CULTURES OF *RHIZOBIUM* INOCULANTS WITH THOSE OF *BEIJERINCKIA* AND *AZOTOBACTER*

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The survival of *Rhizobium japonicum* cultured in peat with *Beijerinckia indica* and *Azotobacter chroococcum* was examined with special reference to nodulation and yield of soybean var. *Clark* in Delhi soil. There was better nodulation and increase in yield of the crop as compared with culture containing *R. japonicum* alone.

INTRODUCTION

Workers with rhizobia have made detailed studies on the production of inoculant, its application to seed and its survival in storage and in soil (Vincent 1958; Roughley and Vincent 1967; Roughley 1968; Date 1968; Iswaran *et al.* 1969, Iswaran 1970). It is of interest that Dudman (1968) found that capsulated *Rhizobium-trifolii* TA1 could enhance nodulation compared with a non-capsulated culture and suggested that attention should be given to strains or cultural conditions which induced capsulation in *Rhizobium* spp intended for legume inoculation. It has also been reported that *Azotobacter* and other organisms increased the nodulation efficiency of rhizobia (Krassilinikov and Korenyakov 1944) and it could be that if rhizobia and *Azotobacter* together are used for legume seed inoculation the *Azotobacter* polysaccharide would enhance the survival of *Rhizobium*. It is with this object in view that an experiment was undertaken to see if soybean rhizobia when grown together with *Beijerinckia indica* a highly capsulated organism improved the yield of soybean var. *Clark*.

MATERIALS AND METHODS

Strains of *Rhizobia, Beijerinckia* and *Azotobacter* used for seed inoculation were those maintained in the culture collection of the Division of Microbiology, I.A.R.I., New Delhi.

Sources of peat

The extensive peat deposit surrounding the Nilgiri hills (Wellington) consists of black structureless peat up to a depth of several feet. The peat is acidic (*p*H 4.5–5.0) and requires 10 per cent of (w/w) of $CaCO_3$ to adjust the *p*H to 6.5. Peat from this deposit was used, after neutralisation with $CaCO_3$. Peat used was dried in layers, 1 inch thick at 40°C for 2 days when its water content was 10 per cent. After drying, the peat was milled to 200 mesh.

Preparation of peat cultures

The soybean *Rhizobium* SB 16 was grown in yeast-extract-mannitol medium (Fred *et al.* 1932) at 28°C to provide > 100 × 10^6 viable bacteria/ml. *Azotobacter* (strain 41) used for inoculation was grown in 100 ml medium in 500 ml flasks on a rotary shaker at 28°C for 2 to 14 days. The medium contained K_2HPO_4, 0.2g; $MgSO_4.7H_2O$, 0.2g; NaCl 0.2g; $FeSO_4.7H_2O$, 0.01g; $CaSO_4$ 0.1g; $MnSO_4$, 0.01g; Na_2MoO_4, 0.01g; $CaCO_3$ 1.5g; mannitol 10.0g; distilled water 1000 ml; *p*H 7.0.

Beijerinckia was grown under the same condition using Becking's glucose phosphate acid medium. In preparing the peat cultures aerated cultures were used in place of static cultures as the counts were more in the former than in the latter. Polysaccharide gums were estimated under static and shake cultures in the case of *Azotobacter chroococcum* and *Beijerinckia indica* using acetone method.

Amounts of equal volumes of *Rhizobium japonicum* SB 16 and *Beijerinckia indica* and *R. japonicum* and *Azotobacter chroococcum* (strain 41) were mixed with neutralised peat while individual strains constituted the controls. The prepared peat cultures were stored at 26°C for a period of 3 weeks before use for inoculating seeds.

Pot culture experiment

The pot culture experiment was carried out in triplicate using Delhi soil having a *p*H of 7.6. Four kg of the soil were placed in pots.

A basal dressing of single superphosphate 500 mg; zinc sulphate 2 mg; manganese sulphate 1 mg; ammonium molybdate 1 mg; and borax 1 mg were thoroughly mixed with the soil in each pot.

Seeds of soybean var *Clark* were inoculated with peat cultures containing the following treatments using 16 per cent gum as adhesive. The treatment consisted of (*i*) uninoculated-check (*ii*) peat containing efficient strain of *Rhizobium japonicum* SB16, (*iii*) peat containing *Beijerinckia*, (*iv*) peat containing equal volumes of *Beijerinckia* and *R. japonicum*, (*v*) peat containing equal volumes of *Azotobacter* and *R. japonicum*, and (*vi*) peat containing *Azotobacter*. A uniform population of 4 plants was maintained for 8 weeks. Plants were separated from soil and the root system examined for nodulation. The yield of pots was recorded. The inoculum used in these pot experiments had a count of 10^8 cells/g in the case of each microorganism.

Results and Discussion

The results of seed inoculation experiments (Table I) indicate greater survival advantage to *Rhizobium japonicum* offered by the presence of *Beijerinckia* probably due to the high polysaccharide content present in this organism (Table II). Again *Beijerinckia* is a highly capsulated and gum forming organism which might have given protection to *R. japonicum* thereby enhancing better survival of the organism on the seed leading to better nodulation and increase in yield.

TABLE I

The effect of seed inoculation on the nodulation and yield of Glycine max *var.* Clark *in the presence of* Beijerinckia indica *and* Azotobacter chroococcum

	Average of four replications (pots)*	
	Number of nodules per plant	yield of beans per pot (g)
1. Uninoculated-check	5.0	10.0
2. Inoculated with peat containing *R. japonicum*	56.2	18.5
3. Inoculated with peat containing *Beijerinckia indica*	8.2	11.2
4. Inoculated with peat containing *Beijerinckia indica* and *R. japonicum*	125.0	25.80
5. Inoculated with peat containing *Azotobacter chroococcum* 41 and *R. japonicum*	66.2	18.4
6. Inoculated with peat containing *Azotobacter chroococcum*	12.0	12.2
C.D. at 0.5 level	31.4	7.0

*Each pot had 4 plants.

TABLE II

Gum production and number of viable cells in culture medium under aerated and stationary conditions

Culture	Cultural conditions	Gum production (g)	Viable counts/ ml $\times 10^8$
Beijerinckia indica	Shake culture	1.30	61.0
	Stationary	1.82	8.0
Azotobacter chroococcum	Shake culture	0.19	48.5
	Stationary	0.42	13.15

CONCLUSIONS

Cultures of *Rhizobium japonicum* prepared from broth in the presence of a highly mucoid strain of *Azotobacter chroococcum* or *Beijerinckia indica* gave greater yields of the crop when used for inoculation of *Glycine max* var. *Clark*. Shake cultures of *Beijerinckia* and *Azotobacter* showed greater counts of these organisms but less of gum production as compared to static cultures.

ACKNOWLEDGEMENT

We gratefully acknowledge the facilities provided by Dr. N. S. Subba Rao, Head of the Division of Microbiology, Indian Agricultural Research Institute, New Delhi.

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