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## Isolation and morphological characterization of indigenous rhizobacteria of Bastar Plateau

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### Abstract

Plant Growth Promoting Rhizobacteria contribute in overall growth of the plant and their use in agriculture is a sustainable and eco-friendly form of reducing harmful chemicals from the environment and decrease their irrational use in agriculture. Thus, the aim of the present investigation was to study the indigenous rhizobacteria of Bastar Plateau. Total of 41 rhizobacteria were isolated from the soil samples collected from five blocks i.e. Tokapal, Laundiguda, Bastar, Bakawand and Jagdalpur of Bastar district. From the observation recorded regarding 41 different isolated rhizobacteria it was found that highest number of rhizobacteria was isolated from sample CH2 [isolate named as CH2(A), CH2(B), CH2(C) and TM4 [isolate named as TM4(A), TM4(B), TM4(C)] consisting of 3 isolates respectively. Through overall observation of all the morphological features of different rhizobacterial isolates it was found that most of the rhizobacterial isolates were circular (27 isolates), had entire margin (33), white color (23), raised elevation (16), smooth texture (35), butyrous consistency (26) and opaque (34) in their colony morphology.

**Keywords:** Plant growth promoting rhizobacteria, rhizosphere, morphology, Bastar

### Introduction

The rhizosphere zone has been defined as the volume of soil directly influenced by the presence of living plant roots or soil compartment influenced by the root (Hiltner, 1904) [9]. "Plant growth promoting rhizobacteria are those bacteria which are living in to the rhizospheric regions and support plant growth by different kinds of biological processes". PGPR is mostly found in bulk soil, rhizosphere (on the root surface), and endo-rhizosphere (inside the root surface). PGPM may be a bacterial, fungal and archaeal population.

PGPR can be categorized into two main types which are e PGPR and iPGPR. ePGPR is the rhizobacteria which promotes the growth of extracellular plants and colonize in the root surface area / rizoplant or in the intercellular space of the root cortex. While iPGPR is the Rhizobacteria which stimulates the growth of extracellular plants that live specifically on the inside of the root surface / rizoplant root cell nodular structure.

PGPR either directly or indirectly boost plant growth and yield. N<sub>2</sub> fixation, the solubilization of mineral phosphate and zinc; the creation of phytohormones such auxins, cytokinins, and gibberellins; the sequestering of iron through the formation of siderophores; the production of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase, which hydrolyzes ACC, the immediate precursor of ethylene in plants are the direct growth- promoting processes. The length of the seedlings roots is stimulated when the ethylene content is reduced. The production of antibiotics, the removal of iron from the rhizosphere, the production of the enzymes that lyses fungal cell walls, such as  $\beta$ -(1,3)-glucanase and chitinase and the synthesis of antifungal compounds like cyanide are all indirect ways through which PGPR support plant growth. (Aeron *et al.*, 2011; Gamalero and Glick, 2011; Jayaprakashvel and Mathivanan, 2011; Saraf *et al.*, 2014) [1, 7, 10, 13].

Various symbiotic PGPR like *Bacillus*, *Pseudomonas*, *Azotobacter*, *Acinetobacter*, *Rhizobium*, *Arthrobacter*, *Alcaligenes*, *Stenotrophomonas*, *Serratia*, *Proteus*, *Acetobacter*, *Azospirillum*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, and *Xanthomonas* colonise in the plant rhizosphere and interact with the root exudates. Allelochemicals such glucanases, chitinases, siderophores, biocidal, detoxifying enzymes, antibiotics, and biocidal volatiles can be produced when PGPR colonises the rhizosphere. The development of numerous plant growth regulators and plant defence against various illnesses and insect pests are both made possible by these allelochemicals.

Additionally, by securing the hazardous substances and metals, these bacteria do bioremediation (Hayat R *et al.* 2010, Ahemad M *et al.* 2011) <sup>[8, 3]</sup>. Exudates and bacteria can communicate with each other and work together to produce osmolytes, disease-fighting compounds, biomass, and other compounds.

The rhizospheric microbial population can be used as ideal biocontrol for inhibiting the disease causing pathogens in plants. The beneficial microbes release pathogen-suppressing metabolites into the root zone that helps in the inhibition of phytopathogens. Hence, these act as frontline protection against the pathogenic attacks in the rhizospheric zone of the plants (Bulgarelli D *et al.* 2013) <sup>[4]</sup>.

To prevent the huge losses caused by phyto pathogens, it is urgent need of researching beneficial microbes of soil to protect the crop from various diseases without the use of harmful chemicals keeping in view the environment safety.

### Material and Methods

The present investigation was conducted in the Plant Pathology laboratory of S.G College of Agriculture and Research Station, Jagdalpur, Bastar (C.G.). Different bacterial isolates was isolated from rhizospheric soil sample collected from different regions of Bastar district.

### Survey and sampling of rhizospheric soil

An extensive survey was conducted in Bastar plateau region of Chhattisgarh in five blocks. From each block, 3 villages were selected and from each village five locations i.e rice crop field was chosen to collect rhizospheric soil sample. In each location, individual rice crop was uprooted carefully and soil adherent to the roots were taken from field. Total of two sample from each location was taken and after uprooting the crop was slightly shaken to remove the excess water and extra soil and after that the soil closely bound to rice roots were collected in a polythene bags labeled with information such as date of collection, village name, latitude, longitude, host information, stage of crop etc. and immediately taken to laboratory and maintained at ambient temperature for further investigation.

### Isolation of rhizobacteria

Isolation of rhizobacteria from collected soil sample was done by serial dilution method in nutrient agar media (Beef extract: 3g/l, peptone: 5g/l, NaCl: 5g/l, Agar: 20g/l, pH: 7.0) using spread plate technique. Firstly 10 gm of each soil sample was taken in 250 ml erlenmeyer flask containing 90 ml distilled water and shaken for desired time. 1 ml of this soil suspension was taken in a sterilized test tube containing 9 ml of distilled water which was then serially diluted using sterile pipette tip by subsequent transferring of 1ml of suspension until it reached 10<sup>-6</sup> dilution. Dilution of 10<sup>-3</sup> was generally taken for the isolation procedure.

From 10<sup>-3</sup> dilution fold 0.1 ml of aliquot was taken using pipette and transferred on pre prepared nutrient agar media petri plates, this aliquot was then spread using sterilized glass spreader following spread plate technique. The prepared plates were then incubated for 48-72 hrs at 28±2 °C to observe the colonies of bacteria. After 72 hrs and proper development of bacterial colonies, individual colony was counted and population of bacteria in original sample was calculated by using formula:

$$\text{CFU/ml} = \frac{\text{No. of colonies} \times \text{dilution factor}}{\text{Volume of culture plated}}$$

### Purification and preservation of isolated rhizobacteria

Morphologically different colonies of each individual sample were subjected to purification procedure. Purification of isolated rhizobacteria was done by streak plate method using nutrient agar media. Individual colonies were picked up by sterilized inoculation loop and streaked on prepared petriplates in a zig-zag manner on four sides of plates. This plates were then incubated for 48-72 hrs at 28±2 °C to obtain purified individual bacterial strains. The isolated individual purified rhizobacterial strains are preserved in nutrient media agar slants prepared by streaking of bacterial culture in sterilized slants and then incubated in BOD at 28±2 °C for 48-72 hrs and after the development of pure culture it is stored in refrigerator at 4 °C for further use.

### Morphological characterization of isolated rhizobacterial strains

The isolated rhizobacterial strains were characterized for morphological features such as colony form, colour, margin, elevation, texture, consistency and opacity.

### Result and Discussion

#### Survey and sampling of rhizospheric soil

An extensive survey was conducted in Bastar plateau zone of Chhattisgarh to collect and examine the soil of various locations regarding the presence of rhizobacteria. Five blocks namely Bastar, Bakawand, Jagdalpur, Lahundiguda and Tokapal were considered and from that total of fifteen village was selected namely Badanji, Balenga, Bhatagaon, Bhond, Chondimetawara, Dhuragaon, Durkiguda, Kumharawand, Kinjoli, Lamker, Mongrapal, Muli, Potanar, Takraguda and Tekameta respectively.

#### Population Dynamics

To assess the rhizobacterial population, rhizospheric soil was collected along with passport data as represented in Table 1. Experiment results revealed that only 30 soil samples produced different bacterial colonies in nutrient agar media plates and the rest of 45 soil samples did not recorded any bacterial colonies. Table 2 represent the highest colony forming unit (CFU g<sup>-1</sup> of soil) (at the dilution of 10<sup>-3</sup>) was of sample BA5 (19.7 X 10<sup>5</sup>) followed by PO5 (18.2 X 10<sup>5</sup>), MO1 (17.2X 10<sup>5</sup>), TM4 (16.0X 10<sup>5</sup>) and BO2 (15.0X10<sup>5</sup>). From the observation recorded regarding different rhizobacterial isolates it was found that highest number of rhizobacteria was observed from sample PO1, CH2 and TM 3 and LM1 consisting of 4 isolates respectively followed by sample PO5, CH5, TM4, B3, B5, LM3, BO5, KU1 and BH3 with 3 isolates and rest of sample PO3, LM3, BA5, BO2, BO3, MO1, KU2 and BH2 gave only 2 bacteria each as depicted in Table 2. Among the 66 observed rhizobacterial isolates only 41 isolates were recovered and maintained for further study and rest of 26 isolates could not be recovered. Studied isolates are PO1, PO 2, PO 3, PO 4, CH 5, CH 6, CH 7, CH 8, CH9, CH 10, TM 11, TM 12, TM 13, TM 14, TM 15, B 16, B 17, B 18, B 19, LM 20, LM 21, LM 22, LM 23, BA 24, BA 25, BA 26, BO 27, BO 28, BO 29, BO 30, KI 31, KI 32, MO 33, MO 34, MO 35, MU 36, KU 37, KU 38, BH 39, BH 40 and BH 41.

Through observation of 41 different isolated rhizobacteria it was found that highest number of rhizobacteria was isolated from sample CH2 [CH 5, CH 6, CH 7] and TM 4 [TM 13, TM 14, TM 15] consisting of 3 isolates respectively followed by sample PO5, CH5, TM3, B3, LM1, LM3, BA5, BO5, KI2, MO1 and BH3 with 2 bacteria respectively and rest of sample i.e. PO2, PO4, CH3, B2, B4, BA1, BO2, BO3, MO2, MU1, KU1, KU2 and BH2 gave only one bacteria each as represented in Table 2.

Similar findings were also reported by Venu *et al.* (2022) [16] that the highest number of bacterial populations were found in the rhizospheres of paddy and pointed gourd respectively, from the 46 bacteria isolated from lateritic regions of West Bengal. They also concluded that when compared to the rhizospheres of other crops, the root exudates of cucurbits harbour the highest number of bacterial populations.

### Morphological characterization of indigenous rhizobacteria

Colonial and cultural characterization of 41 isolates was done microscopically and the characters studied were form, margin, texture, chromogenesis, elevation, consistency and opacity.

Studies on morphology of different rhizobacterial isolates as indicated in Table 3 it was found that in case of shape 27

isolates were circular and 14 were irregular, 33 isolates had entire margin, 5 isolates had filiform whereas 3 isolates had undulate margin, white colour was found in most of isolates including 23 isolates, 10 light yellow to yellow isolates, 4 was orange to light orange and 3 was light pink in colour, 35 isolates had smooth texture and 6 had dry, 16 isolates showed raised elevation, 9 showed umbonate, 10 was of flat elevation and 6 of them had convex elevation, in case of consistency 26 isolates had butyrous consistency, 12 showed sticks to surface and 3 was of mucoid consistency and 34 isolates was opaque and 7 were found to be translucent.

Similarly Agrawal *et al.* (2013) [2] isolated 28 bacterial isolates from several tomato- growing fields close to Dehradun and characterized them morphologically and biochemically. In contrast to the samples from the field and the home, the results showed that the cfu obtained in these cases were seen to be maximum in the rhizosphere layer of garden soil (3.41 X 10<sup>6</sup> CFU gram<sup>-1</sup> soil). Bacteria also displayed wide morphological variation, with the most variation in colony shape being irregular and circular. Most of the bacteria that were recovered from different soil samples had a mucoid texture, were fully edged, and appeared creamy white.

**Table 1:** Geographical locations of different soil sample

S. No	Block	Village No. of Sample	Stage of GPS crop	
			Latitude	Longitude
1	Tokapal	Potanar 5	Tillering 19°7'31.14"	81°53'5.08"
		Chotimetawara 5	Tillering 19°8'2.71"	81°52'41.23"
2	Laundiguda	Tekameta5	Tillering 19°6'16.82"	81°56'6.86"
		Takraguda 5	Tillering 19°9'0.8"	81°48'16.08"
		Badhanji 5	Tillering 19°12'6.26"	81°55'41.07"
3	Bastar	Dhurgaon 5	Tillering 19°9'58.63"	81°45'41.16"
		Lamkaer	Tillering 19°12'41.88"	81°52'23.36"
		5 Balenga 5	Reproductive 19°14'39.34"	81°54'18.79"
4	Bakaband	Bhond 5 Kinjoli 5	Tillering 19°12'52.75"	81°54'45.93"
			Tillering 19°14'27.31"	82°1'11.86"
		Mongrapal 5	Tillering 19°19'18.54"	81°50'26.54"
5	Jagdarpur	Muli 5	Tillering 19°15'58.78"	82°2'13.25"
		Kumharawand 5	Maturity 19°5'27.78"	81°2'13.25"
		Durkiguda 5	Reproductive 19°6'15.76"	81°57'50.13"
		Bhatagaon 5	Maturity 19°6'15.86"	81°57'49.53"

**Table 2:** Total no of rhizobacteria isolates observed in soil sample

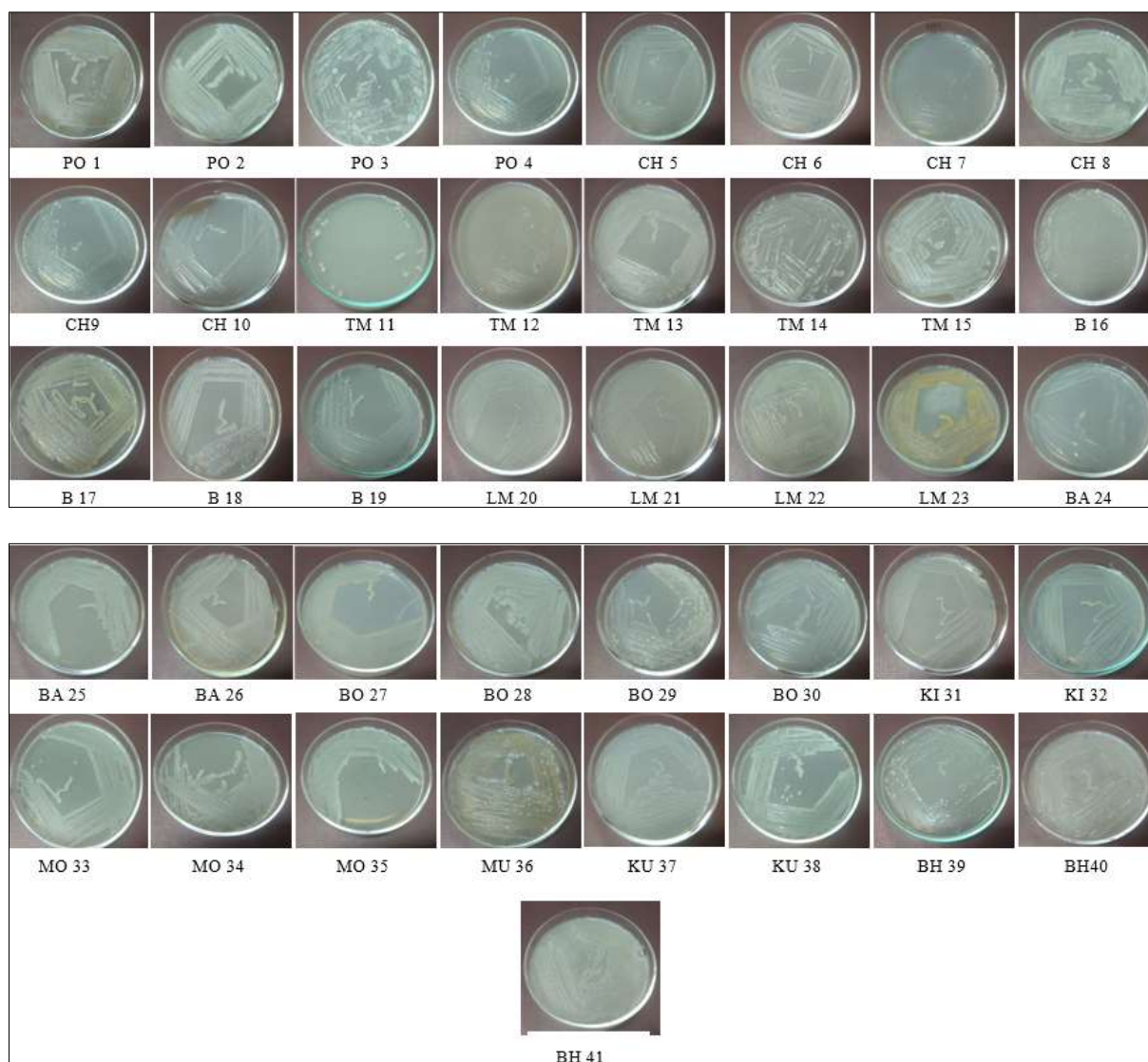
S. No	Sample	CFUg <sup>-1</sup> of soil	Total no. of rhizobacteria	No. of isolates	Name of isolates
1	PO1	10.9 X 10 <sup>5</sup>	4	0	-
2	PO2	10.5 X 10 <sup>5</sup>	1	1	PO 1
3	PO3	44 X 10 <sup>5</sup>	2	0	-
4	PO4	11.2 X 10 <sup>5</sup>	1	1	PO 2
5	PO5	18.2 X 10 <sup>5</sup>	3	2	PO 3, PO 4
6	CH1	30 X 10 <sup>5</sup>	1	0	-
7	CH2	10.8 X 10 <sup>5</sup>	4	3	CH 5, CH 6, CH 7
8	CH3	70 X 10 <sup>5</sup>	1	1	CH 8
9	CH5	58 X 10 <sup>5</sup>	3	2	CH 9, CH 10
10	TM3	46 X 10 <sup>5</sup>	4	2	TM 11, TM 12
11	TM 4	16.0 X 10 <sup>5</sup>	3	3	TM 13, TM 14, TM 15
12	B2	52 X 10 <sup>5</sup>	1	1	B 16
13	B3	10.6 X 10 <sup>5</sup>	3	2	B 17, B 18
14	B4	13.0 X 10 <sup>5</sup>	3	1	B 19
15	LM 1	59 X 10 <sup>5</sup>	4	2	LM 20, LM 21
16	LM3	10.5 X 10 <sup>5</sup>	2	2	LM 22, LM 23
17	BA 1	30 X 10 <sup>5</sup>	1	1	BA 24
18	BA5	19.7 X 10 <sup>5</sup>	2	2	BA 25, BA 26

19	BO2	15.0 X 10 <sup>5</sup>	2	1	BO 27
20	BO3	70 X 10 <sup>5</sup>	2	1	B 28
21	BO5	10.0 X 10 <sup>5</sup>	3	1	BO 29, BO 30
22	KI2	72 X 10 <sup>5</sup>	1	2	KI 31, KI 32
23	MO 1	17.2 X 10 <sup>5</sup>	2	2	MO 33, MO 34
24	MO2	70 X 10 <sup>5</sup>	1	1	MO 35
25	MU1	10.0 X 10 <sup>5</sup>	1	1	MU 36
26	KU1	60 X 10 <sup>5</sup>	1	1	KU 37
27	KU2	45 X 10 <sup>5</sup>	2	1	KU 38
28	DU1	50 X 10 <sup>5</sup>	3	0	
29	BH2	68 X 10 <sup>5</sup>	2	1	BH 39
30	BH3	60 X 10 <sup>5</sup>	3	2	BH 40, BH 41
Total		30	66	41	

**Table 3:** Morphological characterization of isolated rhizobacterial isolates

S. No	Isolate	Form	Margin	Texture	Colony colour	Elevation	Consistency	Optical feature
1	PO 1	Circular	Filliform	Smooth	Light Orange	Raised	Butyrous	Opaque
2	PO 2	Irregular	Entire	Smooth	White	Flat	Butyrous	Opaque
3	PO 3	Irregular	Filliform	Smooth	White	Flat	Butyrous	Opaque
4	PO 4	Circular	Entire	Sticky	White	Umbonate	Butyrous	Translucent
5	CH 5	Circular	Entire	Smooth	Creamy White	Flat	Sticks To The Surface	Opaque
6	CH 6	Circular	Entire	Smooth	Yellow	Umbonate	Butyrous	Opaque
7	CH 7	Circular	Entire	Smooth	Pink	Umbonate	Butyrous	Opaque
8	CH 8	Circular	Entire	Smooth	Creamy White	Convex	Mucoid	Opaque
9	CH 9	Circular	undulate	Smooth	White	Umbonate	Butyrous	Opaque
10	CH 10	Circular	Entire	Smooth	White	Umbonate	Butyrous	Opaque
11	TM 11	Circular	Entire	Smooth	Light Pink	Raised	Sticks To The Surface	Opaque
12	TM 12	Circular	Entire	Smooth	White	Raised	Sticks To The Surface	Translucent
13	TM 13	Circular	Entire	Smooth	Orange	Raised	Butyrous	Opaque
14	TM 14	Irregular	undulate	Smooth	White	Raised	Sticks To The Surface	Opaque
15	TM 15	Circular	Entire	Smooth	White	Convex	Mucoid	Opaque
16	B 16	Circular	undulate	Dry	White	Flat	Butyrous	Opaque
17	B 17	Circular	Entire	Smooth	Light Orange	Umbonate	Sticks To The Surface	Translucent
18	B 18	Circular	Entire	Smooth	Creamy White	Raised	Butyrous	Opaque
19	B 19	Circular	Entire	Smooth	White	Convex	Butyrous	Translucent
20	LM 20	Circular	Entire	Dry	Yellow	Flat	Butyrous	Opaque
21	LM 21	Circular	Entire	Smooth	Creamy White	Raised	Sticks To The Surface	Opaque
22	LM 22	Circular	Entire	Smooth	Orange	Raised	Butyrous	Opaque
23	LM 23	Circular	Entire	Smooth	Yellow	Umbonate	Butyrous	Opaque
24	BA 24	Circular	Entire	Smooth	Pure White	Convex	Sticks To The Surface	Opaque
25	BA 25	Irregular	Entire	Smooth	Yellow	Convex	Sticks To The Surface	Opaque
26	BA 26	Irregular	Entire	Smooth	White	Raised	Sticks To The Surface	Opaque
27	BO 27	Irregular	Entire	Smooth	Light Yellow	Raised	Mucoid	Translucent
28	BO 28	Irregular	Entire	Smooth	White	Raised	Butyrous	Opaque
29	BO 29	Irregular	Entire	Smooth	Yellow	Raised	Butyrous	Opaque
30	BO 30	Circular	Entire	Smooth	Light Pink	Convex	Butyrous	Opaque
31	KI 31	Circular	Entire	Smooth	Yellow	Umbonate	Butyrous	Opaque
32	KI 32	Irregular	Entire	Smooth	White	Raised	Sticks To The Surface	Opaque
33	MO 33	Irregular	Entire	Dry	White	Raised	Butyrous	Opaque
34	MO 34	Circular	Entire	Smooth	Light Yellow	Flat	Butyrous	Opaque
35	MO 35	Irregular	Entire	Dry	Light Yellow	Flat	Butyrous	Translucent
36	MU 36	Circular	Entire	Smooth	Light Yellow	Raised	Butyrous	Opaque
37	KU 37	Irregular	Filliform	Smooth	White	Raised	Butyrous	Opaque
38	KU 38	Circular	Entire	Smooth	White	Umbonate	Sticks To The Surface	Opaque
39	BH 39	Irregular	Filliform	Dry	White	Flat	Sticks To The Surface	Opaque
40	BH 40	Irregular	Filliform	Dry	Creamy White	Flat	Butyrous	Translucent
41	BH 41	Circular	Entire	Smooth	Light Yellow	Flat	Butyrous	Opaque





**Plate 1:** Isolated rhizobacterial isolates

### Conclusion

Rhizobacteria was found to contribute not only in various plant growth promotion activities but also on disease suppression by production of different secondary metabolites. The aim of the present investigation was to study the indigenous rhizobacterial population present in soil samples and study their morphology. Through the investigation it was found that maximum colony forming unit (CFUg<sup>-1</sup>) was of sample BA5 (19.7 X 10<sup>5</sup>) followed by PO5(18.2 X 10<sup>5</sup>), MO1(17.2 X 10<sup>5</sup>), TM4 (16.0 X 10<sup>5</sup>) and BO2 (15.0 X 10<sup>5</sup>) i.e. of Balenga, Potanar,

Mongrapal, Tekameta and Bond village of Bastar district of Chhattisgarh and through morphological study of rhizobacterial isolates it was concluded that maximum number of isolates were circular in shape and had entire margin, smooth texture, white in colour, raised elevation, butyrous consistency and are opaque in their morphology.

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