ANTIMICROBIAL ACTIVITY AND IDENTIFICATION OF PURIFIED BACTERIA OBTAINED FROM SOIL

Md EA Raghib Khan*
*Amity University, India.
DOI: https://www.doi.org/10.56726/IRJMETS50633

ABSTRACT

The soil surrounding the plant root where root exudate migrates, and microbiological activity is exceptionally high is called the rhizosphere. This soil bacteria associated with the rhizosphere known as plant-growth-promoting rhizobacteria (PGPR) produce great antibiotics with many applications in industries, drugs, and medicines development etc. Numerous studies have demonstrated the antimicrobial activity of PGPR against bacterial and fungal pathogens. Due to the extensive use of antibiotics, drug-resistant microbe infections are increasing globally. There is an urgent need for an alternative therapeutic approach. Bacteria from the rhizosphere and their secondary metabolites can be a promising source of novel antimicrobial compounds. The goal of this project is to isolate bacteria, especially focus on actinobacteria from the rhizosphere of Ocimum tenuiforum and Tinospora cordifolia and to evaluate their potential for growth inhibition against pathogens by dual-culture plate assay. A total of twenty-seven bacteria showing clear zones were isolated and gram staining was performed. All of them were gram negative and rod-shaped except R13 which was gram positive.

I. INTRODUCTION

For human life, both now and in the distant past, trees and plants were crucial. Early man relied on them for both his material requirements, such as supplies for food, housing, clothing, medicine, ornaments, and tools, and his spiritual needs, such as magic or ritualistic acts. When using such local and traditional treatments that have been tried and found to be non-toxic, safe, affordable, and socially and culturally acceptable, there is every reason to do so. Medicinal herbs are typically readily available locally and are normally cheaper (Bannerman et al., 1983).

Many researchers have looked at the genus Tinospora, and it has been claimed that it contains several phytochemicals with notable medicinal effect. About 70 genera and 450 species of plants belong to the Menispermaceae plant family, which inhabit tropical lowland areas. These rarely grow as shrubs and are typically climbing or twining. Flowers, little chimes, and seeds that are typically hooked or uniform. Leaves that are alternating or lobed. Terpenes and alkaloids are abundant in this family. Both the traditional medical system and Ayurveda highlight the plant's healing properties (Sharma et al., 2010).

Tinospora, often known as "Giloy," is an herbaceous, glabrous, and deciduous plant that serves as a multifunctional medicinal plant. It is a plant that spreads readily throughout India and is referred to as "Amrita" in ancient ayurvedic texts. The entire plant has significant medical value. It is used as a bitter tonic to treat illnesses of the digestive tract, irritation, diabetes, and skin infections. It has anti-inflammatory, antiviral, antiallergic, and anticancer properties (Mittal et al., 2014).

There are several advantages and benefits to using medicinal plants, the most important of which are their low cost and global availability. Another evident benefit is their safety in comparison to other therapeutic drugs, as well as the absence of serious adverse effects. Aromatic herbs are a rich source of physiologically active substances that may be used in both agriculture and medicine (Yamani et al., 2016). Due to its supposed therapeutic powers, Ocimum tenuiforum, commonly known as Ocimum sanctum, Tulsi, or Holy Basil from the Lamiaceae family, has been dubbed the "Queen of Plants" and the "Mother Medicine of Nature." It is one of the most valuable and holistic herbs used in traditional medicine in India for many years, and practically every component of the plant has been shown to have medicinal effects. Tulsi aqueous extracts have traditionally been used to treat many forms of poisoning, stomach pains, common colds, headaches, malaria, inflammation, and heart disease (Pattanayak et al., 2010). Tulsi oil has been reported to have a variety of beneficial characteristics, including expectorants, analgesics, anti-emetics, and antipyretics; stress reducers and...
inflammation relievers; and anti-asthmatic, hypoglycaemic, hepatoprotective, hypotensive, hypolipidemic, and immunomodulatory agents.

Natural products, due to their structure, continue to be the most promising source of secondary metabolites and exhibit antibacterial action against pathogenic bacteria. Natural compounds derived from microbes continue to be the most promising source of future antibiotics. Among the several unknown habitats, dirt is regarded one of the most appropriate conditions for microbial development, and microbes isolated from soil are a primary source of antibiotic discovery (Yunus et al., 2016). Despite the fact that the soil is inherently abundant in bacteria capable of producing antibiotics, the frequency with which synthesis happens at ecologically important levels has remained unclear. Even though the standard technique of random screening has been used for the past 50 years to create novel antibiotics that are beneficial to humans. Keeping this in mind, the goal of this work was to extract and characterise antimicrobial activity generating microorganisms from the rhizosphere.

II. REVIEW OF LITERATURE

Priti Duhan et al. claim that the culturable bacterial endophytes that are found in T. cordifolia have the ability to produce a variety of bioactive compounds and have strong antibacterial properties.

Duraipandiyan V, Ignacimuthu S state that the plant species tested have antifungal characteristics. Which illustrates how these plants are used in traditional medicine to treat a variety of illnesses whose symptoms may include fungus infections and emphasizes the value of using an ethnobotanical approach to choose plants while looking for new bioactive chemicals.

According to Pratibha Vyas and Amandeep Kaur, five bacterial isolates were tested for antagonistic effects on with the phytopathogens Fusarium moniliforme, Fusarium verticillioides, Curvularia lunata, and Alternaria alternata as well as the abiotic stress conditions of salinity, temperature, pH, and calcium salts showing all plant growth-promoting activities. A bacterial isolate T1B1 identified as Bacillus sp. was examined and chosen as a promising candidate to be employed as a microbial inoculant for plants growing in a stressful environment. All of the tested stressors were tolerated by the isolate. Additionally, it demonstrated a variety of actions that helped plants flourish.

According to Gagandeep Kaur et al., preliminary phytochemical analysis of T. cordifolia revealed the presence of sugars, glycosides, flavonoids, phenols, tannins, and amino acids in the crude medication. For the management and treatment of infectious diseases, T. cordifolia stem extracts showed significant dose-dependent antibacterial activity in vitro against both gramme positive and gramme negative bacteria.

According to Fatima et al., secondary screening results and the activity of the crude extracts from solid and submerged state fermentation showed that Gram negative organism (E. coli) was effectively suppressed in comparison to Gram-positive E. faecalis. It is possible to isolate a bioactive substance that can be utilized as a medicine using the actinomycete isolate FA9.

Anupama Sapkota reports that 41 isolates of actinomycetes were found in 11 soil samples; of them, 70.7% were Streptomyces spp., 19.5% were Nocardia spp., and 9.5% were Micromonospora spp. From the initial screening, 43.34% of actinomycete isolates were determined to be active antimicrobial producers, of which 46.34% were effective against Gram-positive test organisms and 12.19% against Gram-negative test organisms. The most effective broad-spectrum antibacterial activity was shown in isolate C7 (Micromonospora spp.) during secondary screening.

Gislín and colleagues, (2018) reported that the microbes present in the rhizosphere shows high level of antagonistic activity. Since soil is a source of several bacteria that produce antibiotics, such as Actinomycetes. Many scientists have chosen it for the isolation of novel antibiotics. Soil heterogeneity also leads to a large range of biological niches and a significant diversity of soil microorganisms (Lacerda et al., 2018).

Ranjita and collaborators. (2018) demonstrated the presence of potentially bioactive actinobacteria, particularly Streptomyces, in the microbiologically unexplored soil habitats of Pobitora Wildlife Sanctuary ecosystems. Streptomyces is capable of producing a variety of bioactive extracellular secondary metabolites that are specifically antibacterial or antifungal toward pathogens that are both antibiotic-non-resistant and antibiotic-resistant. More than 55% of the isolated actinobacterial strains showed antimicrobial activity against...
at least one of the pathogens put to the test. Only a few isolates could be considered to be effective producers of antimicrobial metabolites based on their potential bioactivity towards both test microbial strains and antibiotic-resistant test strains, even though 24 isolates demonstrated production of antimicrobial metabolites with efficient antimicrobial activity. Furthermore, strain PWS52 may be an unique strain with strong antibacterial activity that may be further studied to discover additional antibiotic metabolites for use against different other diseases (Das et al., 2018).

Bacteria are microscopic organisms that generate a wide variety of secondary metabolites, many of which have already been successfully isolated. Some of these secondary metabolites have even contributed to the development of significant antibiotics with diverse medical, veterinary, and pharmaceutical applications. High antibacterial activity has been demonstrated by actinobacteria from soil and marine sediments against Gram-positive and -negative bacteria and fungus. They discovered a high level of antibacterial activity against Gram-positive bacteria, which may be explained by the fact that these bacteria's cell walls are 90% peptidoglycan, as opposed to Gram negative bacteria’s wall, which is considerably more complicated and hence more antibiotic-resistant (Lacerda et al., 2018).

Stephen, (2014) reported that soil bacteria isolated from bare sandy soil were classified into six colony types. The transparent zones were visible in several bacteria isolates.

### III. MATERIALS AND METHODS

#### Study area

The sample was collected from Birsa Agriculture University (BAU), Kanke, district of Ranchi, in the state of Jharkhand. Geographically located at 23°19’N latitude and 85°31’E longitude, and at 625 meters of altitude.

#### Soil sample collection

At BAU, Kanke, Ranchi, Jharkhand, India, Giloy plant location, soil samples from the rhizosphere were taken. Prior to collection, the debris from the soil samples was removed. About 100 g of the rhizosphere soil was taken from the location, dug up to a depth of 15-20 cm, and placed in a sterile, airtight bag before being transferred to the lab and kept at 4 ºC.

#### Isolation and maintenance of soil bacteria

With the help of the standard serial dilution plate method, soil bacteria were identified. A dirt sample weighing 1 g was measured out and dissolved in 9 ml of sterile distilled water. After then, the samples were serially diluted. Out of the 5 dilutions, 100 µl from each dilution (10⁻³, 10⁻⁴, and 10⁻⁵) of each sample were used to prepare nutrient agar spread plates. To detect bacterial colonies that were hostile, the plates were cultured for up to 3 days. In order to obtain pure isolated colonies, the colonies that displayed antagonism were picked out and streaked separately on nutrient agar plates. For future research, pure culture was kept at 4 ºC.

#### Morphological characterization of isolated bacteria

Primary characterization has been done by visualizing the mother plate culture showing antagonistic behavior. Then Gram's Staining was used to characterize the morphology of the isolated hostile bacteria. Each isolate's 24-hour-old nutrient agar plate was employed for Gram's staining.

#### Antimicrobial Activity

Perpendicular streaking has been performed for those bacteria which have shown well defined structured in Gram's staining. All the samples have been tested against the three-test organism i.e., *Escherichia coli*, *Pseudomonas aurcius*, and *Lacto bacillus*. 

---

**Fig 1:** Serial dilution of soil sample  
**Fig 2:** Microphotograph of rod-shaped gram-negative bacteria
Table 1: Strains showing their morphology along with the gram’s staining property.

<table>
<thead>
<tr>
<th>Strains</th>
<th>Morphology of the microorganism</th>
<th>Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC1</td>
<td>Rod shaped</td>
<td>Gram’s negative (-)</td>
</tr>
<tr>
<td>TC2</td>
<td>Rod shaped</td>
<td>Gram’s negative (-)</td>
</tr>
<tr>
<td>TC3</td>
<td>Rod shaped</td>
<td>Gram’s negative (-)</td>
</tr>
<tr>
<td>TC4</td>
<td>Rod shaped</td>
<td>Gram’s negative (-)</td>
</tr>
<tr>
<td>TC5</td>
<td>Rod shaped</td>
<td>Gram’s negative (-)</td>
</tr>
<tr>
<td>TC6</td>
<td>Rod shaped</td>
<td>Gram’s negative (-)</td>
</tr>
<tr>
<td>TC11</td>
<td>Rod shaped</td>
<td>Gram’s negative (-)</td>
</tr>
<tr>
<td>TC12</td>
<td>Rod shaped</td>
<td>Gram’s negative (-)</td>
</tr>
<tr>
<td>TC13</td>
<td>Rod shaped</td>
<td>Gram’s positive (+)</td>
</tr>
<tr>
<td>TC14</td>
<td>Rod shaped</td>
<td>Gram’s negative (-)</td>
</tr>
<tr>
<td>TC15</td>
<td>Rod shaped</td>
<td>Gram’s negative (-)</td>
</tr>
<tr>
<td>TC16</td>
<td>Rod shaped</td>
<td>Gram’s negative (-)</td>
</tr>
</tbody>
</table>

IV. RESULTS AND DISCUSSION

Isolation and Maintenance of microorganism

The samples were taken in the air seal tight bag. Standard serial dilution has been done and each (10⁻³, 10⁻⁴, and 10⁻⁵) of the soil sample spread in the nutrient agar plate and stored at room temperature for 48 hours.

Discussion

A total of 27 antagonistic property showing sample has taken and streaked, out of 27 taken sample, 20 sample developed colonies. Out of 20 samples screened, only 1 sample shows gram’s positive, and the rest had shown gram’s negative property. All the microorganisms have morphologically rod in shaped. Out of 20 samples, 10 samples have been tested against the Escherichia coli, Pseudomonas aureius, and Lacto bacillus.

Fig 3: Showing growth of isolated colonies

Fig 4: Showing Sample tested against test E. coli, P. aureius, and L. bacillus.
V. CONCLUSION

In the present study, we purified ten bacteria from the rhizosphere of Ocimum tenuiflorum and Tinospora cordifolia. Preliminary data indicated the inhibitory potential of isolated bacteria. The findings of this study unlock the door for a more thorough assessment of these isolates against various pathogenic bacteria and fungi. The identification and classification of the metabolic substances these isolates create will be helpful. These substances may provide new metabolites for the development of innovative antimicrobial medications.

VI. REFERENCE


