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Tiny defenders: Isolating antibiotic producers from soil samples

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ABSTRACT

This study explored the soil as a rich source of microorganisms capable of creating novel antibacterial chemicals in an effort to address the growing problem of antibiotic resistance. The isolation process involved careful collection and laboratory cultivation of a variety of soil samples from garbage, gardens, and agricultural settings. The techniques used for characterization included biochemical examination of metabolic characteristics, spore staining, lactophenol cotton blue staining, and Gram-tag staining. Using the well diffusion technique, the antibiotic-producing capacities of the strains were evaluated. Notable antibacterial activity was found for four bacterial isolates (B2, B5, B6, and B9) and seven fungal strains (2F, 3F, 4F, 5F, 7F, 9F, 10F, and 11F). Precise soil sampling and complex microbe cultivation and characterization are major obstacles. The unique aspect of the work is how well antibiotic-producing bacteria were isolated and described from a variety of soil samples, underscoring the possibility of using natural habitats as sources of novel antimicrobial agents. The detected antibacterial activity emphasizes how crucial it is to carry out additional research to combat antibiotic resistance. This study provides opportunities for additional research into the unexplored potential of soil microorganisms for the development of novel antimicrobial agents.

Introduction

Antibiotics are secondary metabolites produced by microorganisms that have antibacterial capabilities. For decades, they have been employed as chemotherapeutic agents against pathogens that spread illness and cause infections. Utilizing antibiotics, maintaining good hygiene, and getting vaccinated significantly reduce mortality from infectious diseases, which were previously the main cause of death (Procopio *et al.,* 2012). Screening of "wild isolates" taken from soil and other natural habitats has led to the discovery of the great majority of new antibiotics. Antibiotics are low-molecular-weight (nonprotein) compounds made mostly by soil-dwelling bacteria as secondary metabolites. Despite the fact that it is well known that there are several antibiotics available, research on finding new antibiotics is ongoing. This has led to ongoing research into the ability of numerous organisms, such as Streptomyces, Bacillus, and Penicillium, to manufacture antibiotics (Brock & Madogan, 1991). Due to the features of chemical structures and affinities for specific target areas within bacterial cells, different antibiotics have varying modes of action. Based on its mode of operation, an antibiotic may fit into one of the following categories: inhibitors of cell wall production, which include penicillins, cephalosporins, bacitracin, or vancomycin. Colistin and polymyxin B impede the activity of cell membranes. Inhibitors of protein synthesis include aminoglycosides, macrolides, lincosamides, streptogramins, chloramphenicol, and tetracyclines. Quinolones, metronidazole, and rifampin are a few examples of drugs that prevent the production of nucleic acids (Dixon, 2006). In this

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genus, *B. brevis* (which produces gramicidin), *B. cereus* (which produces cerexin), *B. circulans* (which produces circulin), *B. laterosporus* (which produces laterosporin), *B. licheniformis* (which produces bacitracin), *B. polymyxa* (which produces polymyxin), and *B. pumum* (e.g., the bulk of these antibiotics are polypeptides, contrary to popular belief. (Marahiel *et al.,* 1993; Béahdy *et al.,* 1974; D'Ave *et al.,*1997). A relatively limited number of bacteria from the genera *Penicillium, Streptomyces, Cephalosporium, Micomonospora,* and *Bacillus* are responsible for the majority of those currently in use (Zinsser *et al.,* 1988). The antibiotic resistance of *Staphylococcus aureus* is a bacterium that is a serious concern in the management of human infections. Concern over the potential harm is shared by scientists and medical experts. Researchers are conducting new research to identify distinct and novel antibiotics against drug-resistant infections (Adwan *et al.,*2008 &Aghamirian *et al.,*2009). Due to their broad and narrow range of inhibitory effects on gram-positive and gram-negative bacteria, antimicrobial compounds are a novel class of medicines that have drawn increased amounts of attention in recent years. The use of promoter-inducible reporter assays is a promising alternate strategy for the discovery of antibacterial drugs (Shapiro *et al.,*2002 & Fischer *et al.,*2004).

Material and Methods

Collection of samples

Three soil samples were taken from various locations at Dolphin (PG) College in Chunni Kalan, Punjab. With the use of a spatula, all of the samples were taken 4 to 5 cm beneath the surface and put into sterile zip-lock bags. The samples were subsequently moved to the Dolphin (PG) College Microbiology Laboratory in Chunni Kalan, Punjab, where they were held at 4°C for further processing.

Processing of samples

One gram of soil was dissolved in 10 ml of sterile distilled water to prepare the samples for testing. To remove debris, boulders, and dirt, the test tube was vortexed for two to three minutes. Tenfold serial dilutions were performed following the transfer of the supernatant to another test tube. Then, potato dextrose agar (PDA), which is used for fungal screening, rather than nutritional agar (NA), which is used for bacterial screening, was used to apply 100 µL of the supernatant from each dilution. Pour plating was used in a recent study. The plates were incubated for 24 hours at 37°C for bacterial growth and for 6–7 days at 25°C for fungal growth. (Bizuye *et al.,*2013)

Characterization of pure isolates of bacteria and fungi

Gram staining: On a clean glass slide, a smear of the selected strain was applied. The smudge was then removed by heating the slide. The heat-fixed smear was washed with water and then inundated with mordant Gram's iodine after being saturated with crystal violet for one minute. The smear was decoloured with 95% ethyl alcohol, washed with water, and then counterstained with safranin for 45 seconds. The smear was cleaned with water, dried with tissue paper, and examined after being magnified 100 times (Williams *et al*., 1993).

Biochemical test-

Indole test: The indole test looks for indole produced from the amino acid tryptophan. A bacterium colony was introduced into a test tube of glucose that contained 1% tryptophan soup. After 48 hours of incubation at 37°C, one millilitre (1 ml) of Kovac's reagent was added to the broth. After gentle shaking, the test tube was left to stand for 20 minutes. The production of red coloration in the top layer indicated a positive test, whereas yellow coloration indicated a negative test (Fay and Barry, 1974).

MRVP test: To assess whether the bacteria underwent mixed acid fermentation, a pH indicator called methyl red (MR) was used. Acetoin production is detected by VP (Voges-Proskauer). One millilitre (1 ml) of MR VP broth was then placed into a small tube after being infected with the test organism in five millilitres (5 ml) of the broth and cultured for 48–72 hours at 37°C. A small quantity of methyl red (two to three drops) was added. When the indicator was applied, a red tint developed, denoting a successful methyl red test, whereas a yellow tint denoted an unsuccessful methyl red test. The remaining soup in the original tube received 4–5 drops of 4% KOH, followed by the addition of 5% naphthol in ethanol. After being closed with a cotton plug, the test tube was shaken and placed in an inclined position. In contrast to a

negative VP test, which had no effect, a positive VP test caused the liquid-air contact to produce a red color within an hour. (MacFaddin, 1980).

Citrate utilization test: This test determines whether the bacteria can use sodium citrate as their only source of carbon. The test organism was incubated in a test tube using Simon's citrate medium for 24 to 72 hours. After incubation, a bright blue tint emerged that indicated a favorable outcome (Abdulkadir and Waliyu, 2012).

Nitrate reduction: The test isolates were added to a prepared nitrate reduction broth and subsequently cultured for 5 days at 30°C. Nitrate reduction was detected by the production of red following the addition of 0.5 ml of reagents A and B.

Urease test: The test isolates were combined with test tubes containing urea broth medium, and the tubes were subsequently cultured at 30°C for 2–5 days. In the test tube, urease activity was visualized as a vivid pink tint.

Screening for antibacterial activity of bacterial and fungal isolates

The antibacterial activities of the bacterial and fungal isolates were determined according to the following methods:

For the well-diffusion method, nutrient agar plates were prepared and autoclaved at 121°C for 15 min. Afterwards, 0.1 ml of 24-hr culture mixture (*i.e. B. cereus, K. pneumoniae*, *E. coli* and *P. aeruginosa*) was poured on an agar plate, and a thin layer was prepared by spreading. The extra test culture was discarded, and the solution was allowed to absorb into the plates. After complete adsorption, the plates were prepared on agar plates with a copper-sterilized borer with a diameter of 1 cm. After the culture was sterilized, the supernatant of the nutrient broth from the isolated pure bacterial culture was collected after centrifugation at a high speed and extracted with the help of a micropipette. A solvent layer containing the active compound was added to the wells on agar plates (100 microlitres) by micropipette. The plates were incubated for 24 to 48 hrs at 30°C in an incubator chamber. On the other hand, the antibacterial activity of fungal isolates was assessed by isolating fungal specimens from cultured SDA with the help of sterile copper borers with a diameter of 1 cm and placing them in the wells of test culture NAM plates.

Results and Discussion

Isolation and identification of microorganisms

In this work, we investigated microbial diversity in three different soil environments: wastelands, gardens, and agricultural areas. We were able to successfully isolate a total of 9 bacterial strains and 12 fungal strains using a number of rigorous approaches, including Gram staining, spore staining, lactophenol cotton blue staining, and biochemical characterization assays. This serves as an example of the diversity and complexity of microorganisms that can be found in different soil sources.

Antibiotic synthesis potential

We carried out extensive testing against four harmful bacteria, namely, *E. coli*, *B. cereus, P. aeruginosa*, and *K. pneumoniae*, to determine the capacity of the isolated strains to synthesize antibiotics. We were able to identify zones of inhibition, a sign of antibiotic activity, using the well diffusion approach. Notably, the findings demonstrated notable antibacterial properties displayed by some strains.

Bacterial and fungal antibacterial activity

The antibacterial activity of four bacterial isolates (B2, B5, B6, and B9) and seven fungal isolates (2F, 3F, 4F, 5F, 7F, 9F, and 10F) was very noteworthy (Table 1-2). These strains exhibited strong antibacterial activity against the target pathogens. Given the specificity of this antibacterial action, these microbes may yield new antibiotics.

Noteworthy performers

With the largest zone of inhibition among the bacterial strains, 2B stood out among the bacterial isolates as the top performer. Isolate 10F had exceptional inhibitory effects on fungi. Table 3-5 and Figure 1 and 2 provide an illustrative picture of the findings by visually summing both of these outcomes.

Relevance of results

Our study's findings offer insight into the world of soil microorganisms and their possible role in the discovery of new antibiotics. The variety of strains that have been identified from different soil settings highlights how adaptable and resourceful these bacteria are and how they have developed defenses against dangerous pathogens. This directly affects how to address the persistent global challenge of antibiotic resistance**.**

Table 1: Morphological characterization of selected isolates of bacteria

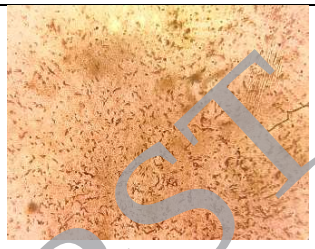
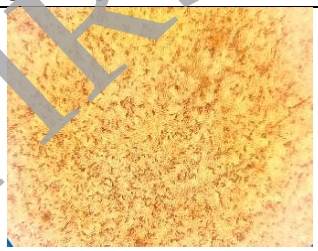
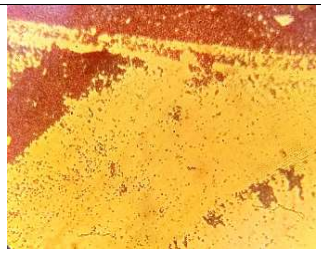
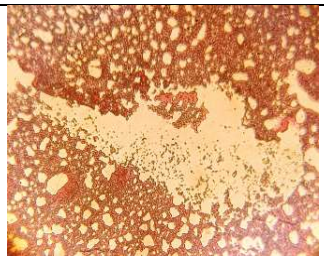
Selected Isolates of different soil samples	Colony character				
	Color	**Shape**	**Opacity**	**Endospore staining**	**Gram- staining**
2B	Transparent	Rod	Translucent	Sub terminal	 gram-negative
5B	Dim yellow	Rod	Opaque	Sub terminal	 gram-negative
6B	White color	Coccid	Translucent	Sub terminal	 gram-negative
9B	Orange color	Coccid	Opaque	Sub terminal	 gram-negative

Table 2: Morphological characterization of selected fungal isolates

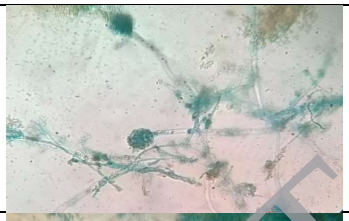
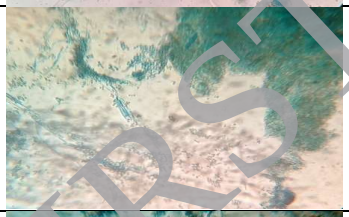
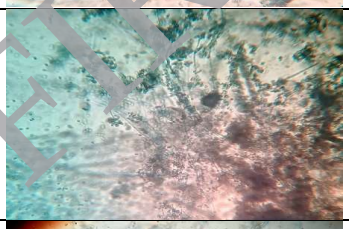
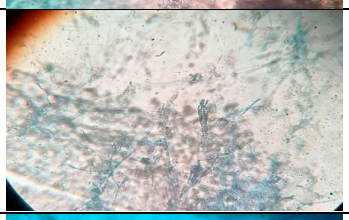
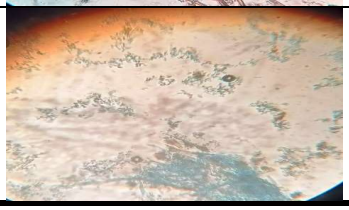
Isolates of fungi	Morphological features	Lactophenol cotton blue staining
2F	It exhibits branched and septate hyphae along with conidiophores that bears chain of conidia	
3F	The presence of spherical conidia near themulti small chain hyphae	
4F	The presence of flask shaped conidiophores bearing clusters of conidia at their tips	
5F	Branched conidiophores that produces oval shaped conidia in chains or clusters	
7F	Typically, darkly pigmented, ellipsoidal or cylindrical in shape, and formed in chains on long and branched conidiophores	
10F	Simple or branched conidiophores bearing whorls of phialides on short basal cells	
11F	The presence of spherical to ellipsoidal conidia with echinulate(spiny) surface	

Table 3: Biochemical characterization of selected isolates from different soil samples

Biochemical Tests		Result			
Color		2B	5B	6B	9B
MR test	Red	-	+	-	-
VP test	Pink–Red color ring	+	-	+	-
Urease test	Pink color	-	+	-	+
Citrate utilization test	Blue color	+	+	+	-
Indole test	Red color ring	-	+	-	-

Table 4: Measurement of the zone of inhibition against different organisms in isolated bacterial samples

Isolated Bacteria Samples	Test samples (zone of inhibition in mm)			
	E.coli	*B.cereus*	*P. aeruginosa*	*K.pneumonia*
2B	18	22	-	-
5B	-	-	19	-
6B	21	-	-	-
9B	-	-	-	25

Table 5: Measurement of the zone of inhibition against different organisms in isolated fungal samples

Isolated Fungi Samples	Test samples (zone of inhibition in mm)			
	E.coli	*B.cereus*	*P. aeruginosa*	*K.pneumonia*
2F	-	19	-	-
3F	-	6	-	-
4F	-	6	-	21
5F	7	-	-	-
7F	17	-	22	-
10F	-	-	-	29
11F	-	-	19	5

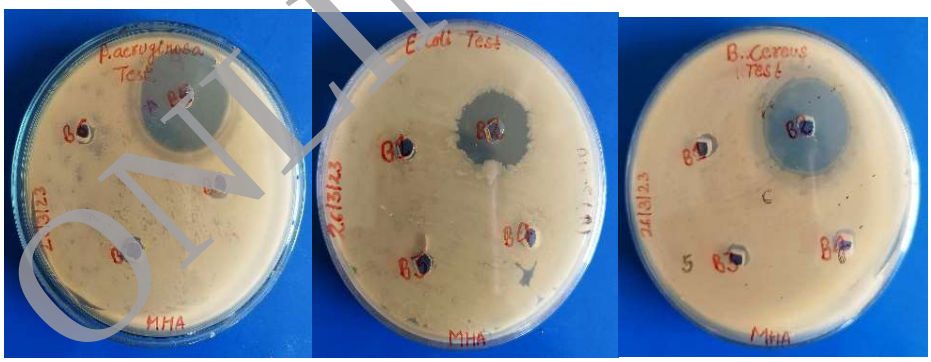


Fig 1: Zone of inhibition of isolated soil bacteria against test organisms

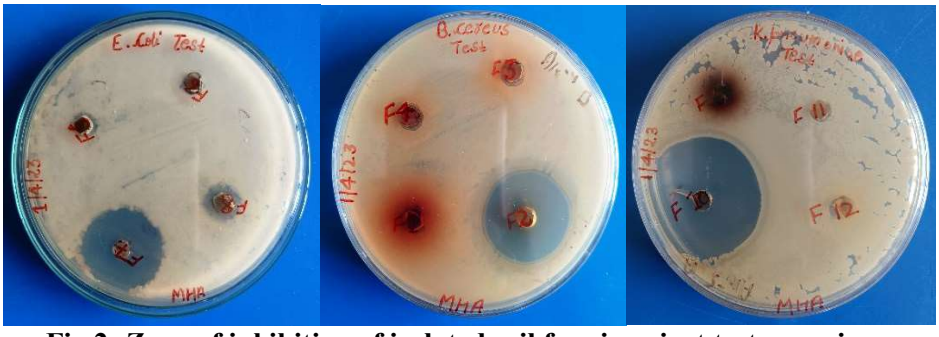


Fig 2: Zone of inhibition of isolated soil fungi against test organisms

Our findings fit into a larger body of work that highlights the importance of soil microorganisms in the development of antibiotics. For example, Genilloud's (2017) research emphasizes actinomycetes, a family of soil microorganisms, and their continued significance as active antibiotic markers. Our study adds to and broadens this body of knowledge by showing that various soil samples are home to a variety of microorganisms that produce antibiotics.

Consistency with Prior Research

Researchers discovered that soil microorganisms were susceptible to antibiotic treatments. This agreement lends weight to our own findings and points to a general pattern in the antibacterial capability of microorganisms originating from soil. A wide range of antibacterial activities was displayed by the twelve isolates. Rahman *et al*. (2011) studied 150 Actinomycetes, and 20 of those isolates (13.30%) exhibited antibacterial activity against the test pathogens. Additionally, Dehnad *et al*. (2010) examined the bioactivity of Iranian Streptomyces isolates. Twenty isolates (36.36%) were shown to have active effects on the test microorganisms by Arifuzzaman *et al*. (2010).

Implications and future directions

Our findings have important ramifications. Future medicinal uses of several strains with strong antibacterial activity look promising. In light of antibiotic resistance, the discovery of new sources of antibiotics is especially important. These results provide a starting point for additional research into the bioactive substances that have antibacterial effects as well as possible mechanisms of action.

Conclusion

In conclusion, separating antibiotic-producing bacteria and fungi from soil samples is a crucial and promising strategy in the search for novel antibiotics. Researchers can find important resources that have tremendous promise for battling drug-resistant infections and resolving ongoing problems with infectious disease management by using the enormous microbial variety found in soil ecosystems.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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