International Journal of Research in Pharmacy and Science

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Research Article

Growth response of three indigenous *Bradyrhizobium japonicum* isolates against a few environmental factors

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Received: 17-04-2015
Review completed: 18-06-2015
Accepted: 25-06-2015

Access this article online	
QR Code	**Website:** www.ijrpsonline.com

ABSTRACT

Bradyrhizobiumjaponicum and soybean is a type of symbiotic microbe-plant interaction in which *Bradyrhizobium* fixes nitrogen for the soybean plant and inturn gets nutrients and protection from it. For its adherance to plant root *Bradyrhizobium* depends on its exopolysaccharide production.Nitrogen fixation and exopolysacchride production are dependent on various environmental factors. The present study was focused on the effects of temperature, pH, NaCl concentrations and different carbon sources on three screenedindigenous strains of *B.japonicum* viz., B1, B2 and B3. These strains were isolated from the root nodules of JS-335 cultivar of soybean cultivated in agricultural farms at Ujjain (Madhya Pradesh). Dry weight of cells and exopolysaccharide production were taken as growth parameters. Results indicate that, all the three isolates showed significant growthat 25°C temperature, 8.0 pH and 2% NaCl concentration. Out of all the tested carbon source for growth and exopolysaccharide production, mannitol was found most suitable. The present study provides the most effective set of environmental conditions for maximum growth of indigenous *Bradyrhizobium japonicum*isolates. The study alsoprovides information for optimum environmental conditions that are critically important for the biofertilizer production at mass scale.

Key words: Biofertilizer, *Bradyrhizobium*, Dry weight, Exopolysaccharide, Soybean cv 335

INTRODUCTION

Leguminous plants, such as Soybean, Pea, Gram,Pigeon pea etc.,obtain nitrogen symbiotically with an unique beneficial group of bacteria known as Rhizobia. Legume isa major source of proteins (human and animal nutrition) for developing countries including India[1]. *Bradyrhizobium japonicum* is a Gram negative bacterium belonging to rhizobia group associated with roots of soybean and have the capacity to fix N_2in the presence of nitrogenase enzyme[2].This bacterium and nitrogenase enzyme both are very sensitive for the environmental conditions. The commercially introduced strains must compete with highly adapted indigenous rhizobia for legume nodulation under specific physiological, biological and environmental soil conditions. Soil acidity limits symbiotic nitrogen fixation by limiting *Rhizobium* survival in soils, as well as reducing nodulation [3].The response of some nitrogen-fixing microbes under adverse environmental conditions such as salt stress, drought stress, acidity, alkalinity, nutrient deficiency, heavy metals, and pesticides were earlier reviewed by various workers[4-10]. The behavior (growth response) of the rhizobia against environmental conditions should be known so that the activities of enzyme can beoptimized for maximum output.It was also noticed that low temperature adversely affects both nodule formation and rate of N_2 fixation [11, 12]. Legume production involves survival and establishment of inoculated rhizobia in the soil environment [13,14].A competitive and persistent rhizobial strain is not expected to express its capacity for nitrogen fixation as these limiting factors force limitations on the vigor of the host legume [15,16]. Thus, keeping above views in mind present study was conducted to observe the growth response of three indigenous *Bradyrhizobium japonicum* isolates (B1, B2, and B3) from JS-335 soybean cv., growing in Black Cotton Soil of Malwa region (Ujjain) against different environmental conditions.

MATERIALS AND METHODS

Growth response study of *Bradyrhizobium* was estimated following exopolysacchride and dry weight (biomass) production under different test conditions. pH, temperature, salt concentration and carbon source were taken as environmental test factors.

Isolation of Bacteria

The bacteria were isolated directly from the active nitrogen-fixing root nodules of JS-335 cultivar [17]and identified[18]. As per earlier study which was based on nodulation capacity, shoot, root lenght and their dry weight only three (B1, B2 and B3) out of total of ten isolates were selected for present study[19].

Test environmental factors:

Growth of three screened *Bradyrhizobium japonicum* isolates was studied for environmental parameters.

Effect of pH: All selected three isolates were grown in 10 ml Yeast Extract Mannitol (YEM) medium at five different pH viz., 2, 4, 6, 8, and 10 and incubated at 27°C for 7 days. After proper incubation, growth was measured on the basis of dry weight of biomass and exopolysaccharide production. Exopolysaccharide production was determined[20]. While dry weight of biomass was determined by centrifugation-dry weight method**.**YEM broth was prepared as follow; Mannitol-10g, K_2HPO_4-0.5 g, $MgSO_4.7H_2O$- 0.3g, NaCl- 0.1g, Yeast Extract- 0.5 g and Distilled Water (D/W) - 1000 ml.

Effect of temperature: The YEM broth was prepared, inoculatedwith bacterial isolates and incubated at 5 different temperatures viz., 15, 20, 25, 30 and 35°C for 7 days. Afterincubation, growth was determined.

Effect of NaCl concentration: The YEM broth with 5 different NaCl concentrations was prepared e.g., 1, 2, 3, 4 and 5%. Inoculatedtubes were kept at 27°C for incubation.

Effect of Carbon source: Different carbon sources – glucose, lactose and sucrose were replaced to mannitol in YEM broth. Growth was determined by exopolysaccharide production and dry weight of biomass.

RESULTS AND DISCUSSION

The major source of nitrogen input in agricultural soils isby atmospheric N_2 fixation. The major N_2-fixing systems are the symbiotic ones, which play a significant role in improving the fertility and productivity of low nitrogen soil [21].The present study was conducted for the assessment of growth response of three indigenous *Bradyrhizobium japonicum* isolates (B1, B2 and B3**,** Photo Plate-1) using different environmental conditions. Within the soil, rhizobia frequently encounter various stresses that affect their growth, initial steps of symbiosis, and the efficiency of nitrogen fixation [4].Environmental conditions affect the growth of bacteria and nitrogen fixation which ultimately affects the plant growth. Effect of change in pH is evident on dry weight and exopolysaccharide production in *B.japonicum*. Maximum dry weight was obtained in B3 isolate at pH value 6, while exopolysaccharide production was highest in B1 isolate at pH 8.At pH value 2 and 10 no growth was observed. Extremes of pH can be a liming factor for microorganisms in soil.There are cases, where pH sensitive stage in nodulation occurs early in the infection process and *Rhizobium* attachment to root hairs is one of the stages affected by acidic conditions in soils. Fast growing *Rhizobium* strains have generally been considered less tolerant to acidicpH than slow growing strains of *Bradyrhizobium* which is also evident in the present study [22]. Though a few rhizobia grow well at pH value less than 5 some strains of *Rhizobium tropici*, *Mesorhizobiumloti*, *Bradyrhizobium* sp. and *Sinorhizobiummeliloti* are very acid-sensitive [23]. In the present study the tested three isolates showed maximum growth (in terms of dry weight) at pH 6 while the exopolysacchride production was highest in all three isolates at pH 8 (**Table-1**).

Figure 1 : Showing the three *B. japonicum* isolates on YEM medium

Table No. 1: Effect of pH on dry weight and exopolysaccharide production

Parameters	Bacterial Isolates	pH 2	pH 4	pH 6	pH 8	pH 10
Dry Weight	B1	0	0.08±0.005	0.2±0.03	0.2±0.01	0
	B2	0	0.12±0.01	0.2±0.02	0.09±0.03	0
	B3	0	0.09±0.01	0.4±0.05	0.06±0.03	0
Exopolysaccharide	B1	0	0.01±0.007	0.02±0.01	0.03±0.04	0
	B2	0	0.01±0.00	0.02±0.04	0.01±0.01	0
	B3	0	0.01±0.00	0.02±0.03	0.01±0.03	0

± *Standard Deviation*

The optimum pH for the growth of root nodule bacteria usually falls between 6.0 and 7.0[23].Acidic soil may have an effect on different stages of growth, from strain survival in soil and on the seed, to root-hair infection, nodule initiation and nitrogen fixation [24].In the present study it was noticed that lower pH was not favorable for growth in terms of cell biomass and exopolysaccharide production. Although, there are reports that rhizobial strains of a given species vary widely in their pH tolerance[25]. However, reports are available that both fast and slow-growing *Bradyrhizobium* strains of *Vigna unguiculata* are tolerant to pH values as low as 4.0 [26]. As regards temperature the most suitable value was 25ºCfor exopolysaccharide and dry weight production **(Table-2)**. Several studies have reported that rhizobial growth is adversely affected by high soil temperature [27, 28]. Some previous workers also confirmed this finding by reporting that optimum temperature for growth of root nodulating bacteria ranged from 25-30°C and high temperature is responsible for inhibition of nodulation process[29].

Table No. 2: Effect of temperature on dry weight and exopolysaccharide production

Parameters	Bacterial Isolates	Temperature 15	20	25	30	35
Dry Weight	B1	0	0.03± 0.04	0.4±0 .09	0.09± 0.08	0
	B2	0	0.02± 0.05	0.4±0 .06	0.07± 0.06	0
	B3	0	0.01± 0.05	0.3±0 .08	0.09± 0.09	0
Exopolysaccharide	B1	0	0	0.03± 0.02	0.01± 0.15	0
	B2	0	0.01± 0.005	0.02± 0.55	0.01± 0.01	0
	B3	0	0.01± 0.006	0.02± .119	0.02± 0.04	0

± *Standard Deviation*

Salinity and drought can adversely affect the nodulation in legume/*Rhizobium* associations which ultimately reduce crop yield. Saline (NaCl/salt) condition affects, plant growth and nitrogen fixation [30-32].Reports are available that salinity plays a major role in agriculture and around 7% of the world's total land area is affected[33], while about 40% of the world land surface have the salinity problem [34]. In the present study it was observed that 2% NaClconcentration was found most suitable for the growth of bacterial isolates (**Figure 1** and **2**) and there was a significant increase in dry weight of all three isolates atthis concentration.

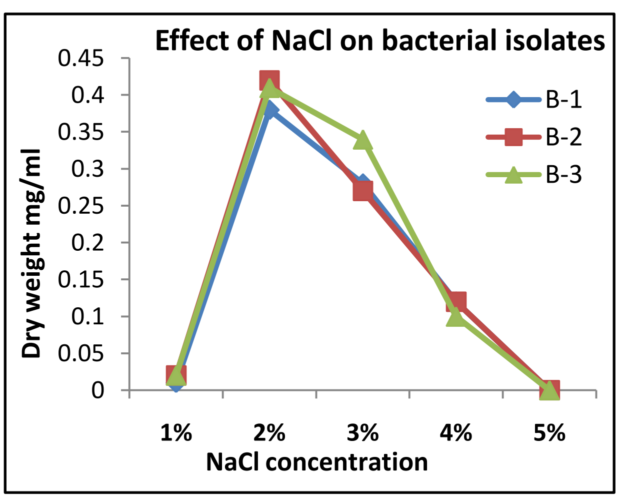

Figure No. 1: Effect of NaCl concentrations on dry weight of three Bradyrhizobial isolates

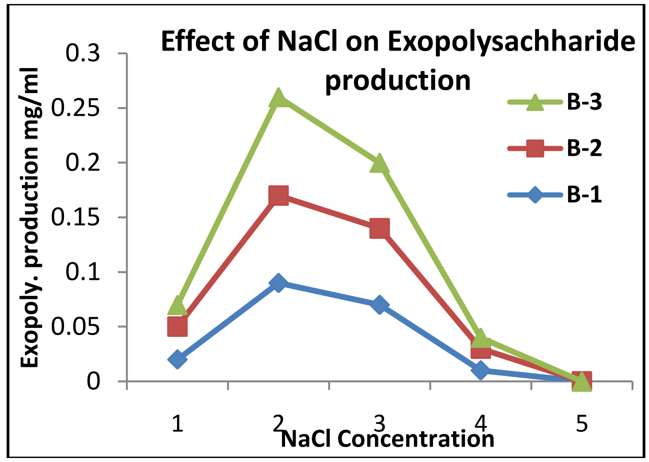

Figure No. 2: Effect of NaCl concentrations on exopolysaccharide production

Nodulation relationship is adversely affected by salinity which can prevent legume establishment and growth, or reduce crop yield [35]. Legumes and the process of nodule initiation both are more sensitive to salt stress than rhizobia. The effect of salt stress on nitrogen fixation has been examined by earlier workers [28, 36-38] and their studies support the present work. Effects of different abiotic factors (acidity, salinity, nitrate and temperature) on growth rate of root nodule bacteria (*Rhizobium* and *Bradyrhizobium)*was also investigated [39]. Effect of carbon source was studied by replacing the source in yeast extract broth and it was observed that the Mannitol was most suitable carbon source for the growth of all three indigenous *Bradyrhizobiumjaponicum* isolates **(Figure 3 and 4).**

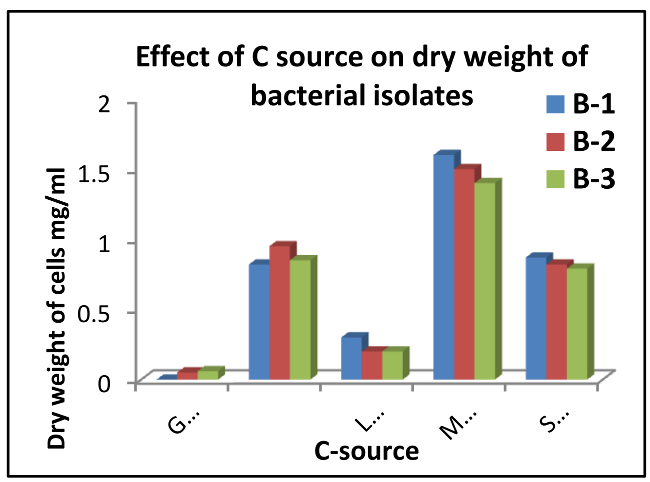

Figure 3 Effect of Carbon source on dry weight of bacterial isolates

Carbohydrate utilization assay indicated that *Rhizobium* isolates obtained from fenugreek roots were able to utilize different carbohydrate sources. But our study differs from the previous work in which*Rhizobium* strains were able to utilize glucose and sucrose more efficiently than normal YEM medium[40].

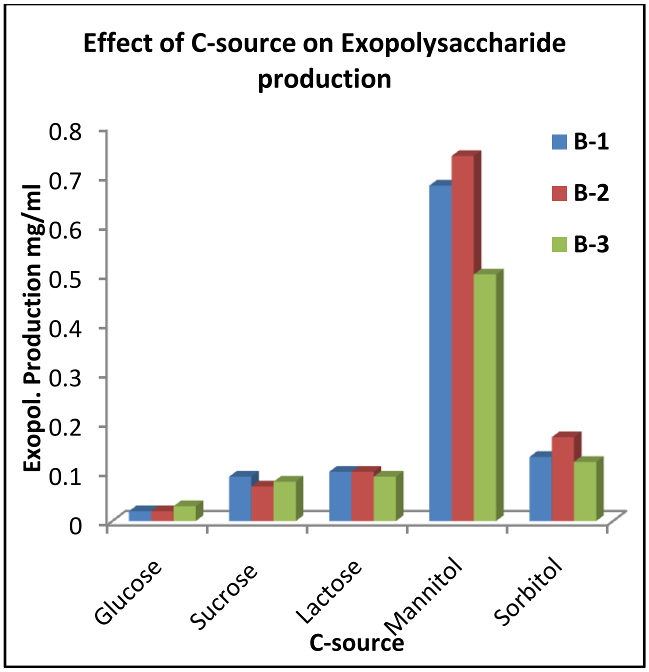

Figure 4 Effect of Carbon Source on exopolysaccharide production

Prime aim of the present agricultural research should be towards assessment of *Bradyrhizobium*response towards various growth governing environmental factors. Since rhizobial population vary in their tolerance to major environmental factors; consequent screening of tolerant strains becomes necessary for getting the maximum output. Indian farmers probably are not taking proper yield from their fields, may be due to excessive use of commercial rhizobial inoculants that are not aquented with the local environmental conditions and an obvious competition from the local strains for space, nutrients and colonization etc. Environmental conditions can also play a crucial role in the determination of activity of *Bradyrhizobium* and these must be considered before the selection of any strain for mass scale cultivation. Present experimental study reveals the importance of local indigenous strains over the commercial ones.

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