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Characterization of *Rhizobium* spp. from Paithan Taluka, dist. Aurangabad

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Abstract
Rhizobium gets an outstanding importance in ecosystem and agricultural production for its capacity of symbiosis and conversion of nitrogen to ammonia. *Rhizobium* biofertilizer is best alternative for chemical fertilizer which is environmentally and economically beneficial to farmers. A critical survey has been carried out for the collection of *Rhizobium* samples from Paithan taluka of Aurangabad district (Maharashtra). Form various locations of Paithan taluka twenty-two samples were collected. These samples were isolated from root nodules of various legume crops like *Arachis hypogea* (Groundnut), *Cicer arietinum* (Chickpea), *Glycine max* (Soybean), *Pisum sativum* (Pea) and *Trigonella foenum* (Fenugreek). Out of 21 collected samples, 05 *Rhizobium* spp. were isolated which are *R. pusense*, *R. ciceri*, *R. japonicum*, *R. leguminosarum* and *R. meliloti*. Out of this *R. pusense* was isolated from groundnut, *R. ciceri* was isolated from chickpea, *R. japonicum* was isolated from soybean, *R. leguminosarum* was isolated from pea, *R. meliloti* was isolated from fenugreek. From the investigation, it can be concluded that *Rhizobium* is mostly host specific for leguminous crops from the study area.

Keywords: *Rhizobium*, paithan, root nodules, biofertlilizer

Introduction
Microorganisms are abundantly present in soil such as bacteria, nematodes, protozoans, fungi etc. Plants and microbes are in association with each other for the growth and development. Plants depend upon microbes for some macro as well as micronutrients. Nitrogen (N_2) is an essential macronutrient for growth and development of host plant (Poonia, 2011) [15]. It is a main constituent of DNA, RNA, Proteins, enzymes and hormones Agah *et al.*, (2013). Soil contains nitrogen in free form but the plants cannot take it directly. Plant needs soil bacteria to convert nitrogen in the available form (nitrates and nitrites).
The soil bacteria involved in N_2 fixation are of two types as symbiotic and non-symbiotic bacteria. Symbiotic bacteria forms root nodules and shows symbiotic association with leguminous host plant. Non-symbiotic N_2 fixing bacteria are freely living in soil. Plants use chemoattractants, antimetabolites to midify microbial population in soil (Cai *et al.*, 2009) [2]. The most common N_2 fixing bacteria are *Rhizobium*, *Azotobactor*, *Azospirillium*, *Rhodopseumonas*, *Clostredium*, *Bacillus*, *Theocapsa*, *Rhodomicrobium*, *Chloromicrobium*, *Enterobactor*, etc.
Rhizobium is one of the important symbiotic N_2 fixing bacteria. *Rhizobium* gets an outstanding importance in ecosystem and agricultural production for its capacity of symbiosis and conversion of nitrogen to ammonia (Vincent, 1981) [21]. The beneficial association between *Rhizobium* and host plant is important for sustainable food system reported by Ochieno *et al.*, (2020) [9]. In sever conditions *Rhizobium* legume association showed superior nitrogen fixing systems with respect to nitrogen fixing potential (Zahran, 1999) [25]. Sharma *et al.*, (2013) [19] studied *Rhizobium* for its drought and salt tolerance capacity in desert environment resulting in growth and nodulation of leguminous hosts. Zhang *et al.*, (1991) [24] reported association of *Rhizobium* with leguminous herbs, shrubs and trees. Mehboob *et al.*, (2009) [7] studied *Rhizobium* for its production of chemical substances as phytohormones, riboflavin, enzymes, chemicals for enhancing plant stress resistance capacity, inhibiting growth of pathogens on associated plant. *Rhizobium* can be served as best alternative for chemical fertilizer which is environmentally and economically cheap for farmers. During the present investigation, various samples of *Rhizobium* spp. were collected and reported from Paithan taluka of Aurangabad district (Maharashtra).

Materials and methods
1. Collection of root nodules
The root nodules of legume plants were collected from different location of Paithan taluka. The locations were Pategaon, Dhorkin, Jambhali, Pachod, Kandewadi, Jayakwadi, Dhangaon, Khadgaon, Dhupkheda. The root nodules were collected from some of the commonly cultivated crops like *Arachis hypogea* (Groundnut), *Cicer arietinum* (Chickpea), *Glycine max* (Soybean), *Pisum sativum* (Pea) and *Trigonella foenum* (Fenugreek). Selected root samples along with root nodule were brought to the laboratory for further investigation.

2. Separation of root nodules
These roots were thoroughly washed under tap water and air dried at room temperature (25±2ºC). The healthy and pink colored juvenile root nodules were selected and carefully removed with sharp and sterile scalpel. These root nodules were surface sterilized with 70% ethanol and then in 0.1 % mercuric chloride (Swami *et al.*, 2020) [20].

3. Isolation of bacteria
A healthy and pink colored juvenile root nodules was cut by using sterile blade in a water drop. Then bacterial samples were incubated on Nutrient Agar medium using streak plate method (Khaitov *et al.*, 2016; Pawar, 2014) [5, 12]. The plates were incubated in incubator for 48 hours at 25±2ºC temperature. For further confirmation of the bacterial samples, these samples were again streaked on special media as Yeast Extract Mannitol Agar (YEMA) w/ Congo Red (HiMedia-M721) or Yeast Mannitol Broth (HiMedia-M716). The pure cultures of the samples were transferred on the slants of the same media.

4. Morphological Characterization

The cell morphology and colony morphology of the isolated bacterial samples were studied (Phalke *et al.*, 2016). Colony morphology was studied by using parameters like color, shape, average colony size, appearance and margins; while cell morphology was studied by using parameters like Gram's staining, average cell length and cell shape.

5. Biochemical Characterization

All the bacterial samples were undergone various biochemical tests for preliminary identification and characterization (Kumari *et al.,* 2018) [6]. Various biochemical tests like gelatin hydrolysis, starch hydrolysis, catalase, urease (urea hydrolysis), citrate, indole production and nitrate reduction were performed in the laboratory.

Results and discussion

Twenty-two *Rhizobium* samples were collected from legume crops from various locations of Paithan taluka of Aurangabad district (Maharashtra) (Table 01). The legume crops were *Arachis hypogea* (Groundnut), *Cicer arietinum* (Chickpea), *Glycine max* (Soybean), *Pisum sativum* (Pea) and *Trigonella foenum* (Fenugreek). The bacterial samples were isolated from root nodules on special media as Yeast Extract Mannitol Agar (YEMA) w/ Congo Red (HiMedia-M721) or Yeast Mannitol Broth (HiMedia- M716). The study of morphological characterization was done using colony morphology and cell morphology which is presented in Table 02. Colony color in almost all the *Rhizobium* samples was creamy white to white; while average colony size of all the samples was 2.9 mm. All the colonies had entire margin with translucent to glistening translucent appearance. Biochemical characterization of all *Rhizobium* samples from Paithan taluka was recorded in Table 03. Various biochemical tests were performed for the preliminary identification and characterization of each species. Gelatin test was found negative for all the samples which indicate that the isolated bacterial samples are of *Rhizobium* spp. Catalase and Nitrate reduction test were found positive for all bacterial samples.

To study the distribution of *Rhizobium* spp. in Paithan taluka, on the basis of colony morphology, cell morphology and biochemical tests, 05 groups were created and presented in Table 4. Group- I comprised of 05 bacterial samples (Sample Code No. PA01, PA05, PA10, PA15 and PA21). These were found positive in starch hydrolysis, catalase, indole production and nitrate reduction tests; while negative in remaining 03 tests *viz.* gelatin hydrolysis, urease and citrate tests. All these morphological and biochemical test results resembled with the characters of *Rhizobium pusense.* Group- II comprised of 05 samples (Sample Code No. PA03, PA07, PA12, PA18 and PA20). The group members were found positive in 03 biochemical tests (catalase, urease and nitrate reduction); while negative in remaining 04 tests *viz.* starch hydrolysis, citrate and indole production. All these morphological and biochemical tests resembled with the characters of *Rhizobium japonicum.*

Group III comprised 04 bacterial samples (Sample Code No. PA02, PA06, PA13 and PA19) which showed positive results for starch hydrolysis, catalase, citrate and nitrate reduction tests. Remaining tests were negative for these bacterial samples. All these morphological and biochemical tests resembled with the characters of *Rhizobium meliloti.* Sample Code No. PA04, PA08, PA11 and PA16 were grouped in Group IV according to similar morphological and biochemical tests resembling with *Rhizobium ciceri.* These were found positive in 04 biochemical tests (catalase, urease, indole production and nitrate reduction); while negative in remaining 03 tests *viz.* gelatin hydrolysis, starch hydrolysis and citrate. Remaining 03 samples (Sample Code No. PA09, PA14 and PA17) were grouped in Group V. The group members were found positive only in catalase and nitrate reduction; while negative in remaining 05 tests *viz.* gelatin hydrolysis, starch hydrolysis, urease, citrate and indole production. All these morphological and biochemical tests resembled with the characters of *Rhizobium leguminosarum.*

Similar results were recorded for bacterial smaples isolated from groundnut by Panday *et al.,* (2011) and introduced a new species of *Rhizobium i.e. R. pusense* which was having round, mucoid, smooth and cream–white colonies when grown on YM medium. Similar results were recorded about the *Rhizobium* species isolated from Groundnut crop from various places of Paithan taluka. Chaudhary *et al.,* (2021) [3] performed various biochemical tests like indole production, catalase and nitrate reductase test, all these tests were responded positive for rhizobial strain which was later confirmed as *R. pusense.* The bacterial samples isolated from Soybean crop in the taluka showed identical result with *Rhizobium* species from Soybean crop isolated by Gachande and Khansole (2011) [4]. They studied it for morphological and biochemical characterization of fast and slow growing species identified as *R. japonicum.* Patil *et al.,* (2014) [11] studied isolation, biochemical tests and salt tolerance activity of *Rhizobium* stains from soybean, groundnut and trigonella.

Paudyl *et al.,* (2021) [13] studied morphological characters of *Rhizobium* spp. isolated from chickpea crop and explained its colony characters as circular colonies with translucent appearance of Gram-negative rod-shaped bacteria. Similar result was recorded such as Nour *et al.,* (1994) [8] reported *Rhizobium ciceri* sp. Nov. nodulate chickpeas (*Cicer arietinum* L.). Roychowdhury *et al.,* (2015) [17] isolated *Rhizobium* from chickpea (*Cicer arietinum*) for its identification, characterization and production of biofertilizer. Wadhwa *et al.,* (2017) [22] reported isolation and characterization of *Rhizobium* from chickpea. Similar results were recorded about biochemical characterization of rhizobial isolates collected from chickpea plant in Paithan taluka. Waheed *et al.,* (2014) [23] also explained similar results about morphological characters of *Rhizobium* spp. isolated from pea plant. Shahazad *et al.,* (2019) performed various biochemical tests to identify *Rhizobium* sp. isolated from pea crop like indole production, catalase, urease, citrate utilization, oxidase etc. and identified it as *R. leguminosarum*. Prajapati *et al.,* (2018) [16] studied biochemical characterization of *R. meliloti* isolated from fenugreek plant from various localities of Jaipur by using biochemical tests like catalase oxidation, starch hydrolysis, potassium hydroxide test.

Table 1: Sample Collection From Different Locations of Paithan Taluka

Sample Code No.	Location	Legume Crop from which root nodules were collected	Number of root nodules recorded per plant	Physical Properties of Soil		
				Soil Type	pH	WHC
PA01	Pategaon (North Side)	*Arachis hypogea* (Groundnut)	69	Clay Soil	7.4	41.25%
PA02	Pategaon (Near Bus Stop)	*Trigonella foenum* (Fenugreek)	28	Black Soil	7.1	40.06%
PA03	Dhorkin (North Side)	*Glycine max* (Soybean)	51	Grey Soil	7.3	35.31%
PA04	Dhorkin (East Side)	*Cicer arietinum* (Chickpea)	40	Grey Soil	7.4	37.47%
PA05	Jambhali (North Side)	*Arachis hypogea* (Groundnut)	65	Black Soil	6.8	41.60%
PA06	Jambhali (Near Bus Stop)	*Trigonella foenum*(Fenugreek)	25	Grey Soil	7.3	36.57%
PA07	Pachod (North Side)	*Glycine max* (Soybean)	54	Black Soil	7.0	38.87%
PA08	Pachod (East Side)	*Cicer arietinum* (Chickpea)	41	Grey Soil	7.2	35.08%
PA09	Kandewadi (East Side)	*Pisum sativum* (Pea)	45	Grey Soil	7.4	36.47%
PA10	Kandewadi (Near ZPSchool)	*Arachis hypogea* (Groundnut)	62	Grey Soil	7.4	34.98%
PA11	Jayakwadi (East side)	*Cicer arietinum* (Chickpea)	46	Black Soil	6.9	41.28%
PA12	Jayakwadi (Near dam)	*Glycine max* (Soybean)	59	Clay Soil	7.3	44.49%
PA13	Jayakwadi (North side of dam)	*Trigonella foenum* (Fenugreek)	22	Grey Soil	7.4	38.67%
PA14	Dhangaon (On Paithan Road)	*Pisum sativum* (Pea)	48	Black Soil	7.0	39.11%
PA15	Dhangaon (Near ZP school)	*Arachis hypogea* (Groundnut)	62	Grey Soil	7.3	35.85%
PA16	Khadgaon (West Side)	*Cicer arietinum* (Chickpea)	43	Black Soil	6.8	42.87%
PA17	Khadgaon (North Side)	*Pisum sativum* (Pea)	43	Grey Soil	7.4	36.48%
PA18	Khadgaon (Near Temple)	*Glycine max* (Soybean)	58	Black Soil	7.1	38.75%
PA19	Dhupkheda (West Side)	*Trigonella foenum* (Fenugreek)	27	Clay Soil	7.2	41.38%
PA20	Dhupkheda (Near Bus Stop)	*Glycine max* (Soybean)	54	Grey Soil	7.3	34.24%
PA21	Dhupkheda (Near Temple)	*Arachis hypogea* (Groundnut)	67	Black Soil	7.0	39.47%

Table 2: Morphological characteristics of samples collected from Paithan Taluka

Sample Code No.	Colony Morphology					Cell Morphology		
	Colony Color	Shape of Colony	Average Colony Size	Appearance of colony	Margins of Colony	Gram's Staining	Cell Length	Cell Shape
PA01	White	Circular	2.2 mm	Glistening Translucent	Entire	-ve	1.1µm	Rod
PA02	Creamy White	Circular	2.8 mm	Translucent	Entire	-ve	1.1µm	Rod
PA03	White	Circular	3.1 mm	Translucent	Entire	-ve	1.0µm	Rod
PA04	White	Circular	2.9 mm	Translucent	Entire	-ve	1.0 µm	Rod
PA05	White	Circular	2.6 mm	Translucent	Entire	-ve	1.2µm	Rod
PA06	White	Circular	3.2 mm	Translucent	Entire	-ve	1.4 µm	Rod
PA07	Creamy White	Circular	3 mm	Translucent	Entire	-ve	0.8 µm	Rod
PA08	Creamy White	Circular	2.8 mm	Translucent	Entire	-ve	1.2µm	Rod
PA09	Creamy White	Circular	2.9 mm	Translucent	Entire	-ve	1.1µm	Rod
PA10	Creamy White	Circular	2.7 mm	Translucent	Entire	-ve	1.0 µm	Rod
PA11	White	Circular	3.2 mm	Translucent	Entire	-ve	1.1 µm	Rod
PA12	White	Circular	3.3 mm	Translucent	Entire	-ve	1.2µm	Rod
PA13	White	Circular	2.9 mm	Translucent	Entire	-ve	1.3 µm	Rod
PA14	Creamy White	Circular	3.1 mm	Translucent	Entire	-ve	1.0 µm	Rod
PA15	White	Circular	2.4 mm	Glistening Translucent	Entire	-ve	0.9 µm	Rod
PA16	White	Circular	3.5 mm	Translucent	Entire	-ve	1.3 µm	Rod
PA17	Creamy white	Circular	3 mm	Translucent	Entire	-ve	0.9 µm	Rod
PA18	White	Circular	3.2 mm	Translucent	Entire	-ve	1.2µm	Rod
PA19	White	Circular	3.1 mm	Translucent	Entire	-ve	1.1 µm	Rod
PA20	Creamy White	Circular	3.1 mm	Translucent	Entire	-ve	1.0µm	Rod
PA21	Creamy White	Circular	2.3 mm	Glistening Translucent	Entire	-ve	1.1µm	Rod

Table 3: Biochemical Characterization of the samples collected from Paithan Taluka

Sample Code No.	Gelatin Hydrolysis	Starch Hydrolysis	Catalase	Urease (Urea Hydrolysis)	Citrate	Indole production	Nitrate Reduction
PA01	-	+	+	-	-	+	+
PA02	-	+	+	-	+	-	+
PA03	-	-	+	+	-	-	+
PA04	-	-	+	+	-	+	+
PA05	-	+	+	-	-	+	+
PA06	-	+	+	-	+	-	+
PA07	-	-	+	+	-	-	+
PA08	-	-	+	+	-	+	+
PA09	-	-	+	-	-	-	+
PA10	-	+	+	-	-	+	+
PA11	-	-	+	+	-	+	+
PA12	-	-	+	+	-	-	+
PA13	-	+	+	-	+	-	+

PA14	-	-	+	-	-	-	+
PA15	-	+	+	-	-	+	+
PA16	-	-	+	+	-	+	+
PA17	-	-	+	-	-	-	+
PA18	-	-	+	+	-	-	+
PA19	-	+	+	-	+	-	+
PA20	-	-	+	+	-	-	+
PA21	-	+	+	-	-	+	+

Table 4: Distribution of *Rhizobium* species in Paithan Taluka

Sr. No	*Rhizobium* group.	Sample code	Rhizosphere crops	Colony morphology	Cell morphology	Positive in biochemical tests	Negative in biochemical tests	*Rhizobium* spp.
1	Group I	PA01, PA05, PA10, PA15 and PA21	*Arachis hypogea* (Groundnut)	White or Creamy white, Circular, Glistening translucent with entire margin	Gram –ve Rod Shaped	Starch Hydrolysis Catalase, Indole Production and Nitrate Reductase	Gelatin hydrolysis, Urease and Citrate	*Rhizobium pusense*
2	Group II	PA03, PA07, PA12, PA18 and PA20	*Glycine max* (Soybean)	White or Creamy white, Circular, Translucent with entire margin	Gram –ve Rod Shaped	Catalase, Urease and Nitrate Reductase	Gelatin hydrolysis, Starch Hydrolysis, Citrate and Indole Production	*Rhizobium japonicum*
3	Group III	PA02, PA06, PA13 and PA19	*Trigonella foenum-graecum* (Fenugreek)	White or Creamy white, Circular, Translucent with entire margin	Gram –ve Rod Shaped	Starch Hydrolysis, Catalase, Citrate and Nitrate Reductase	Gelatin hydrolysis, Urease and Indole Production	*Rhizobium meliloti*
4	Group IV	PA04, PA08, PA11 and PA16	*Cicer arietinum* (Chickpea)	White or Creamy white, Circular, Translucent with entire margin	Gram –ve Rod Shaped	Catalase, Urease, Indole production and Nitrate Reduction	Gelatin hydrolysis, Starch Hydrolysis and Citrate	*Rhizobium ciceri*
5	Group V	PA09, PA14 and PA17	*Pisum sativum* (Pea)	Creamy white, Circular, Translucent with entire margin	Gram –ve Rod Shaped	Catalse and Nitrate Reductase	Gelatin hydrolysis, Starch Hydrolysis, Urease, Citrate and Indole Production	*Rhizobium leguminosarum*

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