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In Vitro Techniques for Screening of *Rhizobia*

R. SWAMINATHAN[1] and N. N. PRASAD[2]

The efficiency of *Rhizobium* sp. for nitrogen fixation can be assessed with ease based on the tolerance to crystal violet dye and the activity of dehydrogenase enzyme. Inefficient isolates tolerated higher concentrations of crystal violet while the efficient ones were highly sensitive. However, no correlation could be drawn in the case of dehydrogenase activity.

The nitrogen fixing capacity of rhizobia varies widely among different strains. Recently, Konde (1975) reported that the efficiency to fix atmospheric nitrogen had a negative correlation with the sensitiveness to the dye, crystal violet. Stevenson(1959) observed that the activity of dehydrogenase might serve as measure of the virulence of a bacterium as it can be traced by the reduction of triphenyl tetrazolium chloride. In the present investigation the efficiency of the root nodule bacteria in respect of nitrogen fixation has been compared with the two methods suggested.

MATERIALS AND METHODS

Yeast extract mannitol agar medium was prepared with the addition of crystal violet at different concentrations. The twenty isolates of *Rhizobium* (cowpea group) from blackgram, greengram, sunnhemp and daincha were streaked on the agar plates incubated at room temperature (26-28°C) for seven days and the growth of the isolates was recorded. The nitrogen fixing efficiency of the different strains of rhizobia was determined by estimating the total N content of the plants. In a pot culture study, sterilized paddy field soil was taken and surface sterilized seeds of the crop plants blackgram, greengram sunnhemp and daincha were treated with the different strains of rhizobia and the plants were grown under pot culture condition for a period of 45 days. The total N content of the plant was determined following the methods of Bremner (1960). The growth of the isolates and efficiency in nitrogen fixation were correlated.

The dehydrogenase activity of rhizobia was worked out and calculated on the lines suggested by Stevenson (1959) using Baush and Lomb colorimeter at 585 nm.

RESULTS AND DISCUSSION

The isolates, AUB. 2, AUG. 5, AUS. 4 and AUD. 4 could tolerate

1, 2 : Faculty of Agriculture, Annamalai University, Annamalainagar, South Arcot District.

TABLE I. Tolerance to crystal violet and relative effectiveness of *Rhizobium* sp.

Host	Isolate No.	Crystal Violet Concentration (ppm)		N-fixed (mg/100g of dry matter) *
		Poor growth	Fair growth	
Phaseolus mungo	AUB. 1	40	13.3	310.0
	AUB. 2	—	13.3	980.0
	AUB. 3	40	13.3	560.0
	AUB. 4	40	13.3	660.0
	AUB. 5	40	13.3	670.0
Phaseolus aureus	AUG. 1	100	40.0	510.0
	AUG. 2	40	20.0	920.0
	AUG. 3	40	20.0	970.0
	AUG. 4	20	13.3	1690.0
	AUG. 5	—	13.3	1820.0
Crotalaria juncea	AUS. 1	40	13.3	1370.0
	AUS. 2	40	13.3	2100.0
	AUS. 3	—	40.0	2430.0
	AUS. 4	20	13.3	3540.0
	AUS. 5	40	20.0	210.0
Sesbania aculeata	AUD. 1	100	40.0	310.0
	AUD. 2	20	13.3	1520.0
	AUD. 3	40	13.3	260.0
	AUD. 4	20	13.3	1950.0
	AUD. 5	40	13.3	1110.0

— Negligible growth
* Mean value

respectively 13.3, 13.3, 20 and 20 ppm concentrations of crystal violet and these isolates were found to fix more nitrogen when compared to other isolates of the respective crops. AUB. 1, AUG. 1, AUS. 5, AUD. 1 and AUD. 3 were able to tolerate higher concentrations of crystal violet dye viz., 40, 100, 40, 100 and 40 ppm respectively but fixed lesser quantities of nitrogen.

As regard the dehydrogenase activity, among twenty isolates, AUS. 5 and AUD. 2 recorded more activity than the rest. The isolates, AUG. 4 and AUS. 1 exhibited the least activity. No relationship exists between the dehydrogenase activity and their nitrogen fixing efficiency.

Tolerance to higher concn. of crystal violet has been compared with relative effectiveness of rhizobia to fix atmospheric nitrogen (Shete, 1965 and Konde, 1975). It was stated that a wide range of tolerance exists to crystal violet concns between the strains within the various cross-inoculation groups. In the present study the most

efficient strains of rhizobia - AUB. 2, AUG. 5, AUS. 4 and AUD. 4 were found to be sensitive to even lesser concentrations of the dye. For example the most efficient strains of rhizobia AUB. 2, AUG. 5, AUS. 4 and AUD. 4 could tolerate 13.3, 13.3, 20 and 20 ppm respectively. On the other hand the less efficient strains AUB.1, AUG.1, AUS. 5, AUD. 1 and AUD. 3 could tolerate 40, 100, 40, 100 and 40 ppm respectively. The results are in agreement with the reports of Shete (1965) and Konde (1975).

The weak reduction of triphenyl tetrazolium chloride (TTC) and higher capsule formation in *Pseudomonas solanacearum* was correlated with the virulance of the pathogen by Kelmen (1954) and Hussain and Kelmen (1958). There appeared to be a marked activity of dehydrogenase (TTC reduction) which paralleled with the virulance of the pathogen. In the present study, three of the four efficient isolates viz. AUB. 2, AUS. 4 and AUD. 4 exhibited a higher dehydrogenase activity. On the other hand, AUG. 5, the efficient isolate from greengram exhibited a low dehydrogenase activity. The remaining fifteen isolates studied have had no correlation between dehydrogenase activity and efficiency in nitrogen fixation.

TABLE II. Dehydrogenase activity of *Rhizobium* isolates

Rhizobium isolates	Dehydrogenase activity*
Blank (methanol)	—
Control	—
AUB. 1	1.27
AUB. 2	1.27
AUB. 3	1.16
AUB. 4	1.15
AUB. 5	1.04
AUG. 1	1.38
AUG. 2	1.15
AUG. 3	0.92
AUG. 4	1.15
AUG. 5	0.81
AUS. 1	1.61
AUS. 2	1.61
AUS. 3	1.27
AUS. 4	1.38
AUS. 5	1.84
AUD. 1	1.15
AUD. 2	1.84
AUD. 3	1.38
AUD. 4	1.61
AUD. 5	1.27

* Expressed in μ l of H/g 24 hr at 28°C.

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