

CHARACTERIZATION OF SUNN HEMP RHIZOBIA

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ABSTRACT: A *Rhizobium* sp isolated from the root nodules of *Crotalaria juncea* was a fast growing, acid-producing strain. Size of the colonies formed on yeast mannitol agar plates ranged between 3 - 5 mm in diameter and were gummy and soft in nature. Its efficiency of utilizing carbohydrates as a carbon source differed widely: glucose, mannitol and sucrose were more efficiently used compared to the other carbohydrates examined. A rhizobial strain isolated from *Mimosa pudica* failed to nodulate Sunn hemp. Symbiotic association formed between Sunn hemp and the rhizobial strain isolated from *Tephrosia purpurea* was found to be inefficient, while strains isolated from *Vigna cylindrica*, *Psophocarpus tetragonolobus*, *Phaseolus mungo* and *Phaseolus aureus* nodulated *Crotalaria juncea* as efficient as its specific rhizobial strain. A specific antibiotic sensitivity pattern was exhibited by the Sunn hemp rhizobial strain. It showed resistance to sulphafurazole and penicillin - G, whilst it was sensitive to chloramphenicol, erythromycin, streptomycin and tetracycline. This pattern clearly characterised the Sunn hemp *Rhizobium* from the other rhizobial strains examined.

Introduction

Host specificity is an important aspect in legume *Rhizobium* symbiosis. Even though the general microbiology of *Rhizobium* sp was clearly stated by Somasegaran and Hoben (1985) there is no information available for the *Rhizobium* sp which nodulates *Crotalaria juncea* especially on the cultivar grown in the Jaffna peninsula. The present study was undertaken with a view to obtaining some basic information about the characteristics of the *Rhizobium* sp which form symbiotic association with Sunn hemp. Also an attempt was made to obtain information regarding the cross infection ability of some rhizobial strains obtained from the nodules of some local legumes to infect Sunn hemp.

Materials and Methods

Colony characteristics and carbon source requirement

Colony characteristics were analyzed by the methods of Vincent (1970). The nutritional requirements of *Rhizobium* sp were determined by using agar plate method (Stowers and Eaglesham 1984). One ml of the bacterial suspension (1.45×10^5 cells/ml) was inoculated into the wells cut in the centre of the plates with a sterile cork borer. Growth and acid-producing ability were scored after the incubation period of two days at room temperature (30°C).

Cross inoculation

Rhizobial strains isolated from the root nodules of some of the well known legumes in Jaffna were used in this study. Legumes used in this investigation were:

Crotolaria verrucosa (Rattle box)

Mimosa pudica (Sensitive plant)

Phaseolus mungo (Black gram)

Psophocarpus tetragonolobus (Wing bean)

Vigna cylindrica (Cow pea)

Tephrosia purpurea (Goat's rue)

Inocula of 4×10^7 cells/ml were obtained from overnight cultures grown in mannitol broth and were used to inoculate one week old seedlings of *Crotolaria juncea* grown in seedling agar slants (Vincent 1970). Two ml from each treatment was added into the tubes containing the test plants. Six replicates for each treatment were maintained under laboratory conditions and the observations were made on the number of nodules and the dry weight of shoot (g) at weekly intervals. Dry weight was obtained after six weeks by drying the materials at 80°C for 24 hours.

Antibiotic sensitivity

Five rhizobial strains isolated from the root nodules of Cowpea, Wingbean, Sunn hemp, Mimosa and Green gram were tested for sensitivity to six antibiotics: Chloramphenicol (10 µg), Erythromycin (10 µg), Sulphafurozole (100 µg), Penicillin G (1.5 IU), Streptomycin (10 µg) and Tetracycline (10 µg). The test was done by the paper disc method using oxoid multidiscs (Carpenter 1977). The antibiotic discs were placed evenly on a mannitol agar plate which was spread uniformly with the rhizobial cultures. The cultures for spreading were obtained from overnight cultures grown in mannitol broth media. The plates were incubated at 30°C and examined after two days incubation for zones of growth inhibition.

Results and Discussion

Rhizobial colonies on YMA medium were dome shaped with smooth margin. The appearance of the colonies was milky to watery and translucent. Colonies were formed in less dense manner and were gummy and soft. Size of the colonies varied, but most were ranged within 3-5 mm in diameter.

Maximum growth was achieved on agar plates within two days of incubation at room temperature showing the characteristics of a fast-growing strain. This is in contrast to the observation made by Somasegaran and Hoben (1985) where they reported a slow-growing *Rhizobium* sp nodulating *Crotalaria* sp. Bromothymol blue (green at pH=6.8) incorporated in YMA plates turned yellow at the end of the incubation period showing the production of acid. Acid producing ability of this strain indicated that it was one of the advanced forms (Norris 1965).

The Sunn hemp rhizobial strain used all the carbon sources tested—maltose, fructose, sucrose, mannitol, glucose and cellulose (Table 1) and produced acid during its growth on the yeast agar medium. When sucrose was added as a carbon source it utilized this disaccharide efficiently and produced an intense yellow colouration around the inoculated well. The same result was also observed in glucose and mannitol incorporated plates, showing the production of acid as a result of abundant growth. In contrast to this, Stowers and Elkan (1984) observed that cowpea rhizobia when inoculated in a disaccharide medium showed little growth. Even though the Sunn hemp rhizobia is generally considered to be a member of the cowpea rhizobia group, this strain was very different.

Table 1. Carbohydrate utilising efficiency by Sunn hemp *Rhizobium* sp.

Carbohydrates	Zone size of acid production on yeast agar medium (mm)	Intensity of yellow colouration
Sucrose	62	+++
Maltose	67	++
Fructose	54	++
Mannitol	63	+++
Glucose	61	+++
Cellulose	46	+

Measurements included the diameter of well (10 mm) cut in the centre of the yeast agar plate.

Figure 1 gives the information about the cross inoculating ability of some rhizobial strains. According to the results, *Rhizobium* sp isolated from the nodules of *Mimosa pudica* failed to nodulate *Crotalaria juncea*.

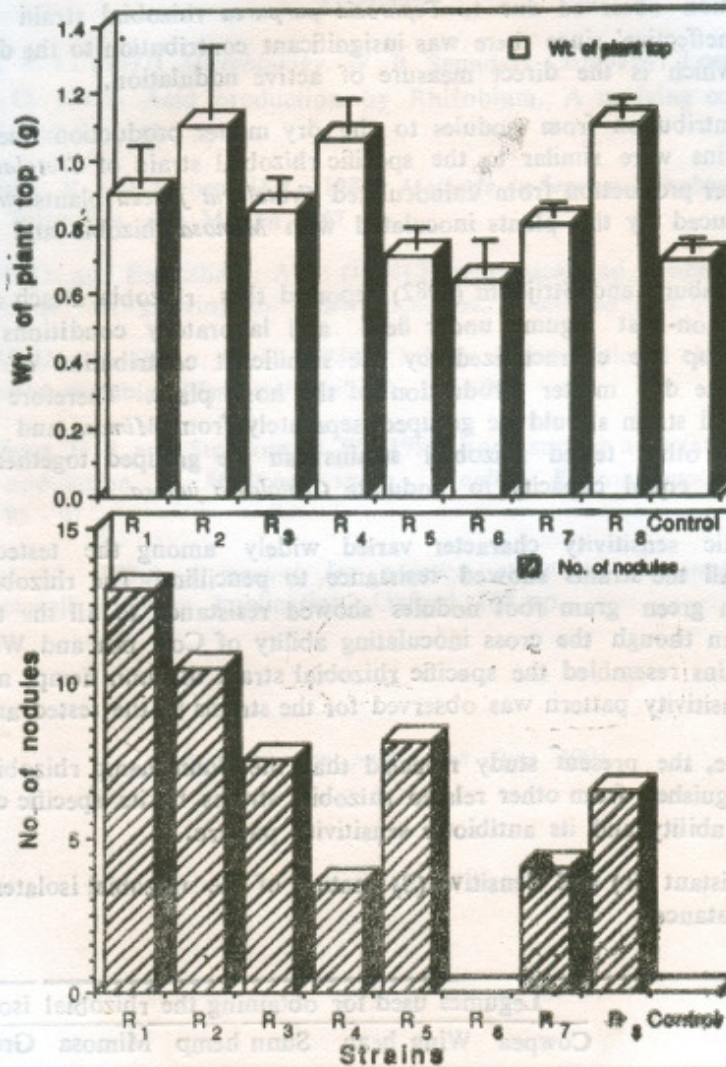


Fig. 1 Cross inoculated ability of *Crotolaria juncea* inoculated with different rhizobial strains, in terms of weight of the plants and the number of nodules formed on the root system after 6 weeks of inoculation.

R₁ (*Phaseolus vulgaris*); R₂ (*Phaseolus mungo*); R₃ (*Psophocarpus sp.*); R₄ (*Vigna cylindrica*); R₅ (*Tephrosia sp.*); R₆ (*Mimosa pudica*); R₇ (Wild *Crotolaria sp.*); R₈ (*Crotolaria juncea*); Control (Uninoculated).

Nodulation observed due to *Tephrosia purpurea* rhizobial strain was considered as 'ineffective' since there was insignificant contribution to the dry matter production which is the direct measure of active nodulation.

The contribution from nodules to the dry matter production due to other rhizobial strains were similar to the specific rhizobial strain of *Crotolaria juncea*. The dry matter production from uninoculated *Crotolaria juncea* plants was similar to that produced by the plants inoculated with *Mimosa* rhizobia and *Tephrosia* rhizobia.

Van Rensburg and Strijdom (1982) reported that rhizobia attach readily to the roots of non-host legume under field and laboratory conditions but, the 'effective' group are characterized by the significant contribution of the fixed nitrogen to the dry matter production of the host plant. Therefore the Sunn hemp rhizobial strain should be grouped separately from *Mimosa* and *Tephrosia* strains, while other tested rhizobial strains can be grouped together because they have the equal capacity to nodulate *Crotolaria juncea*.

Antibiotic sensitivity character varied widely among the tested strains (Table 2). All the strains showed resistance to penicillin. The rhizobial strain isolated from green gram root nodules showed resistance to all the tested antibiotics. Even though the cross inoculating ability of Cow pea and Wing bean rhizobial strains resembled the specific rhizobial strain of Sunn hemp, no similar antibiotic sensitivity pattern was observed for the strains to the tested antibiotics.

Therefore, the present study revealed that the Sunn hemp rhizobial strain can be distinguished from other related rhizobial strains by its specific carbohydrate utilising ability and its antibiotic sensitivity pattern.

Table 2 Resistant (R) and Sensitive (S) nature of the rhizobial isolates for the antibiotic substances.

Antibiotics	Legumes used for obtaining the rhizobial isolates				
	Cowpea	Wing bean	Sunn hemp	Mimosa	Green gram
Chloramphenicol (10 μ g)	S:18	S:30	S:25.5	S:28	R
Erythromycin (10 μ g)	R	R	S:23	S:15.5	R
Sulphafurazole (100 μ g)	R	R	R	S:22	R
Penicillin - G (1.5 I.U.)	R	R	R	R	R
Streptomycin (10 μ g)	S:15	R	S:17.5	S:13	R
Tetracycline (10 μ g)	S:30	S:30.5	S:22.5	S:22.5	R

S : Sensitive; R : Resistant

Values denoted are in mm for the diameters of clear zones.

References

- Carpenter, P. L. (1977) *Microbiology*. W. B. Saunders Company, London.
- Norris, D. O. (1965) Acid production by Rhizobium. A unifying concept. *Plant and Soil* 22, 143 - 166.
- Somasegaram, P. and Hoben, H.J. (1985) *Methods in legume Rhizobium technology*. Nif Talproject and Mircen, 367 pp.
- Stowers, M. D. and Eaglesham, A. J. (1984) Physiological and symbiotic characteristics of fast growing *Rhizobium japonicum*. *Plant and Soil* 77, 3-14.
- Stowers, M. D. and Elkan, G. H. (1984) Growth and nutritional characteristics of cowpea rhizobia. *Plant and Soil* 80, 191 - 200.
- Van Rensburg, H. J. and Strijdom, B. W. (1982) Root surface association in relation to nodulation of *Medicago sativa*. *Applied Environmental Microbiology* 44, 93 - 97.
- Vincent, J. M. (1970) *A manual for practical study of root nodule bacteria*. Blackwell scientific Publication, Oxford, 164 pp.

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