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Physiological Characteristics of Fast Growing *Rhizobium* sp. of *Sesbania*

S. GOPALAKRISHNAN AND H. R. JEEVANAND

Agronomy Division, ICRISAT, Pattancheru-502 324 (A. P.), India

ABSTRACT

A study was conducted to investigate the effective and efficient isolates of *Rhizobium* of *Sesbania bispinosa* from rice-growing areas of Kamarajan district, Tamil Nadu. From about 661 samples, *Rhizobium* was isolated and they were tested for nitrogenase activity by acetylene reduction technique. Out of these the most highly positive isolate (on the basis of acetylene reduction) number DMB R001 was selected. This strain DMB R001 was studied in detail for utilization of various carbon sources, antibiotic resistance pattern and various climatic factors including different pH, temperature and salinity. The observations indicated that the strain DMB R001 could grow well in pH range of 4-11, salinity range of 0–600 millimhos (3% of NaCl) and temperature range of 28-44°C. It was resistant to antibiotics Ch^r, K^r, P^r, A^r and Nal^r. It also grew well at different carbon substitute and generation time varying from 1.9–3.2 h depending on carbon sources. These observations indicated that the strain which we isolated had wide range of adaptability.

Key words : Characteristics, *Rhizobium* sp., *Sesbania bispinosa*, strain DMB R001

INTRODUCTION

In recent years, there has been an increasing awareness of the importance of the process of biological N_2 fixation due to the fact that the production of nitrogenous fertilizers by chemical means is an energy demanding process, cost is more and causes environmental pollution. *Rhizobium* strains are able to form symbiotically nitrogen fixing structures-the nodules-with more than 1100 species of leguminous plants and exhibit specificity in association with their host plants (Vincent, 1974). According to quantitative analysis, leguminous plants in symbiosis with rhizobia can fix nitrogen in a range of 50–300 kg per hectare per year (Phillips, 1980). According to recent classification, root nodule bacteria are split into two genera (Jordan, 1984). The genus *Rhizobium* includes all the fast growers with generation time of 2–4 h and the genus *Bradyrhizobium* includes slow growers with generation time of 6-13 h. The success of selected *Rhizobium* strains in increasing N_2 fixation in legumes has drawn attention because of the increasing need for few long term soil fertility and ecological sustainability. This interest culminated in the identification

of unknown weed legumes that have potential as green manure in rice (Rinaudo *et al.*, 1983). Their productivity depends upon their N_2 fixing potential under unfavourable conditions. However, only very little is known regarding their adaptability to various environmental and physiological conditions (Bordeleau and Prevost, 1994). Such studies in *Rhizobium* of *S. bispinosa* were uninvestigated elsewhere. So, the present study was aimed at the isolation and characterisation of *Rhizobium* from the root nodules of *S. bispinosa*, a fast growing annual legume generally found in the water-logged soils of India.

MATERIALS AND METHODS

About 661 samples of the same legume weeds were collected from different parts of Kamarajan district. The legume plant was identified as *S. bispinosa* with the help of experts from southern branch of Botanical Survey of India, Coimbatore, Tamil Nadu. The efficacy of the collected nodules was determined by measuring the nitrogenase activity (Acetylene reduction activity, ARA) and the Rhizobia was then isolated from the nodules of YEMA slants (Vincent, 1979). Among the isolates, the most efficient one was determined on the basis of ARA and it (DMB R001) was selected for further studies. Utilization of carbon was studied on an enriched medium containing yeast extract with different carbon sources at 1% concentration. Carbon free controls were also used for evaluating the growth. Carbon sources such as dextrose, galactose, maltose, lactose, sucrose, cellobiose, mannitol, glycerol, starch and citrate were sterilized alongwith the medium. One ml of log phase growing cell suspension (10^7 cell ml^{-1}) was added to a 100 ml of YEM broth in 250 ml Erlenmeyer flask and kept on a rotary shaker (150 rev/min) at 32 ± 2°C. Samples were withdrawn at an interval of 4 h and growth was followed for 24 h from zero time in a Spectronic 20 with the absorbance at 600 nm and the generation time was calculated.

Intrinsic antibiotic resistance was determined by observing the growth on antibiotic plates after incubation at 32 ± 2°C for six days. Antibiotic resistance was also determined under liquid cultural conditions. Each tube containing 5 ml of sterile YEM broth and different concentrations of antimicrobial agents like ampicillin, penicillin, nalidixic acid, kanamycin, chloramphenicol, streptomycin, gentamycin and tetracycline and the concentration was determined. The temperature tolerance of rhizobia was determined by inoculating 100 ml of YEM broth in 250 ml Erlenmeyer flasks and incubating at different temperatures viz., 36, 42, 44 and 50°C under stationary conditions. Growth was followed by measuring the absorbance at 600 nm on a Spectronic 20 and the generation time was calculated. Salinity of the medium (YEM broth) was adjusted from 100-1000 mmhos NaCl in steps of 100 mmhos and the medium was inoculated and incubated at 32 ± 2°C for 48 h and

Table 1. Influence of various pH on the *Rbizobium* of *Sesbania* DMB R001

Medium	pH																
	3	3.5	4	4.5	5	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11
Yeast extract mannitol medium	—	—	+	+	+	+	++	++	++	++	++	++	++	+	+	+	+

++ = Good growth, + = Faint growth, — = No growth.

Table 2. Effect of salinity on the growth of *Rhizobium* of *Sesbania* DMB R001

Medium	NaCl concentration in millimhos									
	100	200	300	400	500	600	700	800	900	1000
Yeast extract mannitol medium	++	++	++	++	++	+	—	—	—	—

++ = Good growth, + = Faint growth, — = No growth.

results were noted. The pH of the medium (YEM broth) was adjusted from 3 to 11 with an increment of 0.5 using 1N NaOH as required and inoculated tubes were incubated at 32±2°C for 48°C and the results were noted.

RESULTS AND DISCUSSION

Depending upon the growth of the rhizobial isolate, all rhizobia were classified as *Rhizobium* sp. The colony size was about 1–3 mm in diameter on YEM medium and well established growth appeared within three days inoculation. Similar results were reported by Jordan and Allen (1974) in *R. meliloti*, *R. trifolii* and *R. leguminosarum*. Colony size of more than 1 mm diameter and appearance of colonies within seven days after inoculation are typical of fast growing rhizobia (Graham and Parker, 1966). Effect of different carbon sources on the growth of *Rhizobium* DMB R001 showed that it utilized a variety of mono, di, polysaccharides and polyols. Rhizobia were unable to utilize citrate (Fig. 1). The generation time

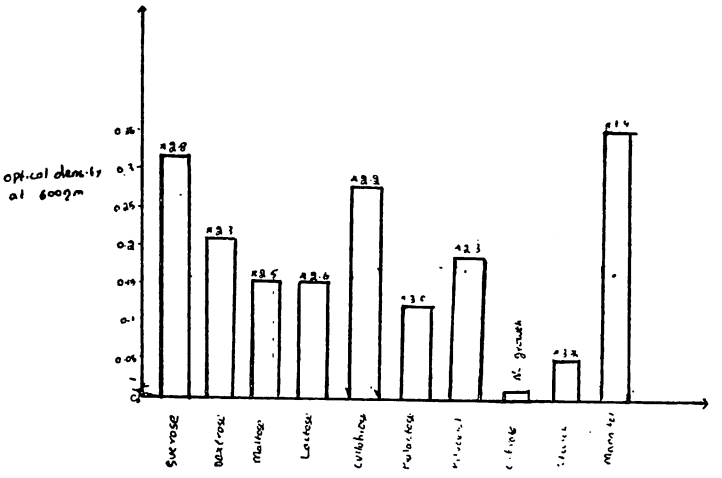

Fig. 1. Utilization of different carbon sources by *Rhizobium* sp. of *Sesbania* DMB R001.

varied between 1.9 to 3.2 h depending on the carbon sources used. The isolate DMB R001 utilizing glucose, galactose, sucrose, lactose, maltose and mannitol was the typical character of fast growing rhizobia (Chakrabarti *et al.*, 1981; Stowers, 1985). According to the reports, the slow growers were unable to utilize or utilized less. Growth studies and ability to utilize different carbons strengthened the view that these rhizobia belonged to fast growing rhizobial group.

The isolate was sensitive to the antibiotics (Fig. 2) like tetracycline (5 μg/m), gentamycin (5 μg/ml) but resistant to ampicillin (180 μg/ml), nalidixic acid (780 μg/ml), chloramphenicol (45 μg/ml), kenamycin (80 μg/ml), penicillin (200 μg/ml)

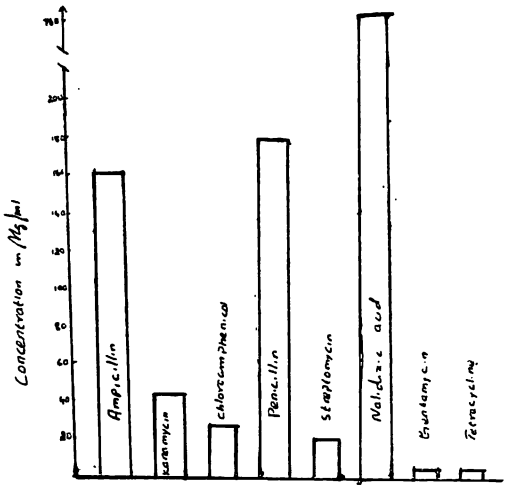

Fig. 2. Antibiogram of *Rhizobium* of *Sesbania* DMB R001.

and streptomycin (20 μg/ml). Similar type of antibiotic resistance pattern has been observed in other cowpea rhizobia infecting clusterbean (Mand, 1987) and pigeonpea (Anand and Dogra, 1990). However, interestingly the present isolate DMB R001 was resistant to very high concentration of nalidixic acid (760 mg/ml). This resistant marker could be used as a selective trait or marker for the identification of the strain from the ecological studies. The effect of different temperatures required for the growth of rhizobia showed that the isolate could grow well at 36, 42 and 44°C (Fig. 3). However, no or very little growth was observed at 50°C. Elevated temperatures might delay nodule formation and development because respiration was increased with high temperature and so less carbon would be available for the symbiosis (Lie, 1981). High temperature adversely affected the survival and persistence of rhizobia, competition, rhizosphere colonization, root hair formation, adsorption of rhizobia and nodulation (Dudeja and Khurana, 1988, 1989a, b).

The isolate DMB R001 was able to grow well between a wide pH range of 5-10. Delayed growth was observed at pH 11. In contrast, the fast growing strain isolated in the present study was able to grow under acidic condition. The growth under acidic condition (pH 5 and 4) was an interesting observation indicating that the isolate

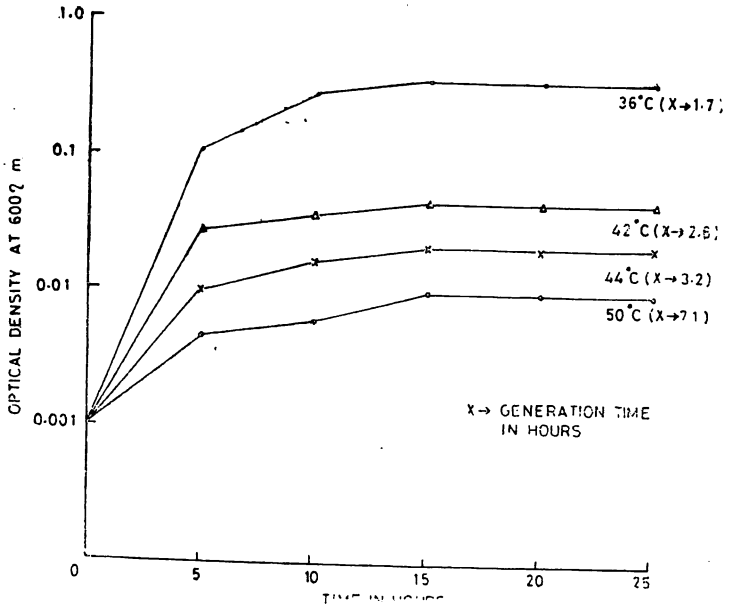

Fig. 3. Growth of *Rhizobium* sp. of *Sesbania* DMB R001 at different temperatures.

could grow well in acidic soils. According to Munns and Keyser (1981), the slow growing *Bradyrhizobium* strains were generally more acid tolerant than the fast growing species especially *R. meliloti*. Yadav and Vyas (1971) in two surveys of 23 rhizobial isolates from eight diverse legume species reported that all grew well as pH values upto 10. But none of the 17 strains of *Bradyrhizobium* tested showed significant growth in liquid media at pH 8.5. So, similar result was observed in our test and it also confirmed that the isolate was a fast growing rhizobia instead of slow growing *Bradyrhizobium*. The isolate could tolerate salinity levels upto 600 mmhos indicating that the isolate was highly salinity tolerant and it could grow well in saline soils. Under saline conditions, micro symbionts was not a limiting factor. However, legumes have been recorded as more sensitive or only moderately resistant to salinity (Zahvan, 1991) because of the accumulation of toxic ions such as Na and Cl in plant tissues, where they disturb enzyme activities. The isolate can grow under extreme environmental conditions of pH, salinity, temperature, different carbon sources and different antibiotics indicating that under natural ecosystem such strain could harvest a good amount of N_2 for legumes in soils.

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