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Comparison of the Pour, Spread, and Drop Plate Methods for Enumeration of *Rhizobium* spp. in Inoculants Made from Presterilized Peat†

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Inoculants prepared with presterilized peat were enumerated by the pour, spread, and drop plate techniques. Results indicated that the three plating methods were interchangeable. The drop plate method was preferred because of its economy in materials and labor.

In the quality control of peat inoculants, the numbers of viable rhizobia are determined by plating on agar medium or by the plant infection method (3). With inoculants prepared from presterilized peat, viable numbers of rhizobia may be determined by the pour, spread, and drop plate methods. When large numbers of samples are plated, as happens during routine quality control checks, the method chosen should economize on the plating media and petri dishes. The method should also allow easy execution of the plating process without sacrificing the accuracy of the results.

The pour and spread plate methods are extensively used for viable counts with pure cultures of *Rhizobium* spp. The drop plate method (2, 3), however, is less frequently used, and no information exists on this method with regard to its relative recovery efficiency in comparison to the pour and spread plate methods.

To compare these plating methods, we recovered rhizobia from 10 different peat inoculants. Each strain of *Rhizobium* was grown individually in 100 ml of yeast mannitol broth (3) in 250-ml Erlenmeyer flasks. Flasks were shaken on a rotary shaker (100 rpm); the incubation temperature was 28°C.

Finely ground (mesh size, 200) peat was purchased from the Nitragin Co., Milwaukee, Wis., and adjusted to pH 6.5 to 6.8 with precipitated calcium carbonate. The peat was then packaged in 50-g quantities in polyethylene bags (16.5 by 11.5 by 0.0038 cm) and sterilized by gamma irradiation (2.5 megarads). A 50-ml sterile plastic syringe (Becton, Dickinson & Co., Rutherford, N.J.) fitted with an 18G 1.5-in. (3.8-cm) needle was used to introduce aseptically 40 ml of a turbid culture (ca. 10^9 cells per ml) into the peat. The needle puncture on each bag was sealed with adhesive tape. The bags were thoroughly massaged to incorporate the culture into the peat and incubated at 28°C for 1 week. At sampling, 1 g of the inoculant was removed aseptically and transferred into 99 ml of quarter-strength yeast mannitol broth in milk dilution bottles. The bottles were shaken (wrist action shaker) for 15 min. For each strain, five dilution series were prepared from the same original suspension. From each series, portions of 0.03 ml, 0.1 ml, and 1.0 ml of the appropriate dilutions were plated (in duplicate) for the drop, spread, and pour methods, respectively. The plating medium was yeast mannitol agar (3) containing Congo red (0.25% [wt/vol]). For the drop method, each plate was divided into eight sectors, and 1 drop (0.03 ml per drop) was delivered to each sector with a calibrated pasteur pipette. The plates were dried at 28°C for at least 2 days before plating by the spread and drop methods. The agar was cooled to 50°C (3) and dispensed at the rate of 15 to 20 ml per plate for pour plating.

The results of viable counts of the inoculants by the three methods are shown in Table 1. There was no general trend in the data; no one method consistently gave the highest or the lowest counts. This suggested that any one of the three methods may be adopted for routine laboratory use.

With pour plates, agar cooled to 45°C may result in selective killing of heat-sensitive strains of bacteria and therefore lower the counts with the pour plates (1). In this investigation, although the agar was cooled to 50°C, the pour plating did not consistently result in the lowest counts, except in four instances.

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TABLE 1. Comparison of drop, spread, and pour plate techniques for the enumeration of rhizobia in peat cultures

Plating method	Log_{10} no. of rhizobia per gram of moist peat[a]									
	TAL22	TAL82	TAL437	ICRISAT3889	TAL1000	TAL182	NIT176A22	CB756	USDA136b	TAL999
Drop	9.84	8.73	9.98	**9.33**	10.08	8.73	9.57	9.56	9.86	9.90
Spread	9.82	8.78	9.93	9.24	10.06	8.81	9.60	9.56	9.88	**9.97**
Pour	**9.68**	**8.65**	**9.80**	9.21	**9.95**	8.80	9.51	9.50	9.77	9.88

[a] Mean of five replications. Means in boldface type within each column are significantly different ($P \leq 0.05$) according to the Duncan multiple range test. Laboratory sources were as follows: TAL, NifTAL Project, University of Hawaii, Honolulu; ICRISAT, International Crop Research Institute for the Semi-Arid Tropics, Hyderabad, India; NIT, Nitragin Co., Milwaukee, Wis.; CB, Commonwealth Scientific and Industrial Research Organization, Brisbane, Australia; and USDA, U.S. Department of Agriculture, Beltsville, MD. The rhizobia were isolated from nodules of the following legumes: TAL22, *Phaseolus lunatus*; TAL82, *Leucaena leucocephala*; TAL437, *Vigna radiata*; ICRISAT3889, *Cicer arietinum*; TAL1000, *Arachis hypogaea*; TAL182, *Phaseolus vulgaris*; NIT176A22, *Vigna unguiculata*; CB756, *Macrotyloma africanum*; USDA136b, *Glycine max*; and TAL999, *Vigna unguiculata*.

Of the three methods, the drop plate procedure was the most preferable because more counts could be made on one plate (Fig. 1). This was because the division of one plate into eight sectors was equivalent to producing eight spread or eight pour plates. Furthermore, with duplicate plating of each dilution, four dilutions could be plated on each plate. There was considerable economy in the use of available incubator space, and the drop method was also less laborious to carry out than the other two methods. These advantages were significant enough to cause us to adopt the drop plate method for quality control of inoculants prepared from presterilized peat. In situations in which inoculants have been exposed to high temperatures, the plant infection method may be preferred over plating methods for a reliable estimate of the numbers of infective rhizobia (4).

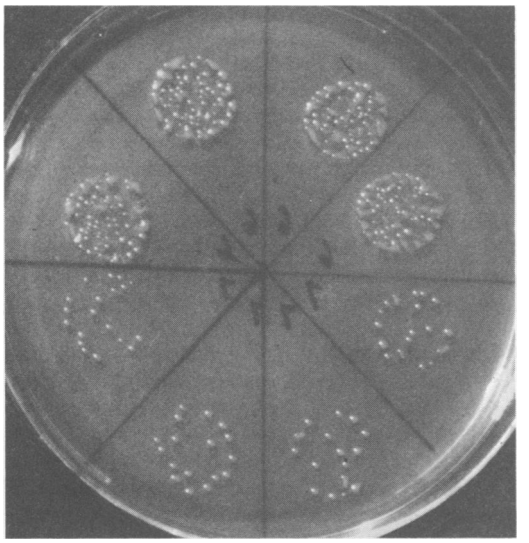

FIG. 1. Growth of colonies of *Rhizobium* sp. (TAL437) from drops plated by the drop method. Two dilutions (10^{-6} and 10^{-7}) of the peat inoculant were plated in quadruplicate and incubated at 28°C for 7 days. Note the reproducibility in all four drops of the 10^{-7} dilution.

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