

## Physiological Properties of Soybean Rhizobia Strains in Relation to Their Nitrogen Fixing Ability : Polysaccharide Production and Nitrate Reduction

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### Abstract

Nine different soybean rhizobia strains were characterized under laboratory condition on the basis of their polysaccharide production and nitrate reduction. The correlation of these physiological properties with their nitrogen fixing ability was established through a field experiment conducted with ten treatments including the nine test rhizobial strains and one un-inoculated control in soybean cv PK-416 under rainfed condition. The highest amount of polysaccharide (246.07 mg/ml) was produced by the strain SB-243 in sucrose as carbon source, whereas, the lowest amount (129.67 mg/ml) was formed by SB-9 in glucose as carbon source. Similarly, maximum amount of nitrite accumulation (9.28 mM) was recorded in case of the strain SB-12 which was at par with the strain SB-16 (9.107 mM) and the minimum amount (7.36 mM) was accumulated by the strain SB-6 during the period of 120 hours of growth. On the basis of efficiency ratio at 60 DAS the strains SB-12, SB-16, SB-102 and SB-271 were classified as efficient, the strains SB-6, SB-9, SB-120 and SB-294 as moderately efficient and the strain SB-243 was found inefficient. Only the nitrate reduction by the strains measured as nitrite accumulation in broth at the end of 120 hours of growth which was positively correlated with the efficiency ratio.

**Key words :** Soybean rhizobia, Polysaccharide production, Nitrate reduction, Efficiency ratio, Physiological properties.

Soybean is characterized to be less responsive to the application of fertilizer N than the symbiotically fixed source, thus emphasizing the need for establishment of efficient symbiotic N<sub>2</sub>-fixing systems in the crop (Mengel et al. 1987). But the usual procedure for screening the efficient strains by inoculation trials is time consuming, expensive and subject to vagaries of nature. Therefore, attempts have been made from time to time by different workers to find out the relationship of N<sub>2</sub>-fixing efficiency of rhizobial strains in different crops with their easily determinable physiological and biochemical characteristics, namely acid production, ability to metabolize nitrates, carbohydrate utilization pattern and polysaccharide production. Keeping this in view, an attempt was made in the present investigation to characterize some soybean rhizobial strains isolated from Nainital tarai soils and other parts of the country on the basis of their physiological properties like polysaccharide production and nitrate reduction and to establish the correlations of the different physiological characteristics of the strains, that may exist with their N<sub>2</sub>-fixa-

tion efficiency.

### Methods

Nine different strains of soybean rhizobia including the three isolated from Nainital tarai soil were procured from the All India Coordinated Research Project on Soybean (ICAR), Pantnagar center and used in the present study.

All cultures were grown and maintained on yeast extract mannitol agar (YEMA) medium. For liquid medium, agar was deleted from the composition of YEMA medium. Strict aseptic conditions were maintained during preparation of media and growth period. The pH of the growth media prior to inoculation was maintained at 7.0. The incubation temperature for all cultures was maintained at 30 ± 1 C throughout the growth period of the organisms. Broth cultures were aerated by placing the flasks on rotary shaker at 140—160 rpm throughout the growth period.

The growth rate of the strains was studied up to 120 hours by periodical serial dilution and plate count method. The mean generation time of the test strains was calculated using the following formula :

$$G = \frac{0.301 \times t}{\log_{10} N - \log_{10} N_0}$$

Where, G = Mean generation time (hours), t = time interval (hours),  $N_0$  = Initial population size (number/ml), N = Final population size after time t (number/ml).

Each strain was tested for polysaccharide production in three carbon sources, namely mannitol, glucose and sucrose. The organisms were grown in 125 ml of liquid medium containing the carbon source under study in 500 ml flasks on a rotary shaker at 30 C for five days. After removal of cells by centrifugation, the culture supernatant was mixed with two volumes of acetone in cold. The crude polysaccharide-containing preparation that developed was collected by centrifugation at 3,500 rpm for 30 minutes. Weights of the preparations were determined after drying at 80 C. The results were reported as milligram of polysaccharide per liter of medium.

To study nitrate reduction by different isolates, nitrite accumulation in the growth medium was measured. For this 1.01 g  $KNO_3$  was added per liter of YEM broth to give a final nitrate concentration of 10 mM per liter. Samples were drawn from the growing cultures at different time intervals and nitrite content was determined in the samples by modified Shinn tech-

nique (Tanner and Anderson 1964).

A field experiment was conducted at the Crop Research Center of the Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, with ten treatments including the nine test rhizobial strains and one uninoculated control in soybean cv PK-416 under rainfed soil condition during *khari* season. Seeds of individual plots were inoculated with freshly prepared carrier based culture of the test strains. The plant samples collected at 60 DAS were analyzed for nitrogen content following modified Kjeldahl method. The efficiency ratio of the strains was calculated as the ratio of N-uptake by shoot in inoculated to that in un-inoculated control plant (Pant and Katiyar 1994).

## Results and Discussion

Table 1 shows that there was a significant variation in extracellular polysaccharide production in different carbon sources irrespective of strain and also among strains irrespective of carbon source. Maximum amount of polysaccharide (224.97 mg/liter) was formed by the strain SB-243 considering the mean of the amounts produced with the three different carbon sources. However, irrespective of the strains, the highest amount of polysaccharide was produced in sucrose and the minimum in glucose as carbon source. The interaction of carbon source and strain was also

**Table 1.** Production of extracellular polysaccharide by the soybean rhizobial strains under different carbon sources.

Strains	Mean generation time (hours)	Amount of polysaccharides (mg/l)			Mean
		Mannitol	Glucose	Sucrose	
1. SB-6	2.43	150.93	147.20	160.20	152.78
2. SB-12	3.02	168.70	166.00	169.03	167.91
3. SB-16	5.65	178.67	179.80	188.17	182.21
4. SB-120	7.30	209.63	200.03	219.90	209.85
5. SB-294	6.50	202.77	197.10	196.97	198.94
6. SB-102	5.61	203.07	209.43	208.43	206.98
7. SB-271	7.12	195.27	183.77	227.83	202.29
8. SB-243	2.85	233.27	195.57	246.07	224.97
9. SB-9	3.68	138.60	129.67	166.73	145.00
Mean	-	186.77	178.73	198.15	-
			SE ±	CD (P = 0.05)	
	Strains		2.13	6.03	
	Carbon source		1.23	3.48	
	Strain × carbon source		3.68	10.44	

**Table 2.** Reduction of nitrate by the soybean rhizobial strains expressed as mM nitrite accumulated per 10 mM nitrate at different time intervals.

Strain	Nitrite accumulation (mM/NO <sub>2</sub> )						Mean
	Time intervals (hours)						
	24	48	72	96	120	144	
1. SB-6	1.325	2.429	4.257	6.776	7.364	7.002	4.859
2. SB-12	1.349	2.487	4.423	7.010	9.283	9.078	5.605
3. SB-16	1.481	2.578	3.777	8.202	9.107	8.576	5.620
4. SB-120	1.608	2.713	4.123	7.929	8.918	8.446	5.623
5. SB-294	1.314	1.473	3.135	5.478	7.351	7.976	4.454
6. SB-102	1.419	2.530	4.541	7.101	8.685	8.229	5.418
7. SB-271	1.351	2.599	4.894	7.841	8.987	8.764	5.739
8. SB-243	1.467	2.417	4.470	6.218	7.696	8.295	5.094
9. SB-9	1.434	2.519	4.649	7.251	7.798	7.224	5.146
Mean	1.416	2.416	4.252	7.089	8.354	8.177	
		SE ±	CD ( <i>P</i> = 0.05)				
	Strains	0.041	0.116				
	Time interval	0.037	0.094				
	Strain × time interval	0.101	0.283				

found to influence significantly the amount of polysaccharide produced. The highest amount of polysaccharide (246.07 mg/liter) was produced by the strain SB-243 in sucrose as carbon source, whereas the lowest amount of polysaccharide (129.67 mg/liter) was formed by SB-9 in glucose as carbon source.

Obviously the data (Table 1) show that the strains with lower mean generation time i.e. fast growing strains, in general, produced fewer amounts of polysaccharides in all the carbon sources than slow

growing. The low amount of polysaccharide production by fast-growers may be attributed to the more effective utilization of carbohydrates by those strains than the slow-growing strains. The variation in the amount of polysaccharides formed in different carbon sources indicated that soybean rhizobia examined in this study were able to utilize glucose most effectively followed by mannitol and sucrose as the carbon source.

The accumulation of nitrite increased continuously up to 120 hours and then started decreasing in all the strains except SB-294 and SB-243 where the nitrite accumulation continued till the end of 144 hours of observation (Table 2). Maximum amount of nitrite accumulation (9.28 mM) was recorded in the strain SB-12 which was at par with the strain SB-16 (9.11

**Table 3.** Response of soybean cv PK-416 to inoculation with rhizobial strains : Effect on N uptake by shoot and efficiency ratio of strains at 60 DAS (mean of two years). \*Efficiency ratio = N-uptake by inoculated plant/N-uptake by uninoculated plant.

Strains	N- uptake by shoot (mg plant <sup>-1</sup> )	Efficiency ratio of strains*
1 SB-6	360.6	1.35
2 SB-12	558	2.09
3 SB-16	486.6	1.83
4 SB-120	411.6	1.54
5 SB-294	380.8	1.43
6 SB-102	523.8	1.97
7 SB-271	442.8	1.66
8 SB-243	300.1	1.13
9 SB-9	417.3	1.57
10 control	266.3	-
Mean	414.8	
SE ±	47.1	
CD (0.05)	140	

**Table 4.** Correlation of physiological properties of the rhizobial strains with efficiency ratio at 60 DAS. \*Significant at 5% probability.

Physiological properties	Correlation coefficients ( <i>r</i> )
1 Nitrite accumulation at the end of 120 hours of growth	0.776*
2 Polysaccharide production in 120 hrs with mannitol as carbon source	-0.267
3 Polysaccharide production in 120 hrs with glucose as carbon source	0.042
4 Polysaccharide production in 120 hrs with sucrose as carbon source	-0.331

mM) and the minimum amount (7.36 mM) was accumulated by the strain SB-6 during the same period of growth. The initial increase in nitrite level in the growth medium up to certain extent and then decrease might be due to the inability of the rhizobial strains to metabolize nitrite in presence of high concentrations of nitrate. Similar observation of inability of rhizobia to utilize nitrite in the presence of nitrate or ammonia was reported earlier in *R. trifolii* strains (Pant and Gangwar 1984).

The relative effectiveness of the strains to fix atmospheric nitrogen was assessed indirectly by computing the efficiency ratio of the strains at 60 days of plant growth. In fact, this stage coincided with the early pod filling stage of the test cultivar of soybean at which  $N_2$  fixation was reported to reach the peak (George and Singleton 1992). The efficiency ratio of the strains ranged from the lowest value of 1.13 in SB-243 to the highest value of 2.09 in SB-12 (Table 3). The strains with efficiency ratio at 60 DAS more than 1.60, within 1.30 to 1.60 and less than 1.30 were classified as efficient, moderately efficient and inefficient, respectively (Pant and Katiyar 1994). Hence the strains SB-12, SB-102, SB-16 and SB-271 were classified as efficient, the strains SB-9, SB-120, SB-294 and SB-6 as moderately efficient and the strain SB-243 was found inefficient.

The amount of polysaccharide produced by the strains within 120 hours in the growth medium containing mannitol/glucose/sucrose as carbon source was not significantly correlated with efficiency ratio. On the contrary, nitrate reduction by the strains measured as nitrite accumulation in the broth at the end of 120 hours of growth was observed to have a significantly positive correlation with the efficiency ratio (Table 4). The higher nitrate reduction by the more efficient strains might be due to the presence of different types of nitrate reductases in the bacteroids which were reported to be related to symbiotic  $N_2$ -fixation to different degrees (Lucinski et al. 2002). It

was also reported that nitrate reductases and nitrogenase share a Fe-Mo cofactor in the bacteroids (Evans and Russell 1971) and hence a positive correlation between  $N_2$ -fixation efficiency and nitrate reduction was possible.

### Conclusion

Thus, it could be concluded that the ability of soybean rhizobia to reduce nitrate could be used as a test as of effectiveness of different strains whereas the differential degree of exo-polysaccharide production by the strains which was not significantly correlated with efficiency ratio could be helpful in characterization of different strains.

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