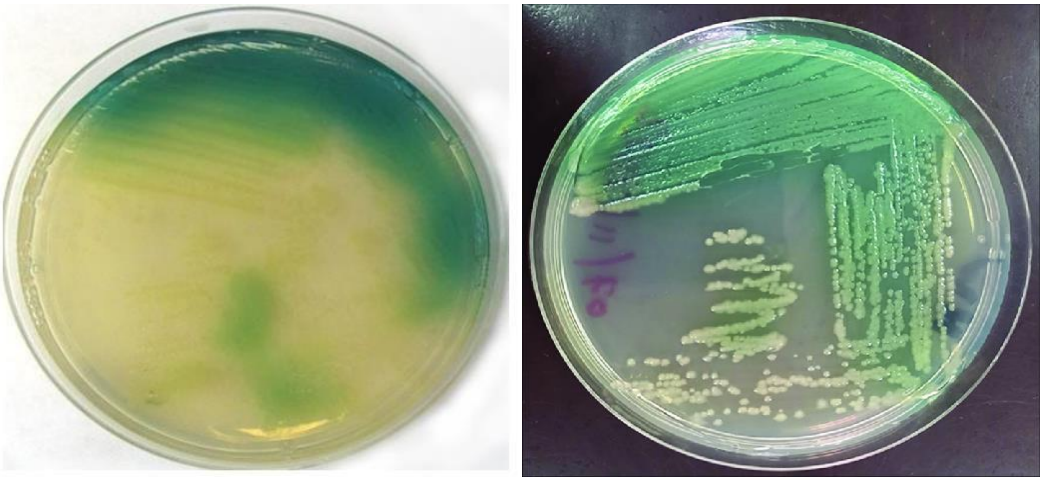

Figure 2. Bacterial plates isolte Pseudomonas species

The GC-MS analysis revealed a wide range of compounds in crude extracts from various bacterial species. The most significant and conspicuous elements of the crude extract subjected to GC-MS analysis were those that disclosed the locations and prior identifications of the compounds found in this study. These substances displayed properties similar to those of bacterial and plant products that are found naturally. The majority were derivatives of volatile substances like ethers, alkaloids, esters, and phenolic compounds, according to the GC-MS data analysis. *Propanoic acid, oxalic acid, phenol, and 1, 3, 5-trioxane* were identified by GC-MS analysis as the main metabolites present in the Bacillus species extracts. Furthermore, compounds with antibacterial, antifungal, and antioxidant properties were found to include *octadeccenoic acid, hexadecanoic acid, cyclobutane, dasycarpidan, 2-hydroxyl-1,3-propanedyl ester, cholestan-3-ol, and 2methylene.*

Crude Bacterial Isolate Extract's Antibacterial Potency

The in-vitro antibacterial potency of crude extracts of bacterial isolates against MDR Gram-positive and Gram-negative bacteria was dependent on whether an inhibition zone was present or absent. The crude extract inhibited twelve MDR strains with inhibition zones ranging from 7 to 28 mm. The bacterial extracts effectively inhibited most Gram-positive and Gram-negative MDR bacterial strains. With an IZ of 28.22 mm, *Paenibacillus dendritiformis* exhibited the highest activity against *Pseudomonas flourfesence. P. aeruginosa, K. pneumoniae, and Acinetobacter baumannii* came in second and third, respectively, at 28 mm, 27 mm, and 21 mm. MRSA, *K. pneumoniae, P. aeruginosa, S. aureus, and S. typhi* were all inhibited by Brevibacillus formosus at 24 mm, 22 mm, 22 mm, 21 mm, and 24 mm, respectively. *Pseudomonas aeruginosa* inhibited *E. Coli, S. typhi, K. pneumoniae, MRSA,* and *S. aureus* at 28 mm, 27 mm, 23 mm, and 22 mm, respectively. All strains are most effective against *E. Coli, S. aureus, K. pneumoniae, Acinetobacter baumannii, and P. aeruginosa*. The least activity was observed against P. aeruginosa and MRSA. The MICs of S. aureus, S. typhi, and E. coli were 1.25, 0.412, and 0.312 mg/mL, respectively. The MIC values of the ethyl acetate extract of bacterial isolates were 0.312, 0.422, 1.25, and 2.5 mg/m for S. typhi, S. aureus, and E. coli.

Identification of PI-2 and PI-3 molecules

Bacterial 16S rRNA PCR products were purified and sequenced. NCBI BLAST was used to analyze the bacterial isolates PI-2 and PI-3. In BLAST, the bacterial isolate PI-2 displayed 98% similarity with the Pseudomonas flourscence database sequence that was currently available. Conversely, Pseudomonas aeuroginosa and isolate PI-3 shared 97% of their similarities. The accession numbers for the deposited BI-2 and PI-3 sequences in GenBank were JF500887.1 and MH298778.1, respectively. Table 4 displayed the PI-2 and PI-3 detailed sequences. By building a

phylogenetic tree with neighbor-joining techniques in MEGA7 software, the phylogenetic positions of the isolates were also assessed [52] (Fig. 3).

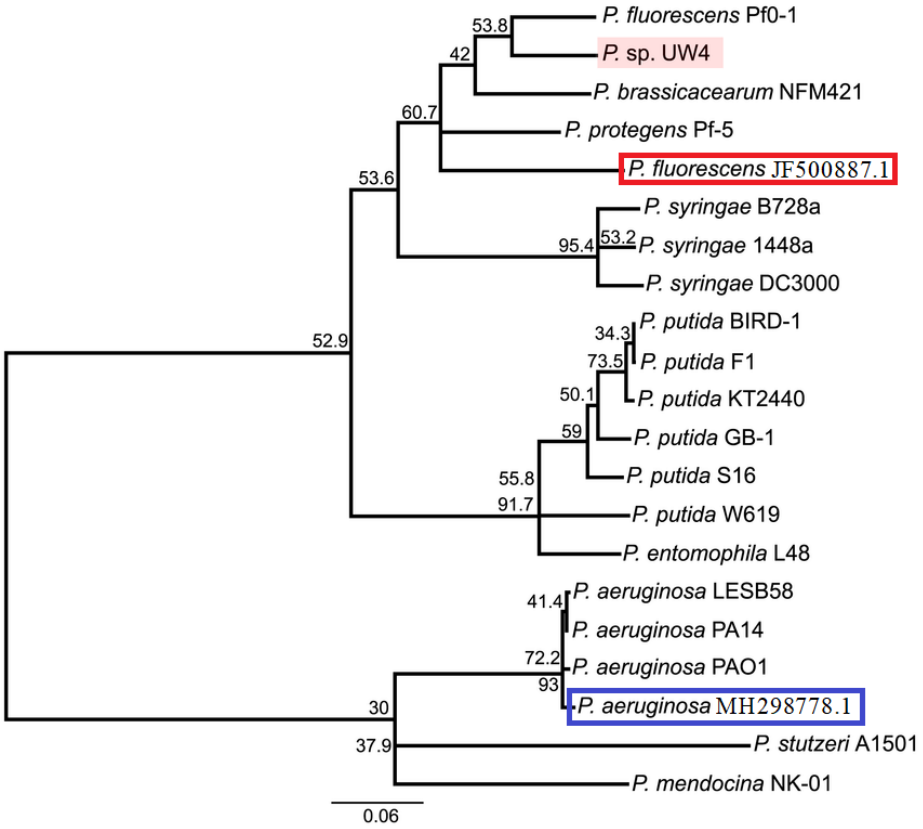

Figure 3 illustrates the evolutionary relationships of bacterial isolates PI-2 and PI-3, determined through the Neighbor-Joining method in MEGA7. Bootstrap test results (500 replicates) are displayed on branches, indicating the percentage of replicate trees where associated taxa clustered together. Evolutionary distances were computed using the number of differences method, with 10 nucleotide sequences analyzed. Gaps and missing data were excluded, and the box highlights deposited sequences of PI-2 and PI-3.

Table 4. 16S rRNA gene sequence of antagonistic bacterial isolates PI-2 and PI-3.

Bacterial	**16s rRNA gene sequence**
PI-2 (*Pseudomonas flourscence*)	>JF500887.1 Uncultured Pseudomonas sp. clone 1 16S ribosomal RNA gene, partial sequence AGAGTTTGATTATGGCTCAGATTGAACGCTGGCGGCAGGCCTAACACATGCAAGTC GAGCGGTAGAGAGG TGCTTGCACCTCTTGAGAGCGGCGGACGGGTGAGTAATGCCTAGGAATCTGCCTGG TAGTGGGGGATAAC GCTCGGAAACGGACGCTAATACCGCATACGTCCTACGGGAGAAAGCAGGGGACCT TCGGGCCTTGCGCTA TCAGATGAGCCTAGGTCGGATTAGCTAGTTGGTGGGGTAATGGCTCACCAAGGCGA CGATCCGTAACTGG TCTGAGAGGATGATCAGTCACACTGGAACTGAGACACGGTCCAGACTCCTACGGG AGGCAGCAGTGGGGA ATATTGGACAATGGGCGAAAGCCTGATCCAGCCATGCCGCGTGTGTGAAGAAGGT CTTCGGATTGTAAAG CACTTTAAGTTGGGAGGAAGGGCATTAACCTAATACGTTAGTGTTTTGACGTTACC GACAGAATAAGCAC CGGCTAACTCTGTGCCAGCAGCCGCGGTAATACAGAGGGTGCAAGCGTTAATCGG AATTACTGGGCGTAA AGCGCGCGTAGGTGGTTCGTTAAGTTGGATGTGAAATCCCCGGGCTCAACCTGGGA ACTGCATTCAAAAC TGTCGAGCTAGAGTATGGTAGAGGGTGGTGGAATTTCCTGTGTAGCGGTGAAATGC GTAGATATAGGAAG GAACACCAGTGGCGAAGGCGACCACCTGGACTGATACTGACACTGAGGTGCGAAA GCGTGGGGAGCAAAC AGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCAACTAGCCGTTGGGAG CCTTGAGCTCTTAG TGGCGCAGCTAACGCATTAAGTTGACCGCCTGGGGAGTACGGCCGCAAGGTTAAA ACTCAAATGAATTGA CGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAAT
PI-3 (*Pseudomonas aeuroginosa*)	>MH298778.1 Pseudomonas sp. strain aeuroginosa 16S ribosomal RNA gene, partial sequence TGATACGTGGAGCGGACGAAGGGAGCTACGCACCCGGATTCCGCGGCGGTAGGGT GAGTAATGCCTAGGA ATCTGCCTGGTAGTGGGGGATAACGTCCGGAAACGGGCGCTAATACCGCATACGTC CTGAGGGAGAAAGT GGGGGATCTTCGGACCTCACGCTATCAGATGAGCCTAGGTCGGATTAGCTAGTTGG TGGGGTAAAGGCCT ACCAAGGCGACGATCCGTAACTGGTCTGAGAGGATGATCAGTCACACTGGAACTG AGACACGGTCCAGAC TCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATCCAG CCATGCCGCGTGTGT GAAGAAGGTCTTCGGATTGTAAAGCACTTTAAGTTGGGAGGAAGGGCAGTAAGTT AATACCTTGCTGTTT TGACGTTACCAACAGAATAAGCACCGGCTAACTTCGTGCCAGCAGCCGCGGTAATA CGAAGGGTGCAAGC GTTAATCGGAATTACTGGGCGTAAAGCGCGCGTAGGTGGTTCAGCAAGTTGGATGT GAAATCCCCGGGCT CAACCTGGGAACTGCATCCAAAACTACTGAGCTAGAGTACGGTAGAGGGTGGTGG AATTTCCTGTGTAGC GGTGAAATGCGTAGATATAGGAAGGAACACCAGTGGCGAAGGCGACCACCTTGAC TGATACTGACACTGA

	GGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTA AACGATGTCGACTAG CCGTTGGGATCCTTGAGATTTTAGTGGCGCAGCTAACGCGATAAGTCGACCGCCTG GGGAGTACGGCCGC AAGGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGG TTTAATTCGAAGCAA CGCGAAGAACCTTACCTGGCCTTGACATGCTGAGAACTTTCCAGAGATGTAGTGGT GCCTTCCGGGAACC TCGTAGTACCTTAGCTGCGCTGCGTGCG

For the reason that so many researchers have chosen to conduct their investigations in soil [53–56], it is considered to be one of the most effective sources for isolating novel antibiotics. In accordance with the findings of certain reports, the heterogeneity of the soil environment is responsible for the presence of a diverse population of soil bacteria [57]. Through the use of soil obtained from a waste dumpsite, the microorganisms that are accountable for the production of antibiotics were successfully isolated in the current study. Using the 16S rRNA of two bacteria that produce antibiotics, we have sequenced them and added them to the BLAST database that is maintained by the NCBI.

The observation of colony morphology and Gram staining were the methods that were utilized to accomplish morphological identification. The Gram staining technique is a well-known and distinctive traditional method that is used to characterize bacteria [9, 58]. The staining procedure revealed that the two bacterial isolates, BI-2 and BI-3, were gram positive. This was demonstrated by the results of the process. Our findings are supported by the report [54], which states that the majority of soil isolates are gram positive in naturally occurring conditions. The antagonistic properties of PI-2 and PI-3 were investigated during the secondary screening procedure by making use of the filtrate obtained from the bacterial culture. It is common practice for researchers to make use of bacterial culture filtrate when employing the agar diffusion method [59, 60]. In order to determine whether or not the isolates PI-2 and PI-3 were active against the human pathogens K. pneumoniae and E. coli, a secondary screening procedure was carried out. According to PI-2, the zone of inhibition against E. coli was at its highest possible level. One of the most common types of harmful bacteria that can be found in soil is E. coli. Recent research has shown that antibiotic-resistant E. coli bacteria can be found in domestic soil [61]. Furthermore, the researchers discovered that 42.3%, 12.6%, and 10% of the E. coli isolates were potentially pathogenic, resistant to multiple drugs, and resistant to a single antibiotic, respectively. The bacterial isolate that we have, PI-2, may therefore be able to inhibit the pathogenic E. coli bacteria.

For the purpose of molecularly characterizing the bacterial isolates, the 16S rRNA amplification and sequencing tests were carried out. When it comes to identifying bacteria in a variety of samples, 16S rRNA gene sequencing was thought to be a more accurate method [62]. It was determined that the sequences of Pseudomonas flourscence and Pseudomonas aeuroginosa were identical to those of the bacterial isolates BI-2 and BI-3, respectively. There are reports that indicate that Pseudomonas species is one of the most prevalent bacterial species that can be found in soil [63, 64]. This is due to the composition of the outer surface of gram-positive bacteria, which makes them more susceptible to antibiotics than gram-negative bacteria. Compared to K. pneumoniae and E. coli, our isolated strains appeared to be more effective, as demonstrated by the larger inhibited zones that were observed against these two bacteria.

The overuse of antibiotics as a treatment is one of the primary factors that contributes to the development of antibiotic resistance. There will always be a need for novel antibiotics that are effective against a wide variety of viruses and bacteria. Secondary metabolites in the natural world are primarily derived from natural products as their primary source. As a result, our objective was to locate and isolate bacteria from the soil that exhibit antibiosis activity. The soil at waste dump sites was used to isolate Pseudomonas flourscence and Pseudomonas aeuroginosa, which we have successfully isolated. It has been demonstrated that these two species possess antibacterial properties against K. pneumoniae and E. coli, respectively. It is our expectation that the results of our research will be of assistance in the

commercial production of more innovative antibiotic drugs, following the appropriate downstream processing. There were three different bacterial genera that were identified and investigated in relation to MDRS in a previous study [65]. There was another study that came to almost identical conclusions [66]. Brevibacillus ssp. [67] was found to have powerful antimicrobial activity against pathogenic bacteria and fungi, according to the findings of a number of investigations. Another study that was conducted on the topic of the inhibitory power of Brevibacillus on MDRS was referred to as [68]. It was determined which compounds were present in the bacterial extracts by using GC-MS in the analysis. These compounds were derived from volatile substances such as ethers, phenolics, alkaloids, and esters. The majority of these compounds were derived from these substances. Additionally, they shared structural similarities with naturally occurring products that originated from plants and bacteria. Propanoic acid, phenol, oxalic acid, and 1,3,5-trioxaneas are the primary metabolites of the particular that was used in this study. These substances have the ability to inhibit the growth of bacteria and act as antioxidants.

Multiple cases reported findings that were comparable to one another. One example is the bacteriocin-like inhibitory substance (BLIS) known as Pecilocin Bb, which is resistant to heat (100 degrees Celsius for thirty minutes), detergents, and organic solvents. This substance is derived from soil and is produced by the Brevibacillus. When the pH is between 1.0 and 9.0, it does not change. Research conducted by Kim and Bae [70] demonstrated that Paneibacillus possesses antimicrobial activity that is broad-spectrum. A number of secondary metabolites, including chondrillasterol, stigmasterol, benzedicarboxylic acid, and octadeconoic acid, were also identified and analyzed by GC-MS as part of the investigation. Similar compounds that were isolated from plants and demonstrated antimicrobial activity against E. coli and MRSA were utilized in a study that was related to the one that was being discussed. Phuong and Han [71] conducted research that was very similar to this particular study on the marine Bacillus subtilis strain HD16b, which is capable of producing benz dicarboxylic acid and octadecanoic acid.

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