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The effect of location in soil on protozoal grazing of a genetically modified bacterial inoculum

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ABSTRACT

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Short-term laboratory experiments were performed to investigate the effect of location on protozoan grazing of a genetically modified bacterial inoculum in soil. *Pseudomonas fluorescens* (strain 10586, containing chromosomally borne genes encoding bioluminescence and antibiotic resistances) was introduced into varying pore size classes by adjustment of the soil matric potential with reference to the moisture release characteristic. The soil ciliate protozoan *Colpoda steinii* was subsequently introduced to the soil at conditions close to field capacity to ensure initial location in larger pores.

When the *Ps. fluorescens* was predominantly located in small pores (less than 6 μm pore neck diameter), the decline in viable cell concentration was less than that when located in larger pores. This suggests that the bacterial inocula introduced into soil may be protected by spatial compartmentalisation. This protection may be from protozoan grazing, in which case the predator activity of the introduced *Colpoda* inoculum was not significant in comparison to that of the indigenous protozoa. Further work is therefore required to determine the mechanism of protection but the findings demonstrate that the antecedent matric potential and pore size characteristics will be critical in determining the survival characteristics of microbial inocula in soil.

INTRODUCTION

The introduction of bacterial populations into the soil environment is frequently followed by a decline in viable cell concentration. This decline ceases when a characteristic survival concentration is reached, which appears to be independent of the initial inoculum size (Crozat et al., 1987). The grazing

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activities of indigenous protozoa are considered to be a significant component in the survival and establishment of bacterial inocula (Habte and Alexander, 1978).

The predatory activities of protozoa are considered to be affected by the matric potential of the soil, since protozoa are dependent upon water for their dispersal and movement through the soil (Sleigh, 1973). The largest protozoan populations are found to exist in saturated soils, and the lowest concentrations in dry soils (Darbyshire, 1976). Postma et al. (1989) observed an increased survival rate of bacterial cells introduced into relatively dry soils in comparison to those inoculated into wetter soils, and suggested that this resulted from reduced protozoan predation.

The heterogeneous and discontinuous structure of soil provides a number of distinct or temporally discrete microhabitats, which are influenced by environmental fluctuations (Hattori and Hattori, 1976). Alexander (1981) proposed the importance of such microsites in the survival of bacterial inocula where they exclude the predator and protect the prey.

At low matric potentials soil water will be restricted to those pores with small size diameters. The predatory activities of the protozoa under such conditions are thought to be restricted, since they are denied access to their prey due to their larger size. Postma and Van Veen (1990) described the habitable and protected pore space in the soil matrix by varying the matric potential of the soil with reference to the moisture release characteristic. Heijnen and Van Veen (1991) found that pores with neck size diameters less than 6 μm positively affected the survival of introduced bacteria, whereas pores with neck size diameters greater than 6 μm had a negative effect.

This article describes short-term laboratory experiments performed to investigate the effect of location on protozoal grazing of a genetically modified bacterial inoculum in soil. Bacterial inocula were located in distinct pore size classes with reference to the soil moisture release characteristic. Protozoa were introduced at matric potentials sufficient to ensure their location in larger pores. Introduction of bacteria into pores with small neck size diameters (<6 μm) was intended to spatially compartmentalise the predator and prey.

MATERIALS AND METHODS

Soil type and preparation

A sandy loam soil from the Insch series (Grid ref. NJ659223) was used. Soil samples were taken from grass ley sites ensuring minimal pesticide contamination. The soil was sieved to collect the fraction of particle size less than 3 mm and was air dried and stored at 4°C. The soil moisture release characteristic was determined by equilibration of initially saturated soil on pressure membrane apparatus. Figure 1 shows the relationship between soil matric

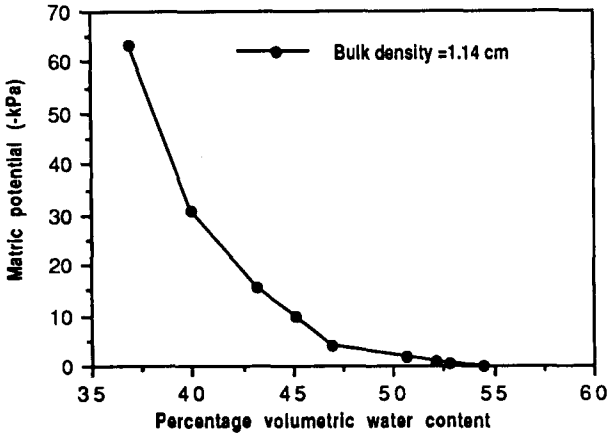


Fig. 1. Moisture release characteristic of repacked Insch soil.

potential and moisture content of repacked Insch soil with a bulk density of 1.14 cm^{-3}.

Bacterial strain and growth conditions

Pseudomonas fluorescens 10586s FAC510 was kindly donated by S. Amin-Hanjani and contains chromosomally borne *lux* A and B genes and genes encoding resistance to kanamycin, spectinomycin, ampicillin and rifampicin. Incorporation of these genes has no detectable effects on the specific growth rate or fitness of the host strain (S. Amin-Hanjani, pers commun., 1991). The bacterium was routinely cultured on LB-broth containing 20 μg kanamycin ml^{-1} at 30°C on a rotary shaker (150 rpm) for 48 h. Prior to inoculation, cells were grown to the mid-exponential phase and harvested by centrifugation (8800*g*). The cells were resuspended in sterile phosphate buffer (15 mM) and starved overnight at room temperature.

Protozoan strain and growth conditions

Colpoda steinii, an indigenous soil protozoan, was kindly donated by Dr. J. Darbyshire. The protozoa were cultured on *Pseudomonas fluorescens* cells in Stanier's medium (Stanier, 1947) supplemented with 0.1% peptone and 0.1% glucose.

Soil microcosm studies

Short-term studies of survival of *Pseudomonas fluorescens* were carried out in soil microcosms, consisting of 50 mm (diameter) sterile Petri dishes into which 9.59 g air-dried soil was placed. The soil formed a 6 mm deep layer

corresponding to the height of two soil aggregates. Microcosms were maintained in polystyrene boxes at 25°C in a constant temperature room for a period of 270 h.

Two treatments were involved in the short-term experiments. In Treatment 1 the bacterial inoculum was located in pores with neck size diameters <6 μm, whilst a buffer zone created by the addition of water separated the bacteria from the protozoa, which were situated in pores with neck size diameters between 30 and 60 μm. In Treatment 2 the small pores (<6 μm) were filled by water. The bacterial inoculum was located in pores with neck diameters 6–30 μm adjacent to the protozoan inoculum, which was located as in Treatment 1 (Fig. 2).

The soil matric potential was raised to -5 kPa over a 30 h period. The bacterial and protozoan inocula, along with the water to create buffer zones, were evenly distributed over the soil surface in a dropwise manner. Even distribution was facilitated by placing a 1 cm^2 grid over the microcosm surface during inoculation and allowing sufficient time for equilibration.

The matric potentials necessary to locate inocula in distinct pore size classes (<6 μm, 6–30 μm and 30–60 μm) were determined by calculating the effective pore neck size diameter (d, μm) as $d=300/$matric potential in kPa. The volumetric water contents required to achieve the desired soil matric potentials were derived with reference to the moisture release characteristic (Fig. 1). The initial matric potential in the microcosms (-50 kPa) was confirmed using the filter paper method described by Graecen et al. (1989), after a 24 h

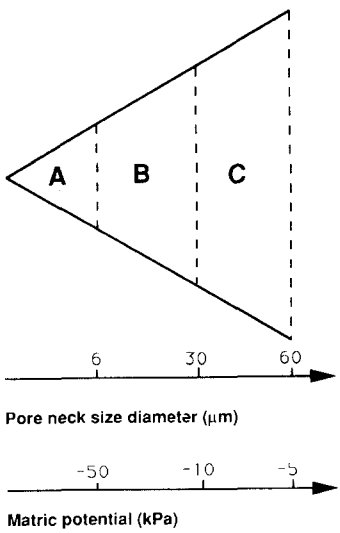


Fig. 2. Schematic representation of the pore neck diameters in which the inocula were located and the matric potential of the soil after equilibration. Treatment 1: A=bacteria, B=water, C=protozoa/water. Treatment 2: A=water, B=bacteria, C=protozoa/water.

equilibration period. The matric potential was raised to -10 kPa over a 6 h period and finally to -5 kPa at 30 h.

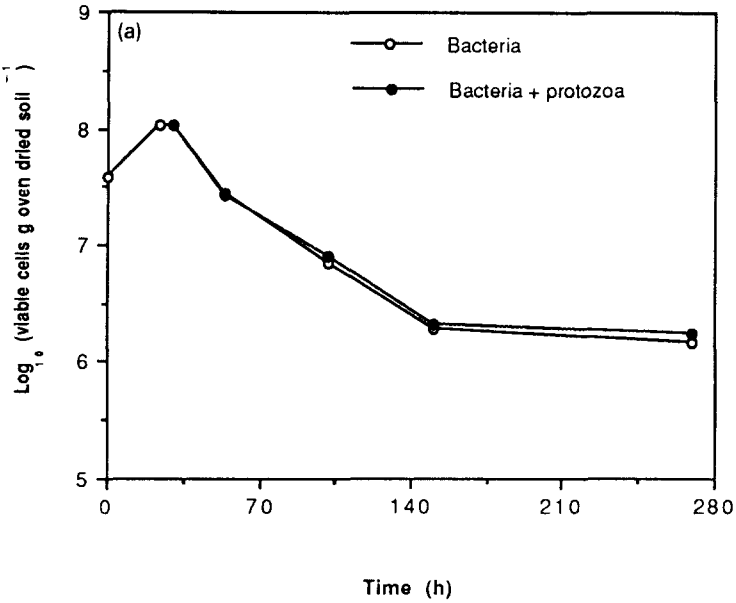
Sampling procedure

Triplicate microcosms were sampled destructively at 0, 24, 30, 54, 102, 150, and 270 h. Soil samples (0.5 g) were transferred to 1 ml phosphate buffer (15 mM) and vortexed for a 10 s period. Viable cell concentrations of *Pseudomonas fluorescens* were determined by dilution plating on *Pseudomonas* minimal medium (Panopoulas et al., 1975) containing 20 μg kanamycin ml^{-1}, 50 μg ampicillin ml^{-1}, 50 μg spectinomycin ml^{-1} and 100 μg cycloheximide ml^{-1}. The effect of location and the presence of protozoa on the bacterial inoculum population dynamics was examined with two way analysis of variance using the Minitab statistics package.

RESULTS

The influence of the location of *Pseudomonas fluorescens* in pores with small neck size diameters (<6 μm) in the presence and absence of a *Colpoda steinii* inoculum (final concentration 5.4×10^2 cells per g oven-dried soil) was studied in Treatment 1. At 0 h the bacterial inoculum (final concentration 8.1×10^7 viable cells per g oven-dried soil) was added to the microcosm. The filter paper method confirmed the location of the liquid component of the inoculum in pores with neck sizes <6 μm, since the water content of the filter paper corresponded to a suction of -54 kPa. It has shown that different distribution patterns of bacterial cells can be achieved by inoculating at different initial moisture contents (Postma et al., 1989). However, adsorption of bacterial cells to soil sites other than those desired may have occurred particularly at low matric potentials. Viable cell concentrations in the sample taken at 0 h were less than those inoculated, due to difficulties in extracting all cells from soil and possible cell death resulting from the different environmental conditions. In the subsequent 24 h the viable cell concentration rose to 1.1×10^8 cells per g oven-dried soil. The increase in matric potential to -5 kPa at 30 h was followed by a decline in viable cell concentration by approximately two orders of magnitude over a 240 h period in the presence and absence of *C. steinii* (Fig. 3a). The survival rate of *Ps. fluorescens* in pores with small neck size diameters was not significantly different in the presence and absence of *C. steinii* (5% level of significance).

In Treatment 2 the decline of *Ps. fluorescens* viable cell concentration located in larger pores (6–30 μm) was studied in the presence and absence of a *C. steinii* inoculum (final concentration 5.4×10^2 cells per g oven-dried soil). At 24 h the bacterial inoculum (final concentration 7.2×10^7 viable cells per g oven-dried soil) was introduced to the microcosm to raise the matric poten-



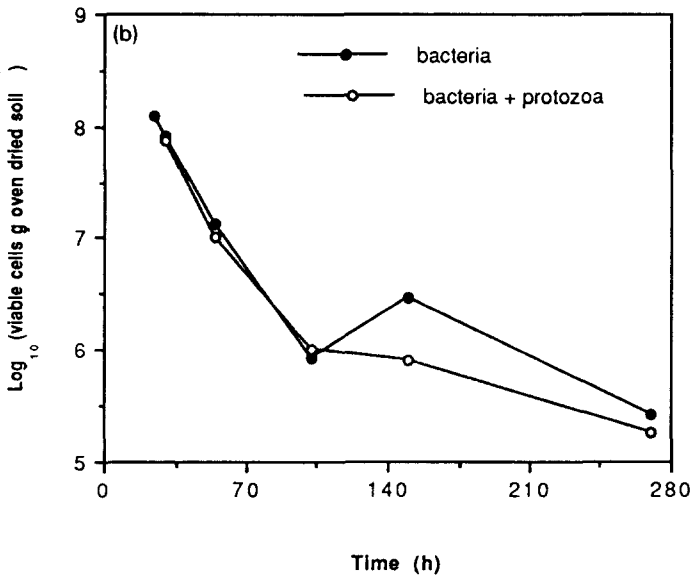


Fig. 3. (a) Population dynamics of *Pseudomonas fluorescens* located in pores with neck diameters less than 6 μm, in the presence and absence of *Colpoda steinii*. The majority of standard error values were less than 15%. (b) Population dynamics of *Pseudomonas fluorescens* located in pores with neck diameters between 6 and 30 μm, in the presence and absence of *Colpoda steinii*. The majority of standard error values were less than 15%.

tial to −10 kPa. The viable cell concentration declined by approximately three orders of magnitude in the presence and absence of *C. steinii* (Fig. 3b). The population dynamics of *Ps. fluorescens* located in larger pores did not differ significantly (5% level of significance) in the presence and absence of *C. steinii*.

The apparently different survival rates of *Ps. fluorescens* located in distinct pore size classes were analysed. It was found that the survival of bacteria located in pores with small neck size diameters was significantly higher, in the presence (5% level of significance) and absence (1% level of significance) of protozoa, than when located in pores with larger neck size diameters.

DISCUSSION

Pseodomonas fluorescens located in pores with neck size diameters <6 μm exhibited greater survival than those situated in pores with neck size diameters between 6 and 30 μm. The addition of *Colpoda steinii* produced no significant effect on the bacterial populations located in the two pore size classes. This lack of influence may be due to the presence of concentrations of indigenous soil protozoa at levels equivalent to that provided by the inoculum, or with greater activities. Sleigh (1973) reported that protozoan numbers in moist soil may range between 10^3 and 10^5 cells per g soil. Postma and Van Veen (1990) found a more pronounced decrease in rhizobial cells in natural soil than in sterile soil at higher soil moisture contents. This decrease was attributed to biotic factors such as protozoan predation. The protozoan inocula provided 5.4×10^2 cells per g soil, which may not have significantly increased predation above that carried out by the indigenous population.

Previous attempts to describe the effects of bacterial location in distinct pore size classes on inoculum survival have relied on the introduction of inocula into soils of varying moisture contents (Postma and Van Veen, 1990; Heijnen and Van Veen, 1991). The observed increase in survival of introduced rhizobia in drier clay treated soils compared to wetter ones may be attributable to a discontinuous water film present in drier soils (Heijnen and Van Veen, 1991). Vargas and Hattori (1986) demonstrated the reduced grazing activity of protozoa in dry soils. This may be considered in terms of their exclusion from pores containing their prey due to their size, or their dependence on sufficient soil water for dispersal and movement. Similar studies were carried out by Kuikman et al. (1990), although in their experiments the final matric potential was much lower than used here, restricting the movement of protozoa. In addition, protozoa and bacteria were inoculated into different portions of soil before mixing. The experiments discussed in this paper were performed at close to field capacity (−5 kPa), thereby eliminating any reduction in protozoal grazing activity due to insufficient soil moisture which may restrict the movement or dispersal of protozoa. The enhanced

survival of bacteria located in pores with small neck diameters is therefore thought to be due to the pore size characteristics rather than the moisture content of the soil. This suggests that the antecedent matric potential and the pore size characteristics will be critical in determining the survival of microbial inocula, genetically modified or otherwise, in soil.

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