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Biochemical and physiological characterization of *Bradyrhizobium japonicum*

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Abstract
Soybean [*Glycine max* (L.) Merrill] is cultivated intensively in India. *Rhizobium* sp. associated with it derives nitrogen (N) by biological nitrogen fixation under field conditions. Out of total 25 isolated sp. from functional root nodules of soybean plants on congo-red yeast extract mannitol agar medium, 20 slow grower *Bradyrhizobium* sp. and 5 fast grower *Rhizobium* sp. were found on growing YEMA media supplemented with bromothymol blue. The purified strains were aerobic, gram negative, non-spore forming and motile rods. The optimum growth kinetics for both sp. was 35 °C at neutral pH (7.0). Both fast and slow growing rhizobia were found to be positive for oxidase, KOH, starch hydrolysis, nitrate reduction and catalase activity. Among all the 25 isolates tested, 23 isolates showed positive reaction to gelatin hydrolysis except Bj-11 and Bj-21 while, 21 isolates were reacted positive to H_2S production whereas, the four isolates *viz*., Bj-1, Bj-2, Bj-8 and Bj-23 were negative in reaction. While all the samples were found negative for IAA production. Pot culture experiment revealed that seed inoculation with these isolates recorded significant improvement in nodule number and plant height ranged from 3.00 to 22.00 nodules per plant and 35.00 to 65.00 cm respectively at flowering stage over uninoculated control. Isolates *viz*., Bj-9, Bj-10, Bj-14, Bj-17, Bj-19, Bj-23, Bj-25 could produce large and pink nodules indicating their effective symbiosis with soybean.

Keywords: *Bradyrhizobium japonicum*, *Rhizobium* sp., characterization, pot culture

Introduction
Microbes are an integral part of natural fertility cycles and play crucial role in the mineralization of nutrients resulting in the formation of organic matter for plants growth. Some soil bacteria and fungi form relationships with plant roots that provide important nutrients like nitrogen or phosphorus. Symbiotic nitrogen fixation is mainly attributed to the legumes and approximately 90% of them fix nitrogen from the atmosphere with Rhizobiaceae. Soybean crop is currently considered as an important crop in India. The most common microsymbionts of soybean are *Bradyrhizobium japonicum* and *Bradyrhizobium elkanii* (Zhang *et al.*, 2011) [18]. *B. japonicum* are the slow growing isolates and currently, the genus of *Bradyrhizobium* consists of 7 species. The genus *Bradyrhizobium* is a rod shaped having 0.5-0.9 µm × 1.2-3.0 µm size. It is commonly pleomorphic, non-spore forming, gram negative motile by polar or sub polar flagellum, aerobic, possessing a respiratory type of metabolism with oxygen as the terminal electron acceptor. Soybean fixes about 49-130 kg/ha nitrogen (Salvagiotti *et al.* 2018) [14] and saves about 15-40% chemical fertilizer under different ecological situation. Soybean offers a variety of possible benefits to production systems, diets and incomes of its producers. The extensive use of N-fertilizers is both harmful to the environment and results in the depletion of fossil fuels. There are also problems of high cost of the commercial inoculants and the inability of the strains to survive the extreme soil conditions. There is variability in the effectiveness and population of indigenous bradyrhizobia in a given location. Therefore, there is need to search strains of *Bradyrhizobium* from among the indigenous soil populations with all the desirable qualities required for the formulation of soybean seed inoculants.

Materials and Methods

Collection of soybean plant samples

Healthy soybean plant samples with effective root nodules were collected in the month of July at the flowering stage of the plant i.e. 40-45 days after sowing.

Isolation of *B. japonicum* from root nodules of soybean

The collected plant samples along with root nodules were carefully washed under tap water. Functional and effective nodules (healthy, bold, unbroken, pink colored) from the roots of field growing soybean plants were detached carefully by giving cut with sharp blade/knife, collected in glass petri plates and surface sterilized with either 75% (v/v) ethyl alcohol for 3 min or 0.5-1% sodium hypochloride for 1-2 min. The nodules were then washed in 3-4 sequential changes of distilled water, blott dried, 5-10 nodules were dispensed in 5 ml distilled water in test tube and crushed with sterile glass rod. The resultant bacterial suspension was streaked with inoculation needle wire loop on solidified YEMA petri plates and incubated at 28±2°C in inverted position. After, 48-72 hrs of incubation, well grown white translucent single colonies of *Bradyrhizobium* and *Rhizobium* sp. developed were picked up and subcultured on YEMA plates (Gachande and Khansole, 2011) [5]. The pure culture obtained was maintained on CR-YEMA test tube slants, for further studies.

Morpho-cultural characters of *B. japonicum*

Morphological and microscopic characteristics of all the isolates were investigated. After an incubation of 3 to 5 days at 28±2°C on yeast extract mannitol agar plate, individual colonies were characterized based on shape, size, color, elevation, texture, margin etc. were observed at 72-96 hrs. of incubation. Fast growing rhizobia generally produced white, semi-transparent / opaque, circular, mucilaginous colonies; while, slow growing strains produced white, opaque, circular, granular colonies microscopically and gram staining reaction were recorded (Pawar, *et al.*, 2014) [10].

Confirmatory tests

Two different tests were performed to confirm the isolate as *Bradyrhizobium* sp. or *Rhizobium* sp. and to differentiate them from other contaminating microbes. In general, *B. japonicum* and *Rhizobium* sp. produce white colonies, whereas many other bacteria take up the dye strongly on CR-YEMA media. While, YEMA supplemented with BTB medium was used for differentiating *Bradyrhizobium* from *Rhizobium* (Maruekarajtinpleng, *et al.*, 2012) [9]. The cultures were streaked on BTB agar plates. After incubation at 28 ± 2 °C for 2-10 days, color change of the medium was observed. The media turns blue for the isolates classified as slow growers and yellow for the fast growers.

Physiological characteristics

Effect of temperature

Yeast extract mannitol broth was inoculated with loopful culture of different isolates of *B. japonicum* and *Rhizobium* sp. and were incubated at different temperature ranging from 5°C to 45°C (5, 10, 15, 20, 25, 30, 35, 40 and 45°C) to find out the effect of temperature on rhizobial growth. The growth of *B. japonicum* and *Rhizobium* sp. was measured turbidiometrically by using spectrophotometer at 600 nm.

Effect of pH levels on growth of *B. japonicum*

Effect of pH on the growth of *B. japonicum* and *Rhizobium* sp. was studied by adjusting pH of the YEMA medium to various levels *viz.*, 4, 5, 6, 7, 8, 9 and 10 using appropriate phosphate buffer. A loop full of 48 hour old bacterial culture was mixed in 100 ml conical flask containing 30 ml YEM broth. Inoculated flasks were incubated at room temperature for 72 hours. After the incubation period observations were recorded for the growth of bacterium turbidiometrically by using spectrophotometer at 600 nm.

Biochemical characteristics

Several biochemical tests *viz.*, gram's staining, catalase oxidation, oxidase, potassium hydroxide (KOH) solubility, starch hydrolysis, gelatin hydrolysis, nitrate reduction, IAA production, H_2S production and mobility test of *B. japonicum* were attempted by adopting standard procedures. (Aneja, 2003) [1].

Pot culture experiment

Inoculum preparation

About 100 ml of broth was taken in a 250 ml conical flask and 1 ml of pure culture suspension containing 6 $\times 10^7$ cells was inoculated and incubated at 28±2°C for 4 to 6 days. It was kept on a rotary shaker to produce heavily turbid suspension. The population of the test isolate was determined by dilution plate method. After the quantitative determination of population in the inoculum suspension, the broth cultures (containing 6 $\times 10^7$ cells ml^{-1}) both effective and ineffective were mixed with sterilized lignite carrier for seed inoculation.

Seed inoculation

Prior to sowing, the seeds of soybean, variety JS-335 were mixed with rhizobial culture carrier based material and made air dry. These inoculated seeds were sown in polythene bags which had already been prepared.

Preparation of bags

Soil from fallow plots was mixed well, sieved and sterilized in a autoclave at 20 lbs pressure for 2 hr. The soil were then filled in pot and allowed to incubate for 4 days and the soil was loosened and mixed well with the help of a stout glass rod and filled it in black polythene bags at the rate of 10 kg per bag. The bags were watered to the optimum moisture holding capacity of the soil and the following experimental conditions were employed. Seeds without test isolate *i.e.* control (C) and seeds treated with different test isolates (T). The *B. japonicum* and *Rhizobium* sp. inoculated seeds were sown in the bag. Each treatment had three replicates for each set along with 10 bags of uninoculated control. Five seeds were sown in each bag in an equal distant manner. Seedlings emerged after 2-7 days after sowing (DAS). After emergence of all the soybean seedlings, seedlings were thinned and only three plants were kept in each bag. Adequate irrigation was given to the growing plants. The plants were uprooted and observations were recorded for plant height, number of nodules, at 45 DAS. The following observations were recorded and the plants were uprooted at flowering stage of growth for morphometric analysis (Kukkamalla and vardhan, 2016) [7].

Host response studies

The isolates were tested for their host response effectiveness by conducting morphometric analysis on the root nodules of host plant.

Number of nodules
Three randomly selected plants were uprooted from each pot at 45 DAS with their root system being intact. The roots were washed in running tap water and nodules carefully detached with forceps. The number of nodules per plant was recorded by taking average.

Plant height
Three plants were randomly selected and uprooted at flowering stage from each bag and the length of shoots and roots was measured from the base to the tip of the shoot.

Statistical analysis
All the data was analysed statistically by applying ANOVA procedure analysis tool, given by O. P. Sheoron, Hisar. The significance of each of the parameter under different treatment were calculated on F-test and standard deviation of all treatments were also calculated.

Result and Discussion

Collection of soybean plant samples
The samples were collected from different locations of Maharashtra and some from Madhya Pradesh (Table-1).

Table 1: Location wise root nodule samples of soybean collected for isolation of *B. japonicum*

Root nodule sample no.	Location of sample	Root nodule sample no.	Location of sample
1	Katol	14	Rahuri
2	Saoner	15	Parbhani
3	Nagpur	16	Nasik
4	Hingana	17	Beed
5	Pauni	18	Sangali
6	Wardha	19	Adilabad
7	Warora	20	Chhattarpur
8	Washim	21	Dewas
9	Pusad	22	Sehore
10	Amravati	23	Bhopal
11	Dhamangaon	24	Plant Pathology Section, COA, Nagpur
12	Akola		
13	Buldhana	25	Market

Characterization of *B. japonicum* and *Rhizobium* sp.
All the rhizobial isolates were studied to carried out the morphological, cultural and biochemical tests, the results were as follows.

Morpho-cultural characters
The cultural and morphological characteristics of the isolates are summarized collectively as under (Table-2, fig-1).

Table 2: Cultural and morphological characteristics of *B. japonicum* and *Rhizobium* sp.

Sr. No.	Characters	Observations
1	Colony shape	Circular
2	Size of colony	2-4 mm
3	Bacterium shape	Rod shaped
4	Color / pigmentation	Whitish pink and glistening
5	Elevation	Convex
6	Margin	Regular / entire
7	Oxygen demand	Aerobic
8	Spore formation	Non spore forming
9	Motility	Motile
10	Opacity	Opaque
11	Gram's nature	Gram '-ve'

Similar results were found by Bhatt, *et al.* (2013) [2] the colony morhology of *Rhizobium* sp. isolated from mungbean shows white, translucent, glistening, elevated, entire margin. Kaur, *et al.* (2012) [6] reported the colony morphology of *Rhizobium* sp. as white, translucent, gummy, glistening, elevated with entire margin isolated from soybean.

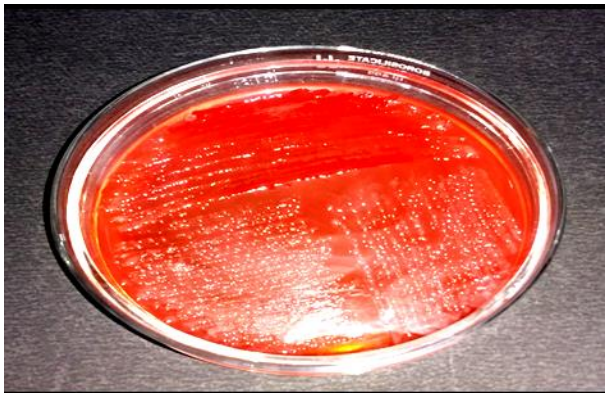

Fig 1: Colonies of *B. japonicum* on CR-YEMA media

Confirmatory tests
Growth of *B. japonicum* and *Rhizobium* sp. on YEMA with congo red medium
The colonies isolated were slimy, creamy white in colored with circular edges and appeared transparent on CR-YEMA plates with marked distinction from red colored *Agrobacterium* colonies.

Differentiation of fast and slow growing rhizobia
For differentiating fast and slow growing rhizobia YEMA medium was enriched with BTB (@ 25µgml^{-1}) to selectively identify slow growing *Bradyrhizobium* sp. from fast growing *Rhizobium* sp. as quoted by Vincent (1970) [17]. All the isolates were subjected to grow on BTB amended medium. The positive isolates of *Bradyrhizobium* sp. remained blue colored on BTB agar medium (fig 2) whereas the negative isolates showed moist and gummy colonies after incubation for 48 hrs at 28°C and medium surrounding the culture turned yellow (fig 2) due to acid production by fast growing *Rhizobium* sp. It was identified that 20 isolates produced blue color colonies, which indicated the presence of alkali producers, considered as slow growing *Bradyrhizobium* spp. and remaining 5 fast growing *Rhizobium* isolates, showed yellow zone along with acid production (Table 3). Among all rhizobia cultures isolated from soybean root nodules, it was found that about 80% of the strains were slow growing *Bradyrhizobium* spp. while only 20% of the strains were fast growing *Rhizobium* spp.

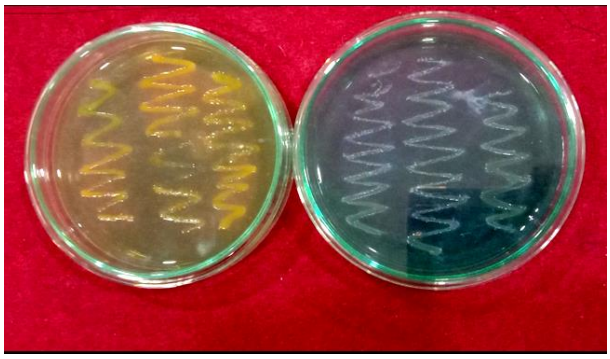

Fig 2: Differentiation of fast grower *Rhizobium* and slow grower *Bradyrhizobium*

YEMA-BTB medium used for categorizing indigenous legume root nodulating rhizobia as fast and slow growing based on acid / alkali production supported by Upadhayay, *et al*. (2015) and Deka and Azad (2006) [16, 3].

Table 3: Differentiation of fast (*Rhizobium* spp.) and slow grower (*Bradyrhizobium* spp.) soybean rhizobia on YEMA (BTB) medium

Isolates	Color produced on BTB agar	Character (grower)
Bj-1	Blue	Slow
Bj-2	Blue	Slow
Bj-3	Blue	Slow
Bj-4	Blue	Slow
Bj-5	Yellow	Fast
Bj-6	Blue	Slow
Bj-7	Blue	Slow
Bj-8	Blue	Slow
Bj-9	Blue	Slow
Bj-10	Blue	Slow
Bj-11	Blue	Slow
Bj-12	Blue	Slow
Bj-13	Blue	Slow
Bj-14	Yellow	Fast
Bj-15	Blue	Slow
Bj-16	Blue	Slow
Bj-17	Blue	Slow
Bj-18	Yellow	Fast
Bj-19	Blue	Slow
Bj-20	Yellow	Fast
Bj-21	Blue	Slow
Bj-22	Blue	Slow
Bj-23	Blue	Slow
Bj-24	Blue	Slow
Bj-25	Yellow	Fast

Physiological characters

Effect of temperature regimes on growth of *B. japonicum* and *Rhizobium* sp.

Temperature is one of the important crucial physical factors affecting microorganisms growth. All the isolated *B. japonicum* and *Rhizobium* sp. were evaluated for tolerance to various temperature regimes- 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, 35°C, 40°C and 45°C (Table 4). At 35°C, all theisolates showed excellent growth, while at 40°C and 45°C, five fast growing *Rhizobium* sp. *viz*. Bj-5, Bj-14, Bj-18, Bj-20 and Bj-25 showed good growth. Whereas, all slow grower *Bradyrhizobium* sp. showed poor growth at 45°C. It revealed from the data that fast growing *Rhizobium* sp. can tolerate high temperature as compared to slow growing *Bradyrhizobium* sp.

Table 4: Effect of different temperature regimes on growth of *B. japonicum* and *Rhizobium* sp. of soybean

Isolates	Optical density at 600 nm								
	Temperature								
	5°C	10°C	15°C	20°C	25°C	30°C	35°C	40°C	45°C
Bj-1	0.06	0.12	0.25	0.38	0.51	0.67	0.82	0.39	0.11
Bj-2	0.04	0.15	0.24	0.42	0.57	0.71	0.80	0.31	0.16
Bj-3	0.09	0.14	0.23	0.41	0.42	0.72	0.76	0.37	0.12
Bj-4	0.08	0.11	0.20	0.39	0.49	0.71	0.78	0.28	0.09
Bj-5	0.01	0.12	0.21	0.31	0.47	0.65	0.82	0.69	0.26
Bj-6	0.05	0.11	0.23	0.38	0.47	0.61	0.80	0.36	0.13
Bj-7	0.05	0.13	0.24	0.41	0.44	0.64	0.81	0.37	0.11
Bj-8	0.03	0.13	0.21	0.38	0.45	0.63	0.93	0.45	0.17
Bj-9	0.09	0.21	0.30	0.51	0.60	0.81	0.92	0.51	0.15
Bj-10	0.07	0.17	0.27	0.48	0.57	0.79	0.89	0.47	0.14
Bj-11	0.04	0.11	0.24	0.39	0.48	0.74	0.79	0.27	0.11
Bj-12	0.05	0.14	0.22	0.40	0.51	0.69	0.80	0.29	0.06
Bj-13	0.03	0.12	0.23	0.32	0.48	0.65	0.83	0.28	0.09
Bj-14	0.07	0.18	0.29	0.43	0.58	0.78	0.88	0.61	0.21
Bj-15	0.04	0.16	0.24	0.41	0.50	0.67	0.78	0.34	0.11
Bj-16	0.03	0.12	0.23	0.39	0.48	0.71	0.75	0.27	0.09
Bj-17	0.08	0.17	0.28	0.47	0.46	0.77	0.90	0.31	0.12
Bj-18	0.07	0.11	0.22	0.30	0.46	0.65	0.82	0.43	0.22
Bj-19	0.08	0.18	0.27	0.48	0.57	0.76	0.86	0.22	0.10
Bj-20	0.08	0.14	0.21	0.32	0.47	0.70	0.80	0.55	0.15
Bj-21	0.02	0.16	0.24	0.39	0.45	0.75	0.84	0.36	0.06
Bj-22	0.05	0.12	0.26	0.39	0.49	0.68	0.88	0.43	0.08
Bj-23	0.06	0.19	0.28	0.48	0.53	0.69	0.83	0.49	0.09
Bj-24	0.05	0.14	0.23	0.36	0.43	0.65	0.79	0.24	0.11
Bj-25	0.07	0.19	0.27	0.46	0.56	0.79	0.91	0.56	0.28

+: 0.01 to 0.30 ++: 0.31 to 0.60 +++: ≥60

Ruiz-Diez, *et al*. (2011) [12] also reported that out of 36 isolates of legume rhizobia, 3 isolates survived at high temperatures and extreme environmental conditions.

Effect of pH levels on growth of *B. japonicum* and *Rhizobium* sp.

Growth of rhizobia in soils is sensitive to pH. The pH has in fact been shown to limit survival and persistence in soils. In the present study all the isolates of *B. japonicum* and *Rhizobium* sp. showed maximum growth at 7 (neutral) pH range with an OD value ≥60 followed by pH 6 and 8 with an OD value ranging between 0.31 to 0.60. The least growth was observed only at 4, 5, 9 and 10 pH with an OD value ranging between 0.01 to 0.30. (Table-5)

Table 5: Effect of pH levels on growth of *B. japonicum* and *Rhizobium* sp. of soybean

Isolates	Optical density at 600 nm						
	pH levels						
	4	5	6	7	8	9	10
Bj-1	0.02	0.17	0.47	0.73	0.46	0.16	0.10
Bj-2	0.06	0.21	0.47	0.77	0.44	0.25	0.12
Bj-3	0.02	0.19	0.45	0.76	0.41	0.19	0.07
Bj-4	0.07	0.23	0.48	0.80	0.43	0.21	0.06
Bj-5	0.05	0.18	0.49	0.81	0.65	0.36	0.19
Bj-6	0.01	0.20	0.38	0.76	0.40	0.21	0.13
Bj-7	0.07	0.24	0.45	0.82	0.43	0.27	0.09
Bj-8	0.05	0.19	0.38	0.79	0.50	0.24	0.13
Bj-9	0.09	0.29	0.61	0.92	0.57	0.30	0.06
Bj-10	0.06	0.27	0.58	0.91	0.55	0.19	0.07
Bj-11	0.04	0.22	0.46	0.84	0.41	0.20	0.09
Bj-12	0.02	0.21	0.50	0.85	0.46	0.26	0.03
Bj-13	0.04	0.18	0.44	0.78	0.47	0.23	0.11

Bj-14	0.06	0.25	0.45	0.89	0.68	0.40	0.22
Bj-15	0.05	0.22	0.49	0.78	0.46	0.25	0.04
Bj-16	0.06	0.21	0.47	0.75	0.42	0.28	0.09
Bj-17	0.07	0.28	0.58	0.92	0.43	0.30	0.10
Bj-18	0.06	0.20	0.48	0.76	0.57	0.37	0.23
Bj-19	0.06	0.26	0.59	0.89	0.43	0.27	0.15
Bj-20	0.03	0.23	0.46	0.73	0.59	0.37	0.20
Bj-21	0.06	0.21	0.45	0.72	0.42	0.23	0.10
Bj-22	0.08	0.22	0.48	0.80	0.49	0.21	0.13
Bj-23	0.05	0.27	0.56	0.89	0.45	0.39	0.09
Bj-24	0.03	0.21	0.49	0.76	0.42	0.23	0.11
Bj-25	0.05	0.27	0.55	0.90	0.62	0.42	0.21

+: 0.01 to 0.30 ++: 0.31 to 0.60 +++: ≥60

These results are similar to what has already been shown by different workers. (Kaur *et al.*, 2012) [6] showed that the best rhizobial growth is in media with pH around neutral. On the other hand, Sadowsky *et al.* (1983) [13] have reported that slow-growing rhizobia such as *Bradyrhizobium*, can have a high level of tolerance to acid conditions (pH 4.5) while fast-growers can tolerate alkaline conditions of pH 9 and 9.5 in growth media.

Biochemical characteristics

In order to identify the test twenty five isolates, it was subjected to the biochemical test. Some of the tests were performed for comparing the characteristics as depicted in Burgey's manual of systematic bacteriology.

Different biochemical tests *viz.*, grams staining, catalase oxidation test, KOH (potassium hydroxide) solubility, oxidase, gelatin hydrolysis, starch hydrolysis, nitrate reduction, etc. were attempted of *B. japonicum* and *Rhizobium* sp. and the results obtained are discussed below (Table-6).

Table 6: Biochemical characteristics of *B. japonicum* and *Rhizobium* sp. of soybean

Biochemical tests									
Isolates	**GR**	**C**	**KOH**	**O**	**SH**	**GH**	**NR**	**IP**	**HP**
Bj-1	-	+	+	+	+	+	+	-	-
Bj-2	-	+	+	+	+	+	+	-	-
Bj-3	-	+	+	+	+	+	+	-	+
Bj-4	-	+	+	+	+	+	+	-	+
Bj-5	-	+	+	+	+	+	+	-	+
Bj-6	-	+	+	+	+	+	+	-	+
Bj-7	-	+	+	+	+	+	+	-	+
Bj-8	-	+	+	+	+	+	+	-	-
Bj-9	-	+	+	+	+	+	+	-	+
Bj-10	-	+	+	+	+	+	+	-	+
Bj-11	-	+	+	+	+	-	+	-	+
Bj-12	-	+	+	+	+	+	+	-	+
Bj-13	-	+	+	+	+	+	+	-	+
Bj-14	-	+	+	+	+	+	+	-	+
Bj-15	-	+	+	+	+	+	+	-	+
Bj-16	-	+	+	+	+	+	+	-	+
Bj-17	-	+	+	+	+	+	+	-	+
Bj-18	-	+	+	+	+	+	+	-	+
Bj-19	-	+	+	+	+	+	+	-	+
Bj-20	-	+	+	+	+	+	+	-	+
Bj-21	-	+	+	+	+	-	+	-	+
Bj-22	-	+	+	+	+	+	+	-	+
Bj-23	-	+	+	+	+	+	+	-	-
Bj-24	-	+	+	+	+	+	+	-	+
Bj-25	-	+	+	+	+	+	+	-	+

GR-Gram's reaction, C-Catalase, O-Oxidase, KOH-Potassium hydroxide, SH-Starch hydrolysis, GH-Gelatin hydrolysis, NR-Nitrate reduction, IP - Indole production, HP-H_2S production

Singh *et al.* (2008) characterized the *B. japonicum*, isolated from soybean root nodules and reported its colonies as milky white, translucent, circular (2-4mm dia.), shiny, raised (convex) and sticky with musty odor. The bacterium was gram negative rods. Deora and Singhal (2010) reported that the bacterium *Rhizobium* on YEMA medium produced sticky and mucoid colonies, gram negative rods and showed positive reactions to starch hydrolysis and negative reaction to methylene blue, lactose and gelatin test.

Effect of *B. japonicum* and *Rhizobium* sp. inoculation on nodulation and plant growth parameters of soybean

A pot culture experiment was laid out (fig 3) to study the effect of inoculation of rhizobia sp. (*Rhizobium* sp./*Bradyrhizobium* sp.) Twenty five isolates were tested for plant height and total number of functional nodules using soybean cv. JS-335 (Table-7).

Table 7: Effect of *B. japonicum* and *Rhizobium* sp. inoculation on nodulation and plant growth parameters of soybean (45 DAS)

Isolates	**Plant growth parameters**			
	Shoot length (cm)	**Root length (cm)**	**Total plant height (cm)**	**No. of nodules per plant**
Bj-1	24	23	47	4
Bj-2	20	35	55	3
Bj-3	28	19	47	6
Bj-4	25	40	65	8
Bj-5	24	28	52	5
Bj-6	24	37	61	3
Bj-7	25	17	42	5
Bj-8	30	27	57	4
Bj-9	34	29	63	22
Bj-10	35	26	61	18
Bj-11	22	25	47	9
Bj-12	23	20	43	7
Bj-13	22	19	41	4
Bj-14	24	25	49	11
Bj-15	26	22	48	5
Bj-16	25	26	51	8
Bj-17	30	33	63	18
Bj-18	30	20	50	3
Bj-19	30	34	64	15
Bj-20	32	28	60	5
Bj-21	34	25	59	6
Bj-22	25	22	47	7
Bj-23	25	19	44	10
Bj-24	24	30	54	8
Bj-25	27	21	48	12
Control	20	15	35	00
SE ±	1.521	1.654	2.235	1.149
C.D.(P=0.01)	4.327	4.707	6.361	3.270

All the *B. japonicum* and *Rhizobium* sp. inoculants recorded higher plant height ranged from 35.00 to 65.00 cm at flowering stage, respectively (Table-7, fig 3). The present results are in agreement with those reported by Kumar *et al*. (1996) [8] who observed that the strains of *Bradyrhizobium* inoculation showed better performance at different stages of growth. Also, similar results were found by Ravikumar (2012) [11] in mungbean studied the inoculated plants with *Rhizobium* possessed greater height, greater fresh weight, greater number of roots, nodules, greater number of leaves, shoots, pods, greater length of pods, greater seed weight, over their respective controls.

Fig 3: Nodulation in soybean

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