ISOLATION OF ANTIBIOTIC PRODUCING BACTERIA FROM SOIL

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Abstract
Antibiotics are a major secondary metabolite produced by a wide range of bacteria. The microbes developed various antibiotics that could be used to treat various infectious diseases. Are useful In vitro isolation, the culture and care of bacteria are quite simple, and we can easily improve their stress. The main soil pathogens of the Bacillus species are caused by important antibiotics such as bactericidal Endospores produced by the Bacillus species are very resistant. They are always found to inhibit the growth of other microbes. In the present research study, soil bacteria with antimicrobial activity have been screened and isolated. Subsequently, various pathogenic bacterial lawns were prepared to check the antimicrobial activity against various pathogens. Different zones are observed against different pathogenic bacteria. Comparison of antimicrobial activity of soil isolation with different antibiotic discs as well as various pathogenic bacteria. A clear zone of soil isolates of 5 mm, 15 mm, 21 mm, 12 mm, 30 mm, 32 mm and 40 mm against germs or pathogenic bacteria. The zones produced by antibiotic discs against pathogenic bacteria were zones of 5 mm, 10 mm, 12 mm, 15 mm, 20 mm, and 21 mm observed.

Keywords: Antibiotics, Pathogenic bacteria, Soil isolates, Zones of inhibition.

1. INTRODUCTION
The problem of antibiotic resistance in bacteria is increasing day by day. Therefore, there is an urgent need to find new antibiotics or new sources of antibiotics. There has been a lot of work over the last few decades, which has seen the development of novel antibiotics from different microorganisms (Mashoria et al., 2013). The term soil refers to the outer loose material of the earth's crust. Soil consists of five major components, among them. Minerals, H, water, organic matter, H, air and living organisms (Abdulkadir and Waliyu, 2012). The soil is a natural reservoir of microorganisms and their antimicrobial products (Dancer, 2004). The production of antibiotics is one of the most important mechanisms for controlling plant diseases with live microorganisms (Chang et al., 2010). Serious bacterial infections that have become resistant to commonly used antibiotics have become a major global health problem in the 21st century (Ceylon et al., 2008). At the beginning of the 20th century, the idea of stopping the growth of microorganisms around each other came up. Later, it was revealed that the inhibition of the growth of the former microorganisms was mediated by the secretion of toxic metabolites. This toxic metabolite was described as 'antibiotic' (Singh and Mishra, 2013). Soil bacteria belonging to the genus Stoptomyces are widely recognized as industrially important microorganisms because of their ability to produce a variety of secondary metabolites, including antibiotics. Actinomycetes have provided important
commercial bioactive compounds of high commercial value and have been regularly tested for new bioactive substances (Methor et al., 2012). Antimicrobial agents play the most important role in the treatment of bacterial infections. Antibiotics can be classified according to their behavior. Antibiotics are classified as broad-spectrum antibiotics when they have the potential to infect a wide range of Gram-positive and Gram-negative bacteria, while antibiotics that are effective only against a specific group of bacteria Spectrum is known as antibiotics. Since Fleming's discovery of *Penicillium notatum* in 1929, and the widespread treatment of this agent in the 1940s under the name of the so-called "golden age antibiotics", many countries around the world of progress. Intensive program to increase the number of antibiotics described or to find someone new (Abussaud et al., 2013). Overtime, the number of multi drug-resistant pathogenic microbes has increased, and there are only a limited number of treatments available to combat these pathogens. Therefore, in order for treatment to be effective under antibiotics, continuous discovery of new, new antibiotics is required (Lehan et al., 2014). Considering the recent emergence of new antibiotics to combat multidrug resistant pathogens, new approaches to antibiotic discovery are needed. One of the richest sources of new antibiotics may be uncultivated soil microorganisms (Gillespie et al., 2002). The isolation and purification methods of antibiotics largely depend on the properties of the antibiotic, its chemical nature, and the environment (El Nagar, 2001). Antibiotics have been described as microbial products that inhibit the growth of other microorganisms (Pivitra et al., 2015). 5,000 antibiotics have been identified from cultures of hot positive and gram-negative organisms, but it contains only 100 antibiotics commercially used to treat human, animal and plant diseases (Basavaraj et al., 2010). About 70 to 80% of all antibiotics found during this time are derived from *actinomycetes*, and from various species of Streptomyces (Norden fuji, 2014). As the most important bacteria in the soil due to the preparation of important antibiotics (Kaur et al., 2014). The amount of antibiotics produced by *Bucillia* was approaching 167. More than 66 and about 23 B-brevis (Jamil et al., 2007) were born from *B. sybils*. Preparations of large-spectrum bacilli and antibacterial antibiotics that are effective against Gram-negative bacteria are relatively rare (Yilmaz et al., 2006). *Actinomycetes* contain a broad and diverse group of Gram-positive, aerobic, mycelial bacteria that play an important ecological role in soil cycles. Many people are known for their economic importance in the preparation of biologically active substances such as antibiotics, vitamins, and enzymes (Basillo et al., 2003). The genus *Streptomyces* is responsible for the formation of more than 60 of the unknown antibiotics (Surwani et al., 2012). In their natural habitat, bacteria use the antibiotics they produce as a protective substance by attacking other bacterial species. Protection is not just about antibiotics. Therefore, antibiotics also act as signaling molecules that are used by bacteria as a means of communication between cells (Lehan et al., 2014). Clearly, antibiotics also contain a chemically heterogeneous group of microorganisms of microbial origin that, at low concentrations, are detrimental to the growth or metabolic activity of other microorganisms (Thomsho et al., 2005). In a sensitive bacterial population, there may be a small number of drug-resistant bacteria that suddenly become resistant to mutations.

2. MATERIALS AND METHODS

2.1. Collection and Preparation of Soil Sample:
Soil samples were collected from different parts of Khyber Pakhtunkhwa, Pakistan. All these samples were collected from the upper layer using some clean dry sterile polythene bags.

2.2. Isolation of Bacteria:
The media used in this study was the nutrition of Agar Medium.

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2.3. **Sample Inoculation:**
Different soil suspensions are inoculated on the growth nourishing agar and then heated to 37 °C for 24 h. After which the colonies were observed.

2.4. **Sub Culturing:**
We pick a colony from the master plate and streak it to another plate that contains nutrient media. After this place in the incubator for 24 hours at 370 C then we do gram staining and various biochemical tests.

2.5. **Gram’s Staining:**
In the colonies that grow on nutrients where the gram is in accordance with the standard method of staining.

2.6. **Biochemical Tests:**
2.6.1. **Indole test:**
Bacteria, which express the enzyme tryptophanase, can hydrolyze the amino acid tryptophan into indole, pyruvic acid and ammonia.

2.6.2. **Citrate use test:**
This was done by inoculating the test organism in a test tube containing Simon Citrate Medium dark blue after incubation indicates a positive result to be used to determine if an organism can use citrate as its sole carbon source.

2.6.3. **Triple Sugar Iron Agar Test (TSI):**
The medium contains three sugars namely: glucose, lactose and sucrose, pH indicator is phenol red and detection system for hydrogen sulphide (H2S).

2.6.4. **Catalase Test:**
This was carried out by putting a drop of hydrogen peroxide on a clean slide. Presence of bubbles indicates positive reaction while absence of bubble indicates negative reaction.

2.6.5. **Oxidase Test:**
In the oxidase test, artificial electron donors and acceptors are provided. When the electron donor is oxidized by cytochrome oxidase it turns a dark purple. This is considered a positive result.

2.6.6. **Coagulase Test:**
Coagulase is a virulence factor of *S. aureus*. The formation of clot around an infection caused by these bacteria likely protects it from phagocytosis. This test differentiates *Staphylococcus aureus* from other coagulase negative *Staphylococcus* species.

2.6.7. **Urease Test**
This test is used to identify bacteria capable of hydrolyzing urea through enzyme ureases.

2.7.1. **Test Bacteria:**
The test bacteria used in this study was obtained from the Microbiology laboratory of Abasyn University including, *Salmonella typi, S. paratyphi, Citrobacter, organella morgani, Proteus, P. areuginosa and E. coli*.

2.7.2. **Pustule Method:**
In this method we make a pathogenic lawn than we take pustule from the old culture of soil isolates and place in a circle on the surface of that pathogenic lawn and then incubate for 24hr at 37°C. After clear zone are observed which indicate antibiotic producing bacteria.

2.8. **Confirmation of Antibacterial Activity**
Bacterial isolates have the potential of producing antibiotic were tested against the test organisms to confirm the antibacterial activity.

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3. RESULTS AND DISCUSSIONS

I collected 150 soil samples from different KhyberPakhtunkhwa (Pakistan) locations. Which shows positive results against 12 to 20 different pathogenic species listed in Table 1. I first apply the gram stain, and then the results of the various biochemical tests mentioned in Table 1. After that we confirm the anti-microbial activity through the Streak method and the Pustule method. Soil bacteria show some activity against gram-positive bacteria separately, while others show activity against gram-negative bacteria as shown in Table 2 along with zones of inhibition. In current study, we have seen a zone of up to 40 mm against proteus, which is the largest zone in all research studies. In my study, the smallest zone against Proteus is 12 mm, which was the largest zone in this study (Abdulkadir and Waliyu, 2012). They also observed that E. coli shows resistance to soil isolation while we observed a clear zone up to 15 mm against the E. coli of our soil isolation and the smallest zone observed. He was 10 millimeters while showing resistance to some antibiotics. Other studies show that the highest zone of isolated areas of the soil against E. coli is 22 mm. In the study conducted by Mashoria et al., 2014 and Kalyani et al., 2012, a zone of up to 12.5 mm was observed against E. coli. Clear zone against P. aerogenos was observed in our study which was similar to the study of (Sandhya et al., 2014). According to (Mashoria et al., 2014), P. aergensa has been observed to have a maximum area of 30 mm. (Nike et al., 2013) The largest area and the smallest area of 10 mm. When studied in the soil, it also shows resistance in some different places. In the case of of S.typhi, 21 mm. Due to review study similar results, which is also found in the study (Mashoria et al.,2013) The highest zone against S.typhi in their study was 28mm while in our study was 21mm. S.typhi also show resistant toward certain soil isolates which also similar to the previous Citrobacter study. The clear zone against P.pyrogen in our study ranges from 5mm to 18mm which is a slight difference in our study and the study conducted by(Kizito and Nwankwo, 2013). The highest zone in their study was 20mm which is almost similar to our study that is 18mm. S.pyogen show resistant to certain soil isolates also similar to the previous study. No study were found about S.paratyphi it show complete resistant to all soil isolates in our study as it is the most pathogenic spp. We also used and Morgenella morganti such pathogenic strain was not used by other researcher as no results were found about it in their study. The highest zone against Citrobacter in our study are 22mm. while other zones of 5mm, 10mm, 12mm and 15mm are also observed. Also show resistant to certain soil isolates. In case of Morgenella morganii highest zone are 15mm and other zones of 5mm, 10mm and 12mm are present. Show resistant to certain soil isolates as mentioned in the above table no 2. In the last we used different antibiotics along with our soil isolates against different pathogenic species. Clear and largest zones was producing by our soil isolates while antibiotic show weak activity against different pathogenic strain. We had done further characterization based on which we identify our soilisolates. Rhizobacteria are present in the soil in an average of about 108 cells per gram (Stein, 2005). B. subtilis is also a Rhizobacterium and an endospore forming bacteria(Sonenshein et al., 2001). The present study was carried out to evaluate the production of antibiotic from newly isolated Bacillus specie from soil-by-soil sprinkling method, which was identified as B. subtilis by performing various. By using soil sprinkle method five various size colonies having zones of inhibition were picked and streaked on nutrient agar plates to get pure cultures. Out of all strains the one showing maximum inhibition zone was selected for optimization (Awais et al., 2010). Antibiotics used in my study are Azithromycin, Amikacin, Nalidixic Acid, Doxycycline and Pipraciline. All the results are present in the above table no 3. According to these B11 and B12 show the highest zone which are 40mm and 32mm and other are 21mm and 15mm are produced against E. coli, P.aerogenosa, Proteus and Citrobacter.

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While in case of B12 30mm is the highest zone and 22mm, 12mm and 5mm of zones are also observed against all the other pathogenic spp. In case of antibiotics Azitheromycin show the highest activity against Proteus and 20mm of zone was observed another 10mm of zone are also measure produced by Azitheromycin against S.aergenosa. Amikacin produced two zones of 5mm while no other clear zone are observed by Amikacin against S.aergenosa and Citrobacter. Doxicycline produced 30mm of zone against E. coli and 12mm of zones against Proteus while show resistant toward Citrobacter and P.aergenosa. Nalidixic Acid show resistant to E. coli except one clear zone of 15mm was observed against Proteus. Pipracilline produced 21mm, 12mm and 5mm of zone against S.aergenosa, Citrobacter and Proteus. In the end we concluded that highest zones were produced by our soil isolates and antibiotic disc against E. coli and Proteus as compare to other pathogenic spp.

In Table- 1 and Table-2 Show the antimicrobial Activity of Antibiotics producing bacteria isolated from soil with different pathogenic microorganisms. While in Table-3 the antimicrobial activity of soil isolates e.g B11and B12 were compared with different antibiotics like Amikacin, Azitheromycin, Doxicyclin, NalIDixic Acid.

Table 1. Morphology and biochemical characterization of bacterial isolation

| BI   | CM | Biochemical Test |
|------|----|------------------|
|      |    | Shape | Gram Reaction | Oxidase | Catalase | Coagulase | Citrate | Indol | Ureases | Sugar Fermentation/Triple sugar iron test |
|      |    |       |               |         |          |           |         |       |          | Glucose | Sucrose | Lactose |
| B1   | Rod| +     | _             | +       | _         | +         | _       | _     | _         | A       | A       | A       |
| B2   | Cocci | +     | _             | +       | _         | +         | _       | _     | _         | A       | A       | A       |
| B3   | Rods | _     | _             | _       | _         | +         | _       | +     | _         | AG      | AG      | AG      |
| B4   | Cocci | _     | _             | _       | _         | +         | _       | +     | _         | A       | A       | A       |
| B5   | Cocci | +     | _             | +       | _         | _         | _       | _     | _         | A       | A       | A       |
| B6   | Cocci | _     | _             | +       | _         | _         | _       | _     | _         | A       | A       | A       |
| B7   | Rods | _     | _             | _       | _         | +         | _       | +     | _         | A       | A       | A       |
| B8   | Rods | _     | _             | _       | _         | +         | _       | +     | A         | G+H2S   | A       | G+H2S   |
| B9   | Rod | _     | _             | _       | _         | +         | _       | _     | _         | A       | A       | A       |
| B10  | Cocci | _     | _             | _       | _         | _         | _       | _     | +         | A       | G+H2S   | A       | G+H2S   |
| B11  | Rods | _     | _             | _       | _         | +         | _       | _     | _         | A       | A       | A       |
| B12  | Rods | _     | _             | _       | _         | _         | _       | _     | _         | A       | A       | A       |

A=Acid Production, AG=Acid and gas production BI = bacterial isolates, CM = colonial morphology

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Table 2. Antimicrobial activity of bacterial isolated from soil

| Screen isolates | Sampled to be inhibited Organisms |
|----------------|----------------------------------|
|                | E. coli  | Salmonella typhi | Salmonella paratyphi | Proteus | P. aerogenosa | S. pyogen | Citrobacter | Morgenella morganii |
| B1             | 12mm     | Nil              | Nil                  | 12mm    | Nil         | 12mm    | 12mm        | 10mm                  |
| B2             | 15mm     | 5mm              | Nil                  | 21mm    | 12mm       | 9mm     | 18mm        | Nil                  |
| B3             | 5mm      | 10mm             | Nil                  | 30mm    | Nil        | 22mm    | Nil          |                       |
| B4             | Nil      | 12mm             | Nil                  | 40mm    | Nil        | 12mm    | Nil          |                       |
| B5             | Nil      | Nil              | 45mm                 | Nil     | Nil        | Nil     | 12mm        |                       |
| B6             | Nil      | Nil              | 25mm                 | Nil     | Nil        | Nil     | 5mm          |                       |
| B7             | 15mm     | Nil              | 15mm                 | Nil     | Nil        | Nil     | 5mm          |                       |
| B8             | Nil      | Nil              | 16mm                 | 15mm    | 15mm       | 15mm    | 10mm        |                       |
| B9             | Nil      | 21mm             | Nil                  | 35mm    | Nil        | 21mm    | 5mm          | 15mm                  |
| B10            | Nil      | Nil              | 12mm                 | 12mm    | Nil        | 10mm    | 12mm        |                       |
| B11            | 10mm     | 15mm             | Nil                  | 40mm    | 21mm       | 18mm    | 15mm        | 15mm                  |
| B12            | 12mm     | 10mm             | Nil                  | 30mm    | 12mm       | 5mm     | 5mm          | Nil                  |
| B13            | 15mm     | Nil              | 21mm                 | 14mm    | 12mm       | 15mm    | 12mm        |                       |

Table 3. Comparison of antimicrobial activity of soil isolate with antibiotic discs

| PS | B11 | B12 | Azithromycin | Amikacin | Doxycycline | Nalidixic Acid | Pipracilline |
|----|-----|-----|--------------|----------|-------------|----------------|--------------|
| E. coli | 32mm | 22mm | R            | 5mm      | 30mm        | R              | R            |
| P. aerogenosa | 21mm | 12mm | 10mm        | R        | R           | R              | 5mm          |
| Citrobacter | 15mm | 5mm | R            | R        | R           | R              | 12mm         |
| Proteus | 40mm | 30mm | 20mm        | 5mm      | 12mm        | 15mm           | 21mm         |

B1= Bacterial Isolates, PS=Pathogenic strains, R= Resistant

Figure 1. Activities of soil isolates against various pathogenic Bacteria

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4. CONCLUSIONS
• It has been concluded that soil is a natural source of many types of microbial populations and plays an important role in production novel antibiotics against various infectious diseases.
• In the present study, we isolate bacterial strains that exhibit more resistant ward pathogenic bacteria than antibiotic discs, as described in Table 3.

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