

Isolation, characterization and symbiotic performance evaluation of soybean (*Glycine max*) nodulating rhizobia from different districts of Bangladesh

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Received 4th December, 2018; Accepted 18th January, 2019

ABSTRACT: Rhizobia can meet up nitrogen requirement of legumes by biological nitrogen fixation through symbiosis. The main objective of this study was to find out the morpho-physiological diversity of indigenous soybean nodulating rhizobia and to find effective rhizobial strains for enhancing better soybean production at different environmental conditions. Fourteen rhizobial strains were isolated and studied their morph-physiological characteristics; evaluated their nodulation and symbiotic efficiency at potted soil. Among the strains, seven were slow growing, four were intermediate to slow growing and three were fast growing. The strains exposed creamy, milky-white and transparent single colonies between 1.17 and 3.67 mm in diameter after 3 to 7 days on growth medium. Twelve strains were alkali producers and only two strains were acid producers. Most of them tolerated both acidic pH (4.0 and 5.0) and alkaline pH (9.0 and 10.0) conditions. All strains tolerated to 1.0% NaCl but none of them survived at 5.0% salt stress. They retained their normal growth up to 37°C but most of them showed growth susceptibility at 45°C and growth was inhibited at 50°C. The strains were inoculated as treatments on soybean plants to compare their performance on growth of soybean along with urea and control treatment. Overall, rhizobial treatments significantly increased nodulation and growth of soybean plants over recommended dose of urea and negative control. Diverse rhizobial strains were associated with soybean root nodules in Bangladesh. The strains SB-27, SB-28 and SB-212 were very effective and produced almost double plant dry matter weight over some other strains, dose of urea and negative control. The physical stress tolerant and highly symbiotic strains deserve to be effective as bio-fertilizer for soybean crop production.

Keywords: Bio-fertilizer, biological nitrogen fixation, nodulation, rhizobia, symbiosis.

INTRODUCTION

Rhizobia are gram negative, non-pathogenic and beneficial soil bacteria. The name rhizobia firstly coined by Frank in 1889, as they form nodules on the root and stem of leguminous plants (Jordan, 1984). More than 98 species of rhizobia have been identified, belonging to 14 genera of α and β -proteobacteria (Berrada and Fikri-Benbrahim, 2014). Rhizobia enriched their value due to some highly important genes which are responsible for nodulation and

biological nitrogen fixation (MacLean et al., 2007). Through a symbiotic relationship, rhizobia fix free atmospheric nitrogen into the usable forms of nitrogenous compounds and supply to the host legume, and the bacteria become benefited by consuming their required carbon sources and other nutrients from the host plants (McNeill and Unkovich, 2007). The plant soybean (*Glycine max* L.) is the world's foremost oil producing legume crop,

deserved the seventh position among world crops by tonnage harvested (Ross-Ibarra et al., 2007). It represents 50% of the global legume crop area and 68% of global legume production (Herridge et al., 2008), having a symbiotic relationship with rhizobia. The plants cannot fix free nitrogen from the environment by their own mechanisms but nitrogen is an integral part of many plants like soybean for making its essential amino acids and other nutrients of the plant (Goormachtig et al., 2004).

Nitrogen fertilization is not only extremely expensive but also contaminates environments such as groundwater by leaching and emission of atmospheric greenhouse gases that contribute to global warming. The screening of rhizobia adapted to local conditions and searching for highly effective strains for use as inoculants represents a promising strategy in overcoming inoculation failure (Chibeba et al., 2017). The efficient rhizobial strains can be used as bio-fertilizer which bears higher efficiency over the chemical nitrogenous fertilizers and contributes to the sustainable agriculture (Anwar, 2010). The main objective of this study was to find out the morpho-physiological diversity of indigenous soybean nodulating rhizobia and to find effective rhizobial strains which can facilitate soybean for better production at different environmental conditions.

MATERIALS AND METHODS

Nodule collection

The soybean root nodules were collected from Chapai-Nawabganj, Lakshmipur, Mymensingh and Noakhali districts of Bangladesh (Figure 1). The fresh, intact and pinkish nodules were collected carefully from field grown healthy soybean plants. Then, the nodules were washed with tap water and dried at room temperature. Later, the nodules were preserved in falcon tubes with silica gel.

Isolation of rhizobia from nodules and their preservation

The juvenile and intact nodules were surface sterilized, firstly with 70% (v/v) ethanol for 45 seconds; secondly with 1% (v/v) sodium hypochlorite (NaOCl) for three minutes, and then nodules were rinsed with distilled water for at least six times perfectly to remove surface disinfectant. The surface sterilized nodules were then crushed in distilled water (50 μ L/nodule) using sterile homogenizer. One loopful of suspension from each crashed nodule was then streaked across CRYEMA (Congo red yeast-extract mannitol agar) medium and incubated at 28°C for 3 to 7 days according to Vincent (1970). After growth, a single colony was re-streaked across CRYEMA medium repeatedly to get the single colony. The selected pure single colony of rhizobia were then inoculated on YEM (Yeast Extract Mannitol) liquid media and kept at 28°C with

90 rpm at shaking incubator for overnight. The regular shaped, uncontaminated single colonies of different nodules were collected and labeled as rhizobial isolates. Finally, each rhizobial isolate was stored at 4°C on agar slant and at -80°C in 50% glycerol for further studies.

Nodulation test at controlled condition by isolated rhizobial strain

The viable soybean seeds were sterilized with 70% ethanol for 45 seconds and then washed gently with 2% NaOCl for 5 minutes. Later, the seeds were rinsed with sterile dH₂O for six times and kept submerged under sterile dH₂O for overnight to moisten the seeds. The moistened seeds were transferred on 1% water agar for germination. After 2 to 4 days, the germinated seeds were transferred to agar slants of 100 mL test tubes and cultivated in controlled condition. Overnight grown pure broth culture of each rhizobial strain was used to inoculate (1mL/plant) 7 to 10 days old soybean plant aseptically. The inoculated plants were grown in the glass house at 28°C with 16 hours day-light and 8 hours dark photoperiod for six weeks and routinely nitrogen free Fåhrens medium (Fåhrens, 1957) was supplied to the plants. The agar slants were wrapped with aluminum foil to keep the root in dark. Three uninoculated plants were grown at the same time as negative controls and as a positive control a previously studied strain of rhizobia, J₁₁ (Anwar et al., 2010), was also inoculated to a plant to confirm the nodulation condition and compare the new results of this study.

Biochemical test

Acid-alkali production by isolated rhizobial strains

To know the acidic or alkaline nature of the isolated rhizobial strains, overnight growth culture (1 μ L) of each isolate was inoculated on YEMA plates containing 10% Bromothymol Blue (BTB) (v/v) and then incubated at 28°C for 3 to 7 days.

Survival capacity of isolated rhizobial strains on acidic and alkaline condition

The YEMA medium with four different pH were prepared, where two of them were acidic (pH 4.0 and pH 5.0) and other two were basic (pH 9.0 and pH 10). The overnight culture of each isolate (1 μ L) was inoculated on the different pH maintained medium and incubated at 28°C for 3 to 7 days.

Salt (NaCl) tolerance test of the isolated rhizobia

To know the salt tolerance of isolated strains, different



Figure 1. Map of Bangladesh: Four pins indicated the locations of nodule collection. (Photo courtesy: www.google.com.bd/maps).

levels of salt (sodium chloride: 0.5, 1, 2, 3, 4 and 5%) were added in YEMA (w/v). Then, pure 1 μ L of overnight culture of each isolate was inoculated on the salt containing media and incubated at 28°C for 3 to 7 days.

Temperature tolerance test of the isolated rhizobia

The temperature tolerance of isolated rhizobial strains were evaluated on YEMA medium by incubating at different temperature. One μ L of overnight growth culture of each isolate was pipetted on the YEMA plates and

incubated at 4°C in refrigerator and 37, 42, 45, 47 and 50°C in dry incubator for 3 to 7 days.

Symbiotic efficiency isolated rhizobia strains at potted soil at field conditions

Soybean seeds were sunk into water overnight for better germination before sowing in potted soil. Viable moistened seeds were sowed in pots (4 seeds per pot) and cultivated properly. Each pot was prepared with 2 kg soil and different fertilizers like Triple super phosphate, Muriate of

potash, Zypsum, Zinc sulphate and boric acid were used following fertilizer recommendation guide (Hassan et al., 2012). Different treatments of rhizobia were prepared with overnight grown pure culture of each rhizobial strain, recommended dose of urea solution, a previously studied strain J₁₁ (Anwar, 2010) as a positive control and negative control (no fertilizer, no nitrogenous fertilizer). Each rhizobial treatment (2 mL culture plant⁻¹) was implied to 5 replications of 10 days aged plants. Soybean plants were cultivated in the natural condition for 6 weeks and harvested. Nodules were collected and counted properly, and then the harvested plants and nodules were dried at 60°C for 72 hours and data were recorded.

Data collection and Statistical analysis

The nodule numbers, nodule dry weight, and plants dry weight were collected carefully and analyzed with the program MSTAT-C developed by Russel (1986). Analysis of variance (ANOVA) for all recorded parameters was performed by F-test. The significance of the differences among the treatment means were evaluated by the least significance difference (LSD) test. After harvesting the plants and nodules, the means and standard errors of nodule numbers and plants' dry weight were analyzed by SPSS program (Version 20.0) with 5% level of significance. The Duncan's Multiple Range Test (DMRT) (Gomez and Gomez, 1984) was performed for comparing the mean values of the different characters.

RESULTS

Nodulation test

Nodulation test is an important test to confirm the host specificity of rhizobia. Although 25 rhizobial strains were isolated from soybean root nodules, only 14 strains (SB-17, SB-20, SB-22, SB-24, SB-27, SB-28, SB-34, SB-35, SB-37, SB-40, SB-42, SB-46, SB-212 and SB-452) were able to induce nodule with soybean root at laboratory conditions along with positive control J₁₁ but none of the negative control showed any nodule with plants' root. The nodules number varied from 10 to 25 per plant and plants dry mass varied from 270 to 530 mg, where the negative control yielded only 140 mg (Table 1 and Figure 2).

Morphological features of nodule forming rhizobial strains

Among 25 isolated rhizobial strains, 14 soybean nodulating strains were selected and characterized (viz., SB-17, SB-20, SB-22, SB-24, SB-27, SB-28, SB-34, SB-35, SB-37, SB-40, SB-42, SB-46, SB-212 and SB-452).

The isolated rhizobial strains showed different morphological features. Based on their growth time, they were categorized into three groups, seven strains of them were slow growers exposed average single colony size 1.17 to 2.00 mm in diameter after seven days; four strains were moderate to slow growers showed 2.00 to 2.33 mm colony after five days; and three strains were fast growers exposed 3.67 to 4.00 mm colony after three days. All single colonies of the strains were circular and convex in shape. The majority strains (64.29%) exhibited creamy color, 21.43% strains were watery transparent and 14.29% strains were milky in color on CRYEMA plates (Table 2, Figure 3).

Biochemical tests

Most of the strains (12 out of 14) were alkali producers; showed blue color on 10% BTB containing YEMA plates. Only two strains SB-42 and SB-452 were acid producers and exposed yellow color in the same test (Table 3).

Isolated rhizobial strains were capable to grow at alkaline pH (9.0 to 10.0) conditions except for the strain SB-46. Most of the strains (10 of 14) could survive at pH 5.0 (Table 3). Only two acid producing strains SB-42 and SB-452 were survived at the pH 4.0. All collected strains tolerated 0.5% salt but five strains of them showed poor growth at 1.0% and became inhibited at 2.0% NaCl. Nonetheless, about 57.14% strains (8 of 14) tolerated up to 3.0% salt and only the strain SB-452 retained its growth up to 4.0% and none of them sustained at 5.0% NaCl (Table 4). In laboratory, the conditions rhizobia grow well on YEMA medium at 28°C but all studied strains were availed to grow at 37°C. However, at least five strains (SB-20, SB-22, SB-34, SB-40 and SB-42) could tolerate 42°C and the strains SB-34, SB-42, SB-212 and SB-452 retained their poor growth up to 45°C and none could tolerate at 47°C. In addition, 4°C inhibited the growth of all rhizobial strains used in present study (Table 4).

The effect of rhizobial treatments on growth of soybean plants

The soybean plants were harvested after six weeks of sowing. The nodule number, dry mass, and plant dry weight were counted and analyzed (Table 5).

Nodule number

The rhizobial strains induced nodules with the root of soybean plants with a significant variation. The strains of SB-27 and SB-28 produced the highest nodule number (60 nodules per plant). The following numbers of nodules were produced by significantly same strains SB-24 SB-34, SB-



Figure 2. Re-nodulation test of rhizobial strains: Picture A,B,C represented three re-nodulated plants which were treated with overnight culture of rhizobia and D represented a negatively controlled plant which was not treated with rhizobia

35 and SB-40 (43, 43, 42 and 43 nodules per plant respectively) which were followed by positive control J₁₁. Nevertheless, the negative control yielded the least nodule number (10 nodules per plant).

Nodule dry matter weight

The nodule dry mass was produced by different treatments ranged between 102.0 mg and 376.0 mg per plant. Like nodule number, the maximum nodule dry matter weight

was found in the strain SB-28 (376.0 mg/plant) and statistically similar result was produced by the strains SB-37 (338.0 mg/plant), SB-27 (324.0 mg/plant), SB-35 (298.0 mg/plant) and SB-24 (288.0 mg/plant). The control treatment yielded the minimum dry weight of nodule (102 mg/plant).

Plant dry mass

The different treatments of rhizobial strains had significant

Table 1. Colony morphology of the isolated soybean rhizobia.

Strainname	Time for single colony formation	Colony size (mm)	Color on CRYEMA
SB-17	5 Day	2.00	Creamy
SB-20	3 Day	3.67	Transparent
SB-22	7 Day	1.17	Creamy
SB-24	7 Day	2.00	Transparent
SB-27	7 Day	2.00	Creamy
SB-28	7 Day	1.67	Creamy
SB-34	5 Day	2.00	Creamy
SB-35	7 Day	1.67	Creamy
SB-37	7 Day	1.67	Creamy
SB-40	7 Day	2.00	Creamy
SB-42	5 Day	2.33	Milky
SB-46	5 Day	2.00	Creamy
SB-212	3 Day	4.00	Transparent
SB-452	3 Day	3.67	Milky
Mean		2.28	
Standard Error		0.23	

5% Level (SPSS Statistics 20).

Table 2. The profile of *in vitro* nodulation of soybean rhizobia.

No.	Strain No.	Nodule numbers	Plant Dry mass (mg)
SB-17	SB-17	10	270
SB-20	SB-20	25	470
SB-22	SB-22	12	340
SB-24	SB-24	14	390
SB-27	SB-27	21	310
SB-28	SB-28	15	260
SB-34	SB-34	22	350
SB-35	SB-35	20	250
SB-37	SB-37	15	440
SB-40	SB-40	18	320
SB-42	SB-42	15	290
SB-46	SB-46	23	350
SB-212	SB-212	21	520
SB-452	SB-452	16	380
J11 (Positive Control)		20	530
Negative Control *		0	140
Mean		6.69	350.63
Standard Error		1.53	25.91

5% Level (SPSS Statistics 20).

influence on dry matter yield of soybean plants. The highest yield (4676.0 mg/plant) was produced by the strain SB-212 which was significantly similar with strains SB-28 (4542.0 mg/plant), SB-27 (4446.0 mg/plant) and SB-35 (4212.0 mg/plant). The control yielded the lowest plant growth (2334.0 mg/plant) which was followed by the treatments of urea and positive control J₁₁.

DISCUSSION

Present study found a slow-growing strain SB-22 exposed the minimum (average 1.17 mm) single colony with creamy color after 7 days but the fast-growing strain SB-212 evolved the maximum (4.00 mm) watery transparent single colony after 3 days of inoculation. Colony size

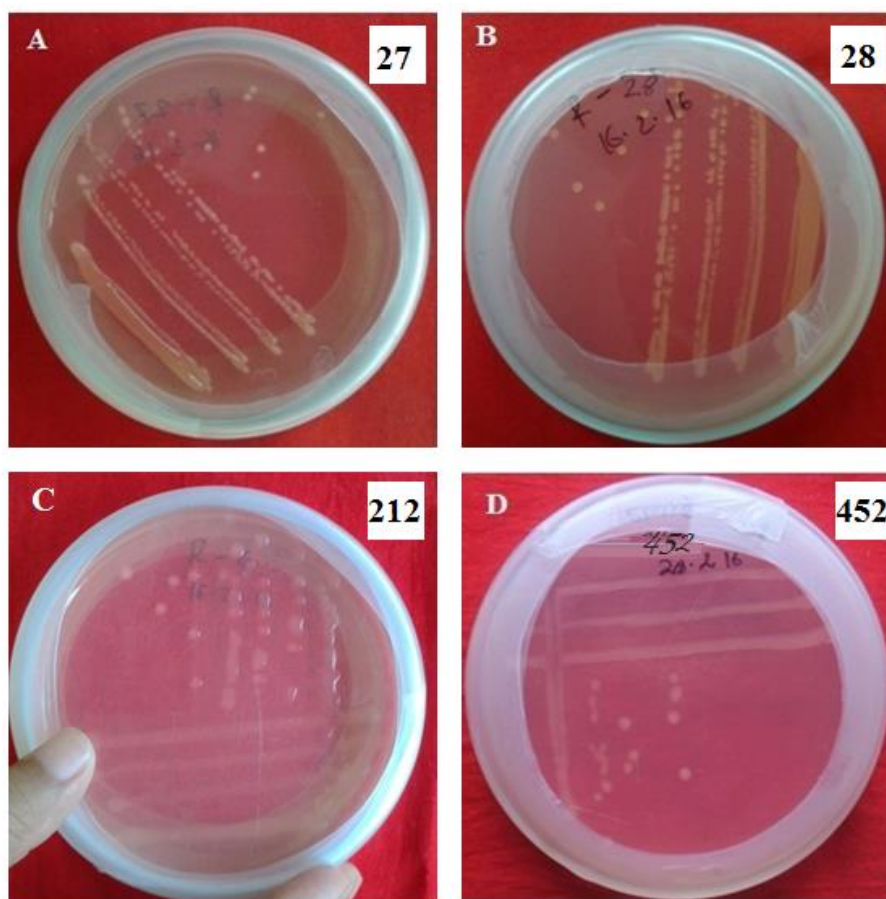


Figure 3. Colony morphology of isolated rhizobia: Plate A and B represented slow growing strains SB-27 and SB-28 with creamy color after seven days; C represented fast growing strain SB-212 with watery transparent color after three days; and D represented another fast growing strain SB-452 with milky color.

Table 3. Acid-alkali tolerance of soybean rhizobia and response at BTB.

Strain name	pH				Color on BTB
	4.0	5.0	9.0	10.0	
SB-17	-	+	+	+	Blue
SB-20	-	±	++	++	Blue
SB-22	-	±	+	+	Blue
SB-24	-	+	+	+	Blue
SB-27	-	-	+	+	Blue
SB-28	-	-	+	+	Blue
SB-34	-	-	+	+	Blue
SB-35	-	-	+	+	Blue
SB-37	-	+	+	+	Blue
SB-40	-	-	+	+	Blue
SB-42	+	++	++	+	Yellow
SB-46	-	+	±	-	Blue
SB-212	-	±	++	++	Blue
SB-452	+	+	+	+	Yellow

[Note: '±' indicated poor growth, '+' indicated normal growth, '++' indicated over growth and '-' indicated no growth].

Table 4. Salt and temperature tolerance profiles of soybean rhizobia.

Strain name	Salt (NaCl)						Temperature				
	0.5%	1%	2%	3%	4%	5%	4°C	37°C	42°C	45°C	47°C
SB-17	+	+	±	-	-	-	-	+	-	-	-
SB-20	++	++	+	+	-	-	-	+	+	-	-
SB-22	+	+	+	±	-	-	-	+	+	-	-
SB-24	+	±	-	-	-	-	-	+	-	-	-
SB-27	+	±	-	-	-	-	-	+	-	-	-
SB-28	+	±	-	-	-	-	-	+	-	-	-
SB-34	+	+	±	-	-	-	-	+	+	±	-
SB-35	+	±	-	-	-	-	-	+	-	-	-
SB-37	+	±	-	-	-	-	-	+	-	-	-
SB-40	+	+	+	+	-	-	-	+	+	-	-
SB-42	+	+	+	-	-	-	-	+	+	±	-
SB-46	+	+	+	±	-	-	-	+	-	-	-
SB-212	++	++	++	+	-	-	-	+	+	±	-
SB-452	++	++	++	+	+	-	-	+	+	±	-

[Note: '±' indicated poor growth, '+' indicated normal growth, '++' indicated over growth and '-' indicated no growth].

Table 5. Statistical analysis of the data of rhizobial strains and soybean plants.

Strains	Average nodule No./plant	Nodule dry mass/plant (mg)	Plant dry mass/plant (mg)
SB-17	29.2 ^{cd}	256.0 ^{def}	3514.0 ^d
SB-22	27.2 ^{cd}	206.0 ^{fg}	3480.0 ^d
SB-24	43.2 ^b	288.0 ^{bcd}	4130.0 ^c
SB-27	60.6 ^a	324.0 ^{bc}	4446.0 ^{abc}
SB-28	60.6 ^a	376.0 ^a	4542.0 ^{ab}
SB-34	42.8 ^b	248.0 ^{def}	3318.0 ^d
SB-35	41.6 ^b	298.0 ^{bcd}	4212.0 ^{bc}
SB-37	31.0 ^c	338.0 ^{ab}	3530.0 ^d
SB-40	43.0 ^b	238.0 ^{ef}	3514.0 ^d
SB-42	16.4 ^{ef}	234.0 ^{ef}	3398.0 ^d
SB-46	14.4 ^{ef}	136.0 ^{hi}	2336.0 ^e
SB-20	24.0 ^d	238.0 ^{ef}	3424.0 ^d
SB-212	31.6 ^c	272.0 ^{cde}	4676.0 ^a
SB-452	17.0 ^e	164.0 ^{gh}	3484.0 ^d
J ₁₁ (Positive Con.)	43.2 ^b	172.0 ^{gh}	2344.0 ^e
Urea	25.2 ^{cd}	108.0 ⁱ	2462.0 ^e
Negative Control	10.0 ^f	102.0 ⁱ	2334.0 ^e
Standard Error	1.698	13.00	95.51
LSD 0.01	6.363	48.71	357.9
Level of significant	**	**	**

LSD= Least Significant Difference, **= Statistically significant.

depends on the generation time of rhizobia and their mucous production ability. Thus, present study got the maximum colony size from the first growing rhizobia. Similar results were also observed by Ansari and Rao (2014) and found white opaque colonies after 7 to 10 days from slow growing rhizobial strains; pink colonies after seven days from moderate slow growing strains and fast growing strains with 4 to 8 mm watery translucent colonies after 2 to 3 days. On the basis of growth, rhizobia were

categorized into two groups: first and slow grower but current study got three groups from soybean nodules from Bangladesh. Similar results also observed by Vincent (1974). Singh et al. (2013) mentioned that the mean generation time of fast-growing rhizobia is between 2 and 4 hours, and 6 hours for slow growing rhizobia.

Although rhizobia grow well on YEMA medium at 28°C but all studied strains were availed to grow at 37°C and five strains (SB-20, SB-22, SB-34, SB-40, and SB-42)

could tolerate 42°C. Even the strains SB-34, SB-42, SB-212, and SB-452 retained their poor growth up to 45°C and none could tolerate at 47°C. In addition, 4°C inhibited the growth of all the rhizobial strains of the study. Ledgard and Steele (1992) and Berrada et al. (2012) also reported that rhizobial strains grew normally between 20 and 37°C temperature, some of them tolerated from 35 to 40°C even the highest 50°C but not over 55°C. This may be happened due to the influence of local environment because generally bacteria can cope up with their ecosystem and become habituated with various environmental responses. However, some strains from current study also tolerated temperature between 40°C and 45°C, which might be for their increased expression of some heat shock proteins, as Michiels et al. (1994) detected 14 heat shock proteins in rhizobial strains, grew within 40 to 45°C. The stress tolerance of rhizobia may depend on indigenous atmosphere because Abo-Aba et al. (2015) isolated three *R. leguminosarum* strains from drought-prone areas from Saudi Arabia those were competent to grow at a wide range of temperature from 30 to 60°C.

Bromothymol blue (BTB) functioned as a weak acid solution and changed its color after reacting with bacterial acidic or basic mucous. Present study identified that the fast growing strain SB-42 and SB-452 were acid producers that is why they exposed yellow color and the rest strains were alkali producers and showed blue color at BTB. Similar result was observed by Sharma et al. (2010) and reported that acid producing rhizobia exposed yellow color and alkaline mucous exposed blue color. Dowdle and Bohlool (1985) and Chen et al. (2000) also observed that the fast growing acid producing strains showed yellow and alkali producing strains showed blue color at BTB. Consequently, the fast growing and acid producing strains SB-42 and SB-452 were competent to grow at acidic pH 4.0 and 5.0. Generally, rhizobia can tolerate a wide range of pH, for instance, Fujihara and Yoneyama (1993), Correa and Barneix (1997) and Reza et al. (2001) reported that *Mesorhizobium sp.* and *Rhizobium fredii* tolerated pH from 4.0 to 10.0. On the other hand, the alkali producing strains tolerated pH 9.0 and 10.0. It is possible because rhizobia have the capacity of tolerance a wide range of pH, from 4.0 to 10.0 (Correa and Barneix, 1997; Sharma et al. 2010; Rahman et al., 2018).

Normally, rhizobia have both of resistance and sensitivity at salt, for instance, Abo-Aba et al. (2015) described that *Rhizobium leguminosarum* strains tolerated salt ranged from 0.5 to 4% NaCl. Moreover, the fast growing rhizobia were more salt tolerant than slow growing *Bradyrhizobia* (Hua et al., 1982; Zahran, 1999). Several genes have been identified in rhizobia which produce response to adapt salinity (Wei, 2004).

The pot experiment conducted at field conditions showed that the strains SB-27 and SB-28 produced almost double growth of soybean plants over urea and control treatment. However, the strain SB-212 yielded the

maximum plant growth according to dry mass though its nodule number and nodule mass were not the highest. Similar results also observed by Chibeba et al. (2017), Habibi et al. (2017) and Kapembwa et al. (2016). In the present study, the nodule dry mass of each plant was not evenly proportioned with their respective nodule number because the nodule size was not similar. This is a very normal phenomenon of rhizobia because it can form different types and size of nodules on legume root system (Ngakou et al., 2009). Similarly, Anwar et al. (2010) observed a large variation by the treatments of different rhizobial strains on soybean plants. Generally, rhizobia are considered as beneficial bacteria which enhance growth and yield of respective host plant. However, the present study found a few numbers of strains functioned efficiently and most of the strains had diverse efficiency. This may happen due to selfish behavior of strains. Ratcliff et al. (2008) explained that there are some selfish rhizobia which can produce nodules and take a sufficient amount nutrient from the host but do not give back better nitrogen to their host and thus, negatively affect the plants growth.

Conclusion

Locally adapted soybean nodulating rhizobial strains had been isolated and characterized from different districts of Bangladesh. Diversity was observed among most of the rhizobial strains based on different characters including colony morphology, growth time; acid-alkali resistance salt and temperature tolerance. Importantly, three strains (SB-27, SB-28 and SB-212) showed the highest symbiotic potentiality and consequently yielded better plant growth than other strains/treatments. This study increased further collection of effective rhizobial strains for inoculants production to enhance soybean growth. The study suggests that the inoculation with locally well adapted strains could be a better avenue for increasing soybean growth and yield in Bangladesh. Further field trails are needed to confirm the findings in this research.

CONFLICTS OF INTEREST

We do not have any conflicts of interest.

ACKNOWLEDGMENT

We eagerly acknowledge to Dr. K.M. Nasiruddin for his valuable instruction and Md. Kamruzaman for reading our manuscript. We are also grateful to the Ministry of Science and Technology, Bangladesh (no.39.009.002.01.00.057. 2015-2016/922/BS168) and the authority of Bangladesh Institute of Nuclear Agriculture for their support to conduct this research.

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