

## SHORT COMMUNICATION

### Bacteriostasis and the inoculation of phosphate-solubilizing bacteria in the rhizosphere

J. A. OCAMPO, J. M. BAREA and E. MONTOYA

Estación Experimental del Zaidin, Avda. Cervantes, No. 1, Granada, Spain

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Bacteriostasis has been described as a factor that can inhibit or reduce the development of bacteria in soil (Brown, 1973). Davis (1975 and 1976) found that many bacteria were sensitive to bacteriostasis. Bacteriostasis is only operative at low nutrient levels, and is overcome by the addition of mineral salts to the soil. The cause of soil bacteriostasis is as yet unexplained, although it seems that it is of biological origin.

In an experimental inoculation of *Azotobacter* into the rhizosphere of lavender, Barea *et al.* (1978) found that *Azotobacter* was sensitive to such factors, and the activity of this seemed to coincide with the decrease in *Azotobacter* numbers in the rhizosphere.

We have examined the effects of soil bacteriostasis on the establishment of phosphate-solubilizing bacteria (phosphobacteria) inoculated into the rhizosphere of lavender (*Lavandula spica* var. *vera* L.).

Microorganisms, plants and treatments used in the glass-house experiment were described by Ocampo *et al.* (1975). Unsterilized field soil No. 1 amended with 2% farmyard manure was used. Three phosphate-solubilizing bacteria, a *Pseudomonas* sp., a *Bacillus* sp. and an *Agrobacterium* sp. were each cultured in Brown's medium (1972). At the time of inoculation the three cultures were mixed. Each seedling received about  $10^8$  phosphobacteria cells.

There were four NPK treatments: 1 = no NPK added; 2 = NPK added at the time of bacterial inoculation; 3 = NPK added at 10 weeks and 4 = treatments 2 + 3. This fertilizer was prepared in the following way: 8.8 g  $\text{KH}_2\text{PO}_4$  and 25.7 g  $\text{NH}_4\text{NO}_3$  were dissolved in 1 l distilled water and 10 ml of this fertilizer were added per kg soil in which lavender was grown at the time stated.

Rhizosphere soil from replicate plants was sampled every 15 days. Two separate measurements of growth of phosphobacteria were made using these samples: (1) the course of development of the bacterial inoculum added to the pots. Bacteria able to solubilize rock phosphate were counted (Ocampo *et al.*, 1975) and (2) the bacteriostatic activity. To assess soil bacteriostasis to phosphobacteria, a technique based on that described by Brown (1973) was used. Rhizosphere soil from each sample was placed in Petri dishes and moistened. Disks of sterile filter paper were either placed on the soil, or in sterile dishes as controls. Agar-disks 7 mm dia (9 replicates/sample), cut from 1.5% sterile water-agar, were placed on the filter paper. Dishes were kept at 26°C for 15 h and separate agar-disks were then inoculated with 10 ml of a suspension of a phosphobacterium (*Bacillus* sp., *Pseudomonas* sp and *Agrobacterium* sp). Three replicates per phosphobacterium were prepared. After a further 48 h incubation at 26°C the disks were removed, stained with 10% dilute carbol fuchsin and examined under the microscope. Bacteriostasis was assessed by comparing the number of microcolonies which grew on disks incubated over soil with those on controls.

As Fig. 1 shows, when the NPK fertilizer was not added

at the beginning of the assay, sensitivity of phosphobacteria to bacteriostasis increased with time and reached a maximum at the middle of the experiment. There was no significant change in the bacteriostatic activity, when NPK fertilizers were applied (treatment 2).

Bacteriostasis was always overcome in pots where an NPK fertilizer was added to the plants at the middle of the assay (treatments 3 and 4) and by the age of plant. Thus, agreeing with Brown (1973), that a certain role can be attributed to the plant which behaved as if supplying substances to overcome bacteriostasis.

Figure 2 shows the course of development of phosphobacteria inoculated in the lavender rhizosphere. Undoubtedly, soil bacteriostasis might have some influence on the establishment of phosphobacteria, but as Fig. 1 (treatment 2) clearly shows, its effect on phosphobacteria remained almost constant until the middle of the assay, whereas the number of inoculated phosphobacteria (Fig. 2) declined at

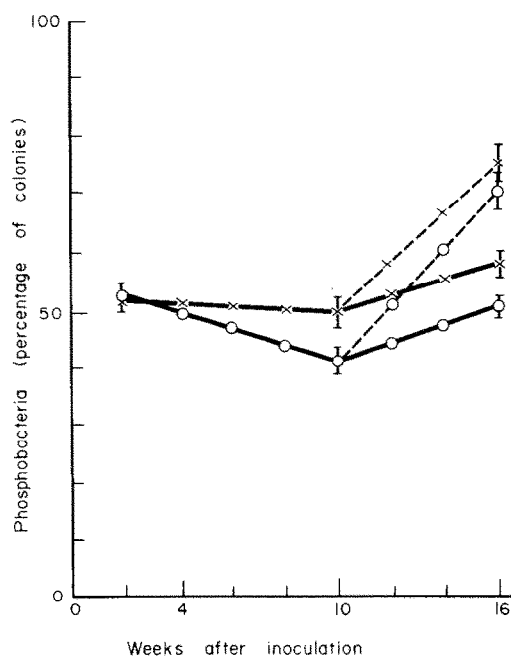


Fig. 1. Course of development of bacteriostatic activity towards phosphobacteria in rhizosphere of lavender given the following NPK treatments: 1. (○—○) no NPK added. 2. (×—×) NPK added at the time of bacterial inoculation. 3. (○---○) NPK added at 10 weeks. 4. (×---×) treatments 2 + 3. Standard errors are shown at representative values.

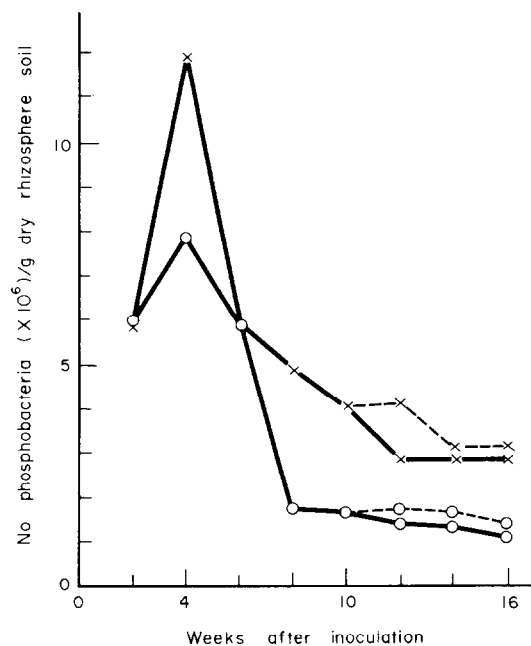


Fig. 2. Numbers of inoculated phosphobacteria in the rhizosphere of lavender receiving different NPK treatments. (See Fig. 1).

this time. Thus, a much more possible explanation for the decrease of phosphobacteria could be the effect of microorganisms acting by producing antibiotics or lytic enzymes (unpublished data).

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