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MICROBIAL DIVERSITY OF FOREST ECOSYSTEM OF ACHANAKMAR TIGER RESERVE IN CHHATTISGARH STATE OF INDIA

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ABSTRACT

A variety of micro-organisms and processes in the rhizosphere profoundly influence the plant growth and the soil quality. Rhizosphere organisms are affected by and contribute to the success ion dynamics of forest communities. Protecting the functional biodiversity of rhizosphere organisms through proper management practice is essential to maintain ecosystem healthy.

Soil sample were collected from rhizosphere region of selected plants *Soymida febrifuga, Acacia catechu, Shorea robusta, Adhatoda vasica, Putranjiva roxburghi* from Achankmar forest of Bilaspur district. A total of 19 bacteria and 7 fungi were isolated. The bacterial isolates identified based on their morphological, physiological and biochemical characterization, *Enterobacter spp. Citrobacter spp, Erwinia spp, Klesiella spp, Shigella spp, Proteus spp,* was isolated. Rhizosphere fungi were isolated on the basis of morphological and physiological characterization. *Penicillium, Alternaria, Mucor, Rhizopus, Aspergillus,* was isolated. Future research on rhizosphere biology will rely on the development of molecular and biotechnological approaches to increase our knowledge in rhizosphere biology.

Keywords: Ecosystem, Rhizosphere, Microbial, Tiger reserve, forest

INTRODUCTION

Biodiversity is essential for the sustainable functioning of agricultural forest and natural ecosystem upon which human survival and health depends. The biodiversity of plants, animals and microbes functioning in many ways to enhance the health and quality of life is enjoyed by human society.

The forest of the state falls under two major forest types i.e. tropical moist deciduous forest and tropical dry deciduous forest. Sal (*Shorea robusta*), saja (*Terminalia tomentosa*), teak (*Tectona grandis*) are the three special in the state. Other are mahua (*Madduca Indica*) bamboo (*Dendrocalamus strictus*), Amla (*Embilica officinalis*) etc. Chhattisgarh forest is spread over an area of 59772 sq. km. In Bilaspur district of state of Chhattisgarh has total forest area of 29750 sq. Km where Achanakmar wildlife sanctuary was constituted in the year 1975 it comprises of 557.55 sq. Km. Forest vegetation mainly comprises of Sal, Saja, Bija & Bamboo. The principal minor forest product Tendu Patta, Sal seed, Harra, Arjuna etc. and many other medicinal plants nearly ten thousand industrial unit depend on forest and the tribal people of this region all dependent on the medicinal plants for their treatments.

Bilaspur lies at 21016' N latitude and 81036'E longitude with an attitude of 298.56 M above the mean sea level.

Achankmar forest of Bilaspur comes under tropical Dry to moist deciduous forest. Out of the mean annual rainfall of 1200-1300mm, about 85% is received. Temperature of this region crosses 60°C, air temperature touch to 48°C and humidity drops up to 3 to 4 percent during summer season **(Anonymous, 1996 and Gupta *et al*, 2000)**.

The general forest degradation process adversely forest the resource base of medicinal and herbal plants so it is very important to study the microbial diversity of this region because it affects the ecosystem functioning and growth and plants.

Almost no work has been conducted on search for microbial diversity of forest plants, in Bilaspur (C.G.) this investigation would help to understand the effect of climate conditions in this microbial population and its diversity in this region.

In present study the attempt have been made to investigate the bacterial and fungal

diversity in rhizosphereic microflora of selected forest plants.

MATERIALS AND METHODS

Sample Collection

Samples were collected from the Achanakmar forest of Bilaspur distt. in Chhattiagarh state. The soil sample was taken from rhizosphere region of *soymida febrifuga, acacia catechu shorea robusta, Adhatoda vasica, Putranjiva Rozburghi*. The Samples were kept in polythene bags to prevent moisture loss and they were properly tagged, sealed and stored in refrigerator for isolation of Bacteria, fungi,

Serial dilution

The samples were serially diluted and plated on nutrient Agar plate for bacteria and SOA (Dextrose Agar) plate for fungus.

Preservation & Maintenance of Bacteria & Fungi

The Bacterial cultures were maintained and sub cultured weekly on nutrient agar slant at 280C fungi were sub cultured weekly on SDA slant at 28ºC. The culture were preserved by freezing at temperature`s well below 0ºC.

Morphological Characterization

The morphological features for bacterial isolates were studied by microscopic examination configuration, elevation, Margin, pigmentation, size, color, mobility, surface color, backside color, hyphal structure, types of spores.

Cotton blue Staining

This stain used for fungi for their identification under the microscope.

Effect of temperature on growth

The bacterial and fungal culture were streaked on nutrient agar and Sabourd dextrose agar respectively and incubated aerobically at 4°C, 28°C, 37°C, 45°C for bacteria and at 37°C, 52°C, 60°C then observed the growth on 4th day.

Effect of pH of medium on growth

The bacterial isolates were streaked on nutrient agar plates of varying pH at (pH - 3.0.5.0, 8.0, 10.0) and incubated at 20°C temperature. Fungal isolates streaked on SDA medium of varying pH (pH 3.0, 5.0, 8.0) and incubate at 28°C temperature. The growth was observed up to 5 days

Effect of different NaCl concentration on growth

Test cultures were streaked inoculated on nutrient agar plates of varying NaCl concentration of (0.1%, 1%, 3% and 5%) and incubated aerobically at 280°C temperature and observed for growth up to 5 days.

Effect of different sugar on growth

This study were done by streaking culture on a Minimal agar media containing varying sugar Sucrose, Lactose, Maltose, Glucose, Mannitol, Inositol and Lactose at pH 7.0+0.2 pH and 25°C. Growth was observed for 5 days.

Biochemical Characterization

Biochemical test such Indole production, Methyle red (MR) and Voges proskauer (VP) test, Citrate Utilization, Starch Casein Hydrolysis, Oxidation fermentation test, Urea Hydrolysis, by standard method given by **Holt *et al.*, 1994, Benson, 200, Cappuccino and Sherman, 2005**.

RESULTS AND DISCUSSION

For the investigation of microbial diversity from forest ecosystem five soil Samples are collected from the rhizosphere of economic and medicinal plant of Achanakmar forest of Bilaspur Distt. Bacteria and fungi are isolated and grown in laboratory condition and characterize. These microbes were characterize on the basis of morphological physiological and biochemical characteristics 19 bacteria and 7 fungi ware isolated form *Putranjiva roxbarghi, Acacia catechu. Adhatoda vasica, Soymida febrifunga. Shorea robusta* platns.

Table 1: Morphological Characteristics of Bacteria

Sample	Colour	Size mm	Configuration	Surface	Elevation Of Growth	Margin	Growth	GM Staining	Motility
S1	Off White	1-2	Filamentous	Shiny	Flat	Smooth	Less	-ve Streptobacilus	-
S2	Off White	1-3	Circular	Shiny	Reised	Entire	Well	-ve Bacili	+
S3	White	4	Filamentous	Rough	Flat	Rhizoidal	Well	-ve, Bacillus	-
S4	Cream	1-2	Irregular	Slimy	Raised	Irregular	Well	-ve, Bacillus	-
S5	Off White	1-3	Irregular	Slimy	Raised	Irregular	Well	-ve, Bacillus	-
S6	Off White	1-3	Irregular	Slimy	Drop like	Smooth	Well	-ve, Bacillus	+
S7	White	6	Irregular	Moist	Flat	Wavy	Well	-ve, Bacillus	+
S8	Off White	6-7	Irregular	Shiny	Convex	Smooth	Well	-ve, Cocous	-
S9	Off White	2-3	Irregular	Rough	Flat	Scalloped	Well	-Ve Coccous	-
S10	Off White	7-6	Irregular	Moist	Flat	Erose	Well	-Ve, Bacillus	+
S11	White	1	Filiform	Slimy	Flat	Wavy	Well	-Ve, Bacillus	+
S12	Off White	8	Irregular	Moist	Flat	Erose	Well	-Ve, Bacillus	-

S13	**White**	**6-5**	**Fillamentous**	**Shiny**	**Raised**	**Irregular**	**Well**	**-Ve, Bacillus**	**+**
S14	**Off White**	**5-6**	**Irregular**	**Moist**	**Raised**	**Undulate**	**Well**	**-Ve, Bacillus**	**+**
S15	**Off White**	**1-3**	**Circular**	**Moist**	**Flat**	**Smooth**	**Well**	**-Ve, Bacillus**	**-**
S16	**White**	**4.5**	**Irregular**	**Moist**	**Flat**	**Irregular**	**Well**	**Ve, Streptobacillus**	**-**
S17	**Yellow**	**1-2**	**Irregular**	**Rough**	**Raised**	**Irregular**	**Well**	**-Ve, Streptobacillus**	**-**
S18	**White**	**4.5**	**Irregular**	**Moist**	**Flat**	**Irregular**	**Well**	**-Ve, Bacillus**	**+**
S19	**Off White**	**3-4**	**Irregular**	**Moist**	**Flat**	**Smooth**	**Well**	**-Ve, Bacillus**	**+**

Table 2: Morphological characterization of fungi

Sample	Surface Colour	Hyphal structure	Type of spores.
1	**Bluish grey**	**Brush like**	**Phialospores.**
2	**Bluish green**	**-**	**Phialospores**
3	**White to dark grey**	**nonseptate root like rhizoids**	**Sporangiospores**
4	**White**	**non septate**	**Sporangiospores**
5	**Grey**	**Brush like**	**Phialospores**
6	**Yellow**	**Septate**	**Sickle shaped macro conidia**
7	**White**	**Non-Septate root like rhizoids**	**Sporangiospores**

Table 3: Effect temperature on Growth of Bacteria

Sample	4ºC	28ºC	37ºC	45ºC
S1	-	+	+	-
S2	-	+	+	-
S3	-	+	+	-
S4	-	+	+	-
S5	-	+	+	-
S6	-	+	+	-
S7	-	+	+	-
S8	-	+	-	-
S9	-	+	+	+
S10	-	+	+	+
S11	-	+	+	-
S12	-	+	+	-
S13	-	+	+	+
S14	-	+	+	+
S15	-	+	+	+
S16	-	+	+	+
S17	-	+	+	+
S18	-	+	+	+
S19	-	+	+	+

Table 4: Effect of different temperature on growth of fungi

Sample	37ºC	52ºC	60ºC
1	+	-	-
2	-	-	-
3	+	-	-

4	-	-	-
5	+	-	-
6	+	-	-
7	+	-	-

Table 5: Effect of pH on the growth of bacteria

Sample	pH-3	pH-5	pH-8	pH-10
S1	**(-)**	+	+	+
S2	-	+	+	+
S3	-	+	+	+
S4	+	+	+	+
S5	-	+	+	+
S6	-	+	+	+
S7	-	+	+	+
S8	-	+	-	+
S9	-	+	+	+
S10	-	+	+	+
S11	-	+	+	+
S12	-	+	+	-
S13	-	+	+	+
S14	-	+	+	+
S15	-	+	+	+
S16	-	+	+	+
S17	-	+	+	+
S18	-	+	+	+
S19	-	+	+	+

Table 6: Effect of pH on growth of fungi

Sample	pH 3.0	pH 5.0	pH 8.0
1	+	-	+
2	+	-	+
3	+	+	+
4	+	+	+
5	+	-	+
6	+	+	+
7	+	+	+

Table 7: Sugar utilization by Bacterial isolates

Sample	Mannitol	Glucose	Maltose	Surcrose	Lactose	Innositol
1	-	+	+	+	-	-
2	+	+	+	+	+	+
3	-	+	-	-	+	+
4	+	+	+	+	+	+
5	+	+	+	+	-	-
6	+	+	+	+	+	+
7	-	+	-	-	-	+
8	+	+	-	-	+	-
9	+	+	+	+	+	-
10	+	+	+	-	-	-
11	+	+	+	+	+	+

12	-	+	-	-	-	-
13	-	+	-	-	-	+
14	-	+	-	-	-	-
15	-	+	-	-	+	-
16	-	+	-	-	+	-
17	-	+	-	+	-	-
18	-	-	+	+	-	-
19	-	+	-	-	-	-

Table 8: Effect of different sugar on the growth of fungi

Sample	Glucose	Sucrose	Inositol	Lactose	Maltose	Mannitol
1	+	+	+	-	+	+
2	+	+	+	-	+	-
3	+	+	-	-	+	-
4	+	+	+	+	-	+
5	+	-	+	-	+	+
6	+	+	+	+	+	+
7	+	+	-	-	+	-

1 (+) = Growth is present; 2 (-) = Dou not shows growth.

Table 9: Effect Of Defferent NaCl Concentration on Growth of Bacteria

Sample	0%	1%	3%	5%
S1	+	+	-	-
S2	+	+	+	-
S3	+	+	+	-
S4	+	+	+	-
S5	+	+	+	+
S6	+	+	-	-
S7	+	+	+	+
S8	+	+	+	+
S9	+	+	+	+
S10	+	+	+	+
S11	+	+	+	+
S12	+	+	-	-
S13	+	+	+	+
S14	+	+	+	+
S15	+	+	+	+
S16	+	+	+	+
S17	+	+	+	+
S18	+	+	+	+
S19	+	+	+	+

Table 10. Biochemical tests on bacterial isolates

Sample	Indol	MR	VP	Simmon Citrate	Lipase Activity	Amylase Activity	Urease Activity	Caseinase Activity	O-F
S1	+	+	-	-	-	-	-	-	-
S2	-	-	+	+	+	-	-	+	+
S3	+	+	-	-	-	-	-	+	+/-

S4	+	-	-	+	+	-	-	-	+
S5	-	+	+	+	-	+	-	+	-
S6	+	-	-	+	+	-	-	-	+
S7	+	+	-	-	+	+	+	+	+
S8	+	-	-	-	+	-	-	-	-
S9	+	+	+	-	+	+	-	+	+
S10	-	+	-	+	-	-	-	+	+
S11	-	+	-	+	+	+	-	+	+
S12	-	-	-	+	-	-	-	+	-
S13	+	-	-	+	-	+	-	+	+
S14	+	+	-	+	-	+	-	+	+
S15	+	+	+	+	+	+	-	+	-
S16	+	-	+	+	+	+	-	+	+
S17	-	-	+/-	-	+	-	-	+	-
S18	-	+	+	+/-	-	+	-	+	-
S19	-	+	+/-	+	-	+	-	+	+

Identification of isolated bacteria: on the basis of identification we concluded that S2, S6 are *Enterobacter spp* ,S10, S11, S14, are *salmonella spp*, S4, S5, S15, S16, are *klebsiella spp*, S1,S12, S17, are *shigella spp*, S11 is *citrobacter spp*, S8, S9, is *nesseria spp*, S13 is *Erwinia spp*, S7 is *proteus spp*, S18 is *listeria spp.*

Identification of isolated fungi: on the basis of morphological characteristics, it was found that 1 and 5 are *penicillium spp*, 3and 7 are *rhizopus spp*, 2 is *aspergillus*, 4 is *mucor*, and 6 is *alternaria.*

RESULTS AND DISCUSSION

The present study is based on the microbial diversity investigation in rhizosphere of selected plants of achanakmar forest of Bilaspur of Chhattisgarh state. This forest having the plants of medicinal importance and also provide large amount of timber. One third of forest comprises of sal, teak and other important medicinal plants. Productivity of these plants depends upon rainfall and on rhizosphere micro flora. There are some barren areas found, so there is need to investigate microbial diversity of Chhattisgarh forest plants. This investigation helps to understand the effects of climate condition on bacterial population and its diversity in this region in order to investigate the microbial diversity, five soil sample were collected from rhizosphere of selected plants of *Soymida febrifuga, Acacia catechu, Shorea robusta, Adhatoda vasica, Putranjiva*

Roxburghi. Further the samples were used for isolation and characterization. Data on **Table 1**, shows morphological characterization. Most isolated bacteria are gram negative Bacillus and Coccus, **Table 2** represents most of fungi are brush like and having phialospore. **Table 3, 5, 7, 9** shows the physiological characterization of bacteria. According to **Table 3**, maximum growth ofbacteria were obsereved at 28°C and 37°C temperature. Bacterial growth was observed at pH 8.0 and pH 10.0 **(Table 3)**. Effect of different concentration of salt and sugar were showed in table 4 and 5. **Table 8, 9, 10** shows the physiological characterization of fungi. Most rhizosphere fungi grown on temperature 28°C and 37°C **(Table 4)**.maximum fungi grow at pH3.0 and pH 8.0 **(Table 6)**. Table 8 represents the effect of different sugar on the growth of fungi.

Biochemical characterization: **(Table 10)** showed the biochemical characterization of bacteria. The rhizosphere bacterial isolates, S2, S3, S4, S6, S7, S8, S9, S11, S15, S16, S17, produce lipase. Isolates S3, S5, S7, S9, S10, S11, S12, S13, S14, S15, S16, S17, S18, S19, showes caseinase activity. Isolates S5, S7, S9, S11, S13, S14, S15, S16, S19, produce amylase and isolates S2, S4, S6, S7, S9, S10, S11, S13, S14, S16, S19, showed positive oxidative fermentation.

The rhizosphere bacteria isolates identified based on their morphological, physiological and biochemical characterization, *enterobacter spp, citrobacter spp, erwinia spp, klesiella spp, shigella spp, proteus spp, salmonella spp* were isolated. Rhizosphere fungi were identified on the basis of mor phological and physiological characterization. *Penicillium, alternaria, mucor, rhizopus, aspergillus,* were isolated from the rhizosphere of selected plants of chhattisgarh forest.

CONCLUSION

A maximum 95% bacterial population exists in the rhizosphere are gram negative bacillus and coccus. Most of the bacteria and fungi utilize, glucose as a energy source and some bacteria and fungi are grown on mannitol, maltose, sucrose, innositol and lactose. Maximum bacteria and fungi are grown at the temperature 28^{o}C and 37^{o}C. Bacteria may grow on the pH range (8-9) and at 0% to 3% NaCl containing medium. On the basis of biochemical characterization it may be concluded that some bacteria having the

capacity to produce amylase, caseinase and lipase. Rhizosphere fungi were identified on the basis of morphological and physiological characterization. *Penicillium, alternaria, Mucor, rhizopus, aspergillus*, were isolated from the rhizosphere of selected plants of chhattisgarh forest. The rhizosphere bacteria isolates identified based on their morphological, physiological and biochemical characterization, *enterobacter spp, citrobacter spp, Erwinia spp, klesiella spp. Shigella spp, proteus spp,* were isolated. Rhizosphere fungi were identified on the basis of morphological and physiological characterization.

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