

Original Article

Effect of *Rhizobium* on Growth and Biomass Production of Rice

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(Received 20 August 2011; Accepted 03 December 2011)

To characterize twenty *Rhizobium* strains isolated from nodules of lentil, grasspea and chickpea, an experiment was conducted in the Soil Microbiology laboratory of the Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh. The isolates were tested for growth on Congo red Yeast Extract Mannitol (YEM) agar, peptone glucose agar, and YEM agar containing Bromothymol blue (BTB). All the strains except Ls 12 absorbed Congo red dye very weakly. Except Le 2 strains, isolated from lentil showed no growth in peptone glucose agar. All the strains isolated from grasspea and chickpea showed moderate growth on this medium. All the strains were fast-growing and showed acidic reaction on YEM agar medium. Among the strains isolated from lentil, Le 1, Le 2 and Le 4 produced moderate turbidity while Le 3, Le 6, Le 7 and Le 8 produced high turbidity in YEM broth. All the strains isolated from grasspea except Ls 3 and chickpea except Ca 1 produced moderate turbidity in YEM broth medium. Strains Ls 3 and Ca 1 produced high turbidity. To assess the effect of ten of these *Rhizobium* isolates viz. Le 1, Le 4, Le 6, Le 8, Ls 1, Ls 2, Ls 6, Ls 7, Ca 3 and Ca 4 on growth and biomass production of rice, a follow-up experiment was conducted in the same laboratory. Root length of rice was significantly increased over control (without inoculation) due to inoculation with different *Rhizobium* strains. The highest root length (9.63 cm) was obtained by inoculation with strain Ls 6 isolated from lentil. All the *Rhizobium* strains produced significantly higher shoot length, fresh and dry biomass over control. The highest shoot length (16.50 cm), fresh biomass (138.3 mg) and dry biomass (27.75 mg) were also obtained from the strain Ls 6.

Key words: Isolation, Characterization, *Rhizobium*, Rice

Introduction

Rice is unquestionably the main cereal food for the people of Bangladesh. It occupies 74.77% of the total cropped area¹, supplies 92% of cereal food. To maintain self-sufficiency in rice, Bangladesh will have to continue to expand rice production by raising yields at a rate that is at least equal to population growth until the demand for rice has stabilized. Meeting the challenge of increasing rice production will become increasingly difficult in Bangladesh as rice areas is continuously shrinking to meet the growing demand for high-value crops and for urban and industrial development. Therefore, it is necessary to increase the production capacity per unit area which would require high inputs. Another alternative to increase crop production is use of beneficial microorganism, which could lead to more productive and sustainable agriculture without harming the environment. Soil-plant-microbe interaction has got much importance in recent decades. Many types of microorganisms are known to inhabit in soil, especially rhizosphere and play important role in plant growth and development. The most inoculation studies have focused on free living diazotrophs, although a few reports indicate *Rhizobium* can act as plant growth promoting rhizobacteria (PGPR)². The PGPR influence the crop growth and development

by releasing plant growth regulators³ and improving morphological characteristics of inoculated roots⁴, which favored nutrient uptake⁵. The growth promoting effects of rhizobacteria may include phytohormone production⁶, N₂ fixation⁷ and more efficient use of nutrients⁶. Feng *et al.*⁸ reported that inoculation of rhizobia to rice produced significantly higher root and shoot biomass; increased their photosynthetic rate, stomatal conductance, transpiration velocity, water utilization efficiency, and flag leaf area (considered to possess the highest photosynthetic activity); and accumulated higher levels of indole acetic acid and gibberellins growth-regulating phytohormones. No work has so far been conducted in our agro-climatic conditions to observe the effect of *Rhizobium* as PGPR on rice. Keeping these facts in mind the present study was under taken to characterize *Rhizobium* strains isolated from root nodules of different leguminous crops, and also to assess their effectiveness on root and shoot development and biomass production of T. Aman rice.

Materials and Methods

Twenty plant samples with rhizosphere soil were collected from different rice growing areas of Bangladesh to isolate *Rhizobium*

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strains. Samples were collected from Kushtia (AEZ-11), Rajbari (AEZ-11), Faridpur (AEZ-12) and Patuakhali (AEZ-13) districts of Bangladesh. Root with nodules from different leguminous crop viz. lentil (*Lens esculentus*), grass pea (*Lathyrus sativus*) and chickpea (*Cicer arietinum*) and rhizosphere soils were collected for isolation and characterization of *Rhizobium* strains. Samples were selected randomly from farmers' field of the respected AEZs. Nodules separated from roots of different leguminous crops were washed in fresh water and preserved in vials containing silica gel. The collected nodules were surface sterilized. The nodules were then crushed and streaked on Yeast Extract Mannitol (YEM) agar medium contained in petridises with the help of sterile loop. Single colonies of the isolates formed on the medium were preserved for further studies.

A preliminary experiment was conducted in the Soil Microbiology laboratory of the Bangabandhu Sheikh Mujibur Rahman Agricultural University to characterize *Rhizobium* strains isolated from nodules of those crops. The strains were assessed for colony characteristics, growth rate and acid/alkali production in laboratory media with a view to know their basic properties prior to more intensive study on their performance in respect of growth and biomass production of rice. YEM containing the following constituents: K_2HPO_4 (0.5g), $MgSO_4 \cdot 7H_2O$ (0.2g), NaCl (0.1g), $CaCO_3$ (3.0g), $FeCl_3 \cdot 6H_2O$ (0.01g), Mannitol (10g), yeast extract (0.5g), agar (Difco) (10g), Congo red (0.25% solution) (10ml), deionized water to 1 litre was used. The initial pH of the medium was 7.3 which was adjusted to 7.0 by adding 0.1 N HCl solution. The medium was inoculated with the *Rhizobium* strains and incubated for one week. Colonies on plates were observed for their morphology and appearance. The YEM agar medium containing bromothymol blue indicator was used for identification of strains. The reaction of the rhizobial strains on this medium was noted every week up to four weeks. Fast-growing rhizobial strains produce acid in this medium, turning the medium yellow and slow growing rhizobia produce alkali which turns the medium blue⁹.

An *in vitro* experiment was conducted in the same laboratory to study the effect of *Rhizobium* isolates on growth and biomass production of rice. Ten of twenty isolates collected from different leguminous crops were used for the experiment. Among them four isolates viz. Le 1, Le 4, Le 6 and Le 8 were taken from lentil, four isolates viz. Ls 1, Ls 2, Ls 6 and Ls 7 were taken from grasspea and two isolates viz. Ca 3 and Ca 4 were taken from chickpea. Those isolates of rhizobia were grown in YEM broth until turbid. Rice variety BSMRAU Dhan 1 was used as the test crop. The composition of rooting solution used in this experiment was ($g L^{-1}$): KNO_3 (0.505), $Ca(NO_3)_2 \cdot 4H_2O$ (0.335), $MgSO_4 \cdot 7H_2O$ (0.37), $NaNO_3$ (0.17), KCl (1.05×10^{-3}), KH_2PO_4 (0.136), Fe-EDTA (3.55×10^{-3}), $MnSO_4 \cdot 4H_2O$ (0.81×10^{-3}), H_3BO_3 (0.57×10^{-3}), $ZnSO_4 \cdot 7H_2O$ (0.22×10^{-3}), $CuSO_4 \cdot 5H_2O$ (0.04×10^{-3}), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.02×10^{-3}) and distilled water- 1000 ml. Stock solution of KNO_3 , $Ca(NO_3)_2 \cdot 4H_2O$, KH_2PO_4 , Fe-EDTA and all other micro nutrients

($MnSO_4 \cdot 4H_2O$, H_3BO_3 , $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$) were prepared separately¹⁰. The pH of the solution was adjusted to 6.8. Fifty five millilitre of seedling media was taken in each boiling tubes (200 mm × 20 mm). Blotting paper (50 mm long) rolls were placed at the top of the tubes and the mouth of the tubes was closed with cotton. These boiling tubes containing seedling media were autoclaved for 15 minutes at 121°C before use. The blotting paper rolls were then slid down towards the rooting solution until the top of the solution reached just below the top of the blotting paper leaving the bottom of the tubes free from blotting paper. Roots of surface sterilized, germinated seedlings (3 days old) were soaked for 30 minutes in 1 ml inoculant of specific bacteria and it was distributed among four respective test tubes (0.25 ml each of 4 test tubes). After that 1 ml of specific bacterial inoculant was added to each test tube. Inoculation of rhizobial strains were done with approximately 10^8 cells ml^{-1} . With the aid of the flamed and cooled end of a forceps the surface sterilized germinated rice seedlings were transferred to each tube into a slit made on the top of the seedling media. These test-tubes were placed on packing foam by making holes on it and were covered by black paper roles from the surface of the packing foam to the top of the filter paper to exclude horizontal light from the roots. The experiment was conducted in CRD design with four replications. The rice seedlings were allowed to grow for four weeks. Data on root and shoot length, fresh and dry biomass were taken.

Data on various characters of the crop were statistically analyzed to find out the significance of variation resulting from the experimental treatments. For this purpose, analysis of variance was worked out for each character of the crop. The difference between treatment means was compared by Duncan's Multiple Range Test.

Results and Discussion

Isolation and characterization of *Rhizobium* strains from different leguminous crops

Growth on Congo red YEM agar

In general, colonies of rhizobial strains absorbed very little of the Congo red dye. Results presented in Tables 1-3 show that all the strains isolated from lentil (Le 1, Le 2, Le 3, Le 4, Le 6, Le 7 and Le 8), chickpea (Ca 1, Ca 2, Ca 3, Ca 4 and Ca 5) and grasspea (Ls 1, Ls 2, Ls 3, Ls 4, Ls 6, Ls 7 and Ls 8) except Ls 12 absorbed the dye very weakly whereas moderate absorption was done in case of Ls 12. This result corroborates with the findings of Trinick¹¹ and Talukder *et al.*¹² who reported that rhizobia absorbed the dye weakly compared with other bacteria.

Growth on peptone glucose agar

Growth in peptone glucose agar as reported by Vincent¹³ indicates that most of the *Rhizobium* strains grow either poorly or moderately in this medium. In the present study, all the strains except Le 2 isolated from lentil showed no growth whereas Le 2 showed poor growth in this medium. All the strains isolated from grasspea and chickpea showed poor growth in this medium (Tables 1-3).

Colony characteristics on YEM agar

Rhizobium strains are classified as fast and slow growing depending on growth on YEM agar. All the strains isolated from lentil, grasspea and chickpea showed fast growth in YEM agar (Tables 1-3). Strains Le 1, Le 6, Le 7, Le 8 and Ls 4 produced bigger colonies and more gum than the rest of the strains on YEM agar medium. Colonies of all the strains except Le 1, Le 6, Le 7, Le 8 and Ls 4 appeared small and separate with slight gum production. The texture of the gum was sticky. This result resembles with that of Talukder *et al.*¹².

Acid/alkali production in YEM agar medium containing bromothymol blue indicator

The importance of production of acid or alkali by the various rhizobia as reported by Norris¹⁴ has been emphasized when considering *Rhizobium* taxonomy. In this study all the *Rhizobium* strains isolated from lentil, grasspea and chickpea showed acidic reactions throughout their four weeks of growth (Tables 1-3). These strains turned green colour of the medium to yellow.

Growth on YEM broth

Results presented in Tables 1-3 indicate that *Rhizobium* strains showed visible turbidity in YEM broth after 5 to 7 days of incubation. Among the strains isolated from lentil Le 1, Le 2, and Le 4 produced moderate turbidity while Le 3, Le 6, Le 7 and Le 8 produced high turbidity in this medium. All the strains isolated from grasspea except Ls 3 and chickpea except Ca 1 produced moderate turbidity in YEM broth. Strains Ls 3 and Ca 1 produced high turbidity. The differences in texture of extra cellular polysaccharide of the fast- and slow-growing rhizobia have been shown to be due to differences in monosaccharide comparison¹⁵.

Effect of inoculation of *Rhizobium* strains on growth and biomass production of rice

Root length

Results presented in Table 4 revealed that root length of rice was significantly increased over control due to inoculation of different *Rhizobium* strains. The highest root length (9.63 cm) was

Table 1. Characteristics of some *Rhizobium* strains isolated from root nodules of lentil

Strains	Growth on congo red YEM agar (absorption of dye)	Growth on peptone glucose agar	Colony characteristics on YEM agar (Fast/ slow)	Acid/ alkali production in YEM agar medium containing BTB	Growth in YEM broth
Le 1	Slight	Nil	Fast	Acid	Moderate turbidity
Le 2	Slight	Poor	Fast	Acid	Moderate turbidity
Le 3	Slight	Nil	Fast	Acid	High turbidity
Le 4	Slight	Nil	Fast	Acid	Moderate turbidity
Le 6	Slight	Nil	Fast	Acid	High turbidity
Le 7	Slight	Nil	Fast	Acid	High turbidity
Le 8	Slight	Nil	Fast	Acid	High turbidity

Table 2. Characteristics of some *Rhizobium* strains isolated from root nodules of grasspea

Strains	Growth on congo red YEM agar (absorption of dye)	Growth on peptone glucose agar	Colony characteristics on YEM agar (Fast/ slow)	Acid/ alkali production in YEM agar medium containing BTB	Growth in YEM broth
Ls 1	Slight	Poor	Fast	Acid	Moderate turbidity
Ls 2	Slight	Poor	Fast	Acid	Moderate turbidity
Ls 3	Slight	Poor	Fast	Acid	High turbidity
Ls 4	Slight	Poor	Fast	Acid	Moderate turbidity
Ls 6	Slight	Poor	Fast	Acid	Moderate turbidity
Ls 7	Slight	Poor	Fast	Acid	Moderate turbidity
Ls 8	Slight	Poor	Fast	Acid	Moderate turbidity
Ls 12	Moderate	Poor	Fast	Acid	Moderate turbidity

Table 3. Characteristics of some *Rhizobium* strains isolated from root nodules of chickpea

Strains	Growth on congo red YEM agar (absorption of dye)	Growth on peptone glucose agar	Colony characteristics on YEM agar (Fast/ slow)	Acid/ alkali production in YEM agar medium containing BTB	Growth in YEM broth
Ca 1	Slight	Poor	Fast	Acid	High turbidity
Ca 2	Slight	Poor	Fast	Acid	Moderate turbidity
Ca 3	Slight	Poor	Fast	Acid	Moderate turbidity
Ca 4	Slight	Poor	Fast	Acid	Moderate turbidity
Ca 5	Slight	Poor	Fast	Acid	Moderate turbidity

Table 4. Effect of *Rhizobium* inoculation on growth and biomass production of rice

Treatment	Root length (cm)	Shoot length (cm)	Fresh biomass yield (mg)	Dry biomass yield (mg)
Le 1	9.13 ab	15.25 ab	92.50 bc	20.00 b
Le 4	9.00 ab	16.38 a	92.25 bc	21.00 b
Le 6	8.75 ab	15.25 ab	93.25 bc	22.75 ab
Le 8	7.38 bc	15.63 a	100.8 abc	21.50 ab
Ls 1	8.00 abc	16.25 a	101.00 abc	20.50 b
Ls 2	8.13 abc	16.13 a	109.50 abc	21.75 ab
Ls 6	9.63 a	16.50 a	138.30 a	27.75 a
Ls 7	9.38 ab	16.00 a	78.50 bc	22.50 ab
Ca 3	6.25 cd	13.75 ab	107.80 abc	21.00 b
Ca 4	8.63 ab	15.63 a	115.30 ab	18.50 bc
Control	5.50 d	12.13 b	69.50 c	13.25 c
LSD (0.05)	1.746	2.882	38.17	5.782

obtained by strain Ls 6 isolated from lentil while the lowest (5.5 cm) was obtained from control (without inoculation). Strains Le 1, Le 4, Le 6, Ls 7, Ca 4, Ls 1 and Ls 2 were statistically identical with Ls 6 regarding root length. This result corroborates with the findings of Afzal *et al.*¹⁶ who reported highest root weight due to inoculation of *Rhizobium* in wheat.

Shoot length

All the *Rhizobium* strains produced significantly higher shoot length over control (Table 4). The highest shoot length (16.45 cm) was obtained from Ls 6 while the lowest (12.13 cm) was from control. This result also supported the findings of Afzal *et al.*¹⁶ who found higher plant height with *Rizobium* inoculation in wheat.

Fresh biomass yield

Fresh biomass of rice increased significantly over control due to *Rhizobium* strains inoculation (Table 4). The highest fresh biomass (138.3 mg) was obtained from inoculation of Ls 6 while the lowest (69.5 mg) was obtained from control. Strain Le 8, Ls 1, Ls 2 and Ca 3 and Ca 4 were statistically similar with Ls 6 regarding fresh biomass yield (Table 4) which correlated with the findings of Francine *et al.*¹⁷ who reported that inoculation of rhizobia to rice produced significantly higher root and shoot biomass.

Dry biomass yield

Dry biomass yield of rice was increased significantly over control due to different *Rhizobium* strains inoculation (Table 4). The highest dry matter (27.75 mg) was obtained from Ls 6 and the lowest (13.25 mg) was from control (Table 4). Strain Le 6, Le 8, Ls 2 and Ls 7 produced statistically similar dry biomass yield with Ls 6 which was at par with the findings of Solaiman *et al.*¹⁸ who reported that *Rhizobium* inoculants increased dry matter yield in

chickpea which also corroborates with the findings of Francine *et al.*¹⁷ as well.

Conclusion

It may be concluded that all the *Rhizobium* strains isolated from different leguminous crops showed standard reactions in the laboratory media and significantly increased root and shoot growth and biomass production of rice. Among the strains Ls 6 which was isolated from grasspea performed best in all the parameters studied.

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