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NOTES

Polyacrylamide-Entrapped *Rhizobium* as an Inoculant for Legumes†

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Pot experiments showed that *Rhizobium japonicum* cells entrapped in a polyacrylamide gel could be used as an inoculant for soybeans and compared favorably to laboratory-made peat base inoculant containing the same bacterial strain.

Peat is most commonly used as the carrier for *Rhizobium* inoculant. Since the composition of peat varies with the location of the deposit, it seemed desirable to devise a synthetic carrier of constant quality that could compete with peat in terms of its capacity to retain the viability of *Rhizobium* over long periods without loss of infectivity and effectiveness. The aim of the work reported here was to investigate the use of polyacrylamide-entrapped *Rhizobium* (PER) as an inoculant for soybeans.

Rhizobium japonicum strain G2Sp, a streptomycin-resistant mutant of United States Department of Agriculture strain 311 b 135, was grown in yeast–mannitol medium (4). To 1 liter of water, the following were added: mannitol, 10 g; K_2HPO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.2 g; yeast extract, 1.0 g; NaCl, 0.2 g; and $FeCl_3$, 4.88 mg; pH was adjusted to 7.0. When the culture was 4 to 5 days old (end of log phase), bacteria were entrapped by a modification of the procedure already described (1, 2).

Four solutions were prepared: (i) phosphate buffer, pH 7 (KH_2PO_4, 1 g; Na_2HPO_4, 2 g; distilled water, 1,000 ml); (ii) 238 g of acrylamide dissolved in 1,000 ml of phosphate buffer; (iii) 12.5 g of *N,N'*-methylenebisacrylamide suspended in 1,000 ml of phosphate buffer (excess *N,N'*-methylenebisacrylamide was eliminated by filtration); (iv) 126 mg of ammonium persulfate dissolved in 1 ml of distilled water (solution iv was prepared just before use from ammonium persulfate kept in a tightly closed vial). For gelation 150 ml of the *Rhizobium* culture, 50 ml of acrylamide solution, 50 ml of the *N,N*-methylenebisacrylamide stock solution, 1 ml of ammonium persulfate solution, and 76 μl of *N,N,N,N'*-tetramethylethylene diamine solution (Merck) were mixed. Gelation was complete in 10 min. The material thus solidified into a block was fragmented with a knife into 20- to 50-ml pieces and washed under running tap water for 24 h. After being washed, the gel, still in a wet state, was fragmented again into 0.1- to 0.2-ml blocks (wet blocks) or crushed in a mortar (wet crush) before being introduced into the soil. The blocks of gel or the wet crush could also be air dried (1 to 3 days) at 25 to 28°C to obtain dried PER blocks, or dried crush, which was then ground into dried PER powder with a Retsch mill.

Laboratory-made peat base inoculant was obtained by mixing 600 ml of *Rhizobium* G2Sp culture with 800 g of neutral sterilized peat so as to obtain the same number of *Rhizobium* in 0.8 g of peat (dry weight) as in 1 ml of PER, i.e., 5×10^8/ml (plate count).

Soybeans (*Glycine max*) cv. 'Chippewa' and 'Jupiter' were grown in pots containing 2 (Table 2, experiment 1 and 2) or 3 (Table 2, experiment 3) kg of soil. Two sandy soils from Senegal, in which soybeans had never been grown before, were used: soil 1 (vernacular name Dior) from the Agronomic Research Center at Bambey, with a low native population of *Rhizobium* that could infect soybeans, and soil 2 from ORSTOM Center at Bel-Air, near Dakar, that had a significantly higher population of native *Rhizobium* infective to soybeans. A 71-mg amount of commercial superphosphate (45% P_2O_5) and 47 mg of commercial potassium chloride (60% K_2O) were added per 1 kg of soil.

Soil inoculation was achieved by applying the inoculant, either peat base or PER (wet or dry),

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at the surface of the soil of each pot and subsequently mixing it with the upper 5-cm soil layer before seeding. The amount of inoculant used in each experiment is indicated in Table 2.

The survival of *R. japonicum* G2Sp in wet PER stored in 0.8% saline (NaCl) solution was compared with survival in liquid culture and in laboratory-made peat base inoculant. Viable bacteria were counted on the yeast–mannitol medium (4) with agar. Table 1 shows that survival was not very different at 4°C for the three types of inoculant. But at 30°C the protective effect of PER was conspicuous.

Since the polyacrylamide carrier might enhance plant growth, the influence of the addition of this material was investigated. The total N content of 6-week-old soybean cv. 'Jupiter' grown in pots with polyacrylamide alone, with autoclaved PER, or without any addition (control) was 38.0, 41.6, and 38.5 mg of N per plant, respectively, indicating that the addition of polyacrylamide to soil had no significant effect on plant total nitrogen content.

Roots easily penetrated the PER blocks without inducing breaks. Ultramicroscopic pictures of soybean roots penetrating PER pieces showed that no gap occurred between the root itself and the polyacrylamide matrix (Fig. 1). Surprisingly, nodules were not formed on the roots as they pierced the block of PER but generally occurred a short distance after the emergence of the root, suggesting that *Rhizobium* escaped from the

TABLE 1. *Survival of R. japonicum contained in different inoculants, stored at 4 and 30°C for 75 days*

Condition	Initial no. of *Rhizobium*[a]	No. of *Rhizobium*[a] after storage	Survival %
Storage at 4°C			
Liquid culture	3.7×10^9	2.5×10^9	67
Laboratory-made peat base inoculant	2.4×10^9	1.5×10^9	62
PER, wet blocks[b]	8.4×10^7	7.0×10^7	83
Storage at 30°C			
Liquid culture	3.7×10^9	$<1 \times 10^6$	<0.1
Laboratory-made peat base inoculant	2.4×10^9	$<1 \times 10^6$	<0.2
PER, wet blocks[b]	8.4×10^7	6.1×10^7	73

[a] Number of viable *Rhizobium* per 1 ml of liquid culture or 1 ml of PER or 0.8 g of peat base inoculant.
[b] Stored as blocks in saline solution.

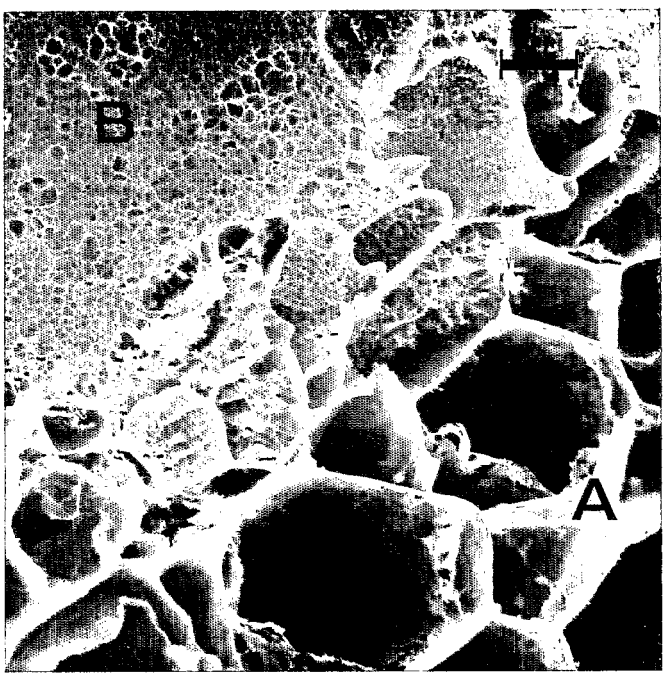

FIG. 1. *Interface root (A)–polyacrylamide gel (B) as viewed in a transversal section of a block of PER penetrated by a soybean root, using a scanning electron microscope JEOL JSM 35 equipped with a cryounit. Bar = 0.01 mm.*

TABLE 2. *Influence of different inoculants on nodulation and total N content of soybeans*

Type of inoculant	Nodule dry wt (mg/plant)	Aerial parts	
		Dry wt (g/plant)	Total N (mg/plant)
Expt 1 (cv. 'Jupiter'; soil 1; 5 weeks; 5 replicates)			
PER, wet blocks (2 ml)	105 (b)[a]	1.72 (cd)	35.3 (b)
PER, wet crush (2 ml)	135 (b)	2.41 (b)	55.3 (d)
PER, air-dried blocks (0.12 g)	60 (a)	1.49 (ac)	19.4 (ac)
PER, air-dried powder (0.12 g)	123 (b)	1.87 (ad)	31.8 (bc)
No inoculum	38 (a)	1.24 (a)	14.9 (a)
Expt 2 (cv. Jupiter, soil 1; 5 weeks; 4–6 replicates)			
Laboratory-made peat base (0.4 g)	134 (b)	2.95 (a)	60.4 (b)
PER, wet crush (0.5 ml)	123 (b)	2.81 (a)	56.2 (b)
PER, autoclaved (0.5 ml)	0 (a)	2.04 (a)	26.2 (a)
Expt 3 (cv. Chippewa; soil 2; 6 weeks; 6–7 replicates)			
Laboratory-made peat base (0.8 g)	483 (b)	6.18 (ab)	204.0 (b)
PER, wet crush (1 ml)	604 (b)	7.09 (b)	213.0 (b)
No inoculation	134 (a)	4.96 (a)	63.5 (a)

[a] Numbers in columns followed by the same letter do not differ ($P = 0.05$) by Mann-Whitney test (3).

matrix, but were not very mobile.

To elucidate the influence of PER size and drying, four formulations were compared: wet blocks, wet crush, air-dried blocks, and dried powder. The results of experiment 1 (Table 2) indicate that crushing wet blocks or grinding dried blocks into powder resulted in better nodulation. Drying PER appeared to be harmful since nodulation and total N of aerial parts were markedly decreased when dried, rather than wet, PER was used as blocks. But this unfavorable effect of drying was much less marked when PER was used as a powder.

Two other experiments (Table 2) showed that the performance of PER (wet crush) was comparable to that of peat base inoculant, since nodulation, dry weight of aerial parts, and total N content of plants did not significantly differ.

PER had the following properties. First, survival of *Rhizobium* cells entrapped in PER appeared to be satisfactory when PER was stored as blocks in a saline solution even at temperatures as high as 30°C. *Rhizobium* survival decreased when PER was air dried. Second, PER could be easily penetrated by roots.

Pot experiments showed that PER make up an inoculant with a performance comparable to that of the laboratory-made peat base inoculant which contained the same strain.

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