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Note

Artificial soil microcosms: a tool for studying microbial autecology under controlled conditions

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Abstract

A novel artificial microcosm containing all the essential chemical components of soil, but with reduced heterogeneity and biological complexity, has been developed. Its utility for supporting realistic microbial populations was demonstrated and an example of how competing bacteria can be studied is illustrated.

Keywords: Artificial soil microcosms; Microbial autecology; Controlled conditions

One of the major terrestrial habitats for microorganisms is soil. Each gram of soil may contain up to 10^{10} microbial cells (e.g. Bundt et al., 2001; Roane and Pepper, 2000) which are vital for a variety of ecologically important processes. Despite this, there is a paucity of knowledge concerning the functions of, and interactions between, microbes in the soil. This is mainly due to the difficulty of studying bacteria in soil, brought about by the heterogeneous nature of the physicochemistry and the complexity of the biological communities that inhabit it (Liesack et al., 1997). The role of individual bacterial types in soil processes is typically determined by adding a marked isolate back to the natural system to facilitate its differentiation from the bulk community. However, for a variety of different reasons the population sizes of introduced microbes tend to decline rapidly (van Veen et al., 1997). Biotic factors such as predation and competition may be important and in some studies soil has been sterilized to reduce biological complexity (Hill and Top, 1998), but this may have major effects on its physicochemical properties. In particular it may cause the breakdown of macromolecules so releasing excess nutrients, and rendering subsequent experiments unrepresentative of natural soil. In contrast, simple single substrates such as sand or vermiculite have been used as model systems (e.g. Brimecombe et al., 2000; Simon et al., 2001), but these typically lack many properties or compounds which will affect microbial function (such as humic acid (Visser, 1985)).

To address this shortfall, an artificial soil substrate was constructed that permitted the study of microbes in a habitat similar to natural soil, but lacking factors that complicate experimental work. The aim was to provide all of the normal components found in soil, such as sand, clay and humic acid, under aseptic conditions, without resorting to steam sterilization procedures.

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Table 1
Typical proportions of dry components added to artificial soil mix

Component	Supplier	Amount (g)
Sand	Sigma-Aldrich (Poole, UK)	35
Kaolinite (1:1 clay)	Sigma-Aldrich (Poole, UK)	10
Bentonite (2:1 clay)	Sigma-Aldrich (Poole, UK)	5
$CaCO_3$	Acros (Loughborough, UK)	0.1
Humic acid	Acros (Loughborough, UK)	1

Individual components of soil can be purchased from chemical suppliers and combined in appropriate proportions. Manufacturing and packaging processes lead to products that have negligible microbial contamination (i.e. below limits of detection; see below), so provided that aseptic techniques are used in the set-up, no further sterilization is required. According to the soil textural triangle a sandy clay loam soil consists of seven parts sand to three parts clay (Burden and Sims, 1999). However, the type of clay (different arrangements of tetrahedral [Si-based] and octahedral [Al/Mg-based] sheets) is also important as this influences water holding capacity and the binding of minerals (for more detail, see Barton and Karathanasis, 2002). Therefore a mixture of two of the more common types (1:1 and

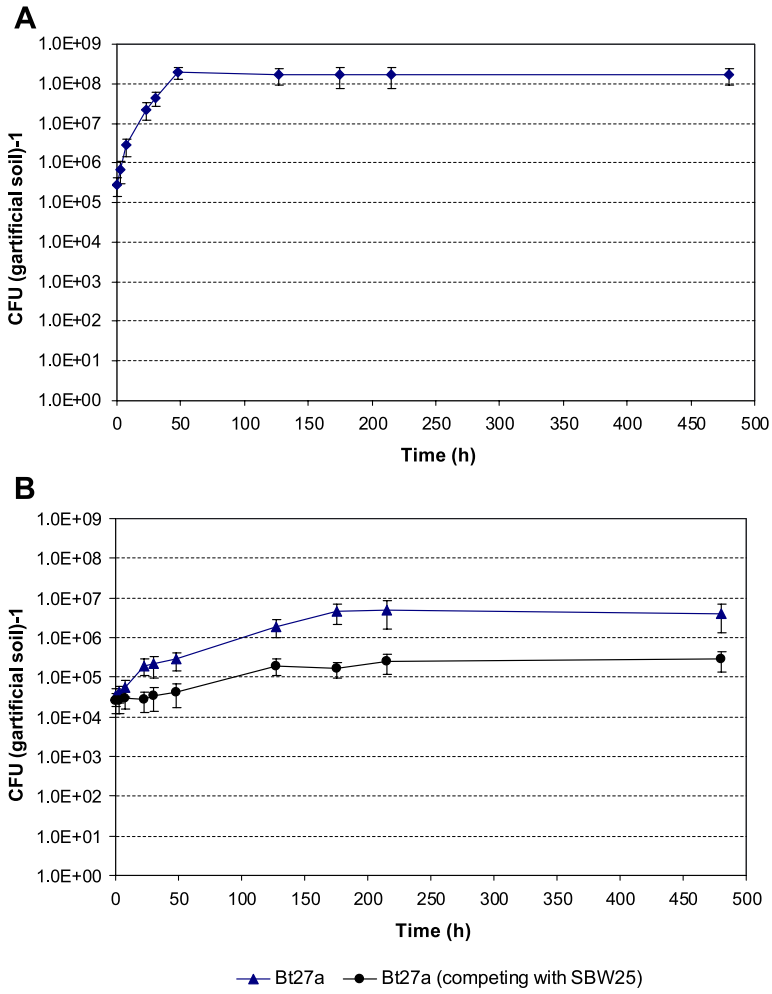

Fig. 1. Dynamics of bacterial populations in artificial soil microcosms. (A) Growth and persistence of *Pseudomonas fluorescens* SBW25. (B) Growth, persistence and effect of a competing pseudomonad population on *Bacillus thuringiensis* BT27a. Error bars indicate standard deviation (SD; $n = 3$).

2:1) was used for the artificial soil described here. In addition, calcium carbonate and a commercial preparation of humic acid were used to provide pH/cation exchange capacity adjustment (Ming, 2002) and nutrients, respectively. The exact proportions of the dry constituents are given in Table 1. These were mixed together by shaking in a sealed container until the resulting blend appeared to be homogeneous. The artificial soil was then hydrated with sterile water (20–30% w/w) and stirred with a sterile spatula. At this point it was possible to add bacteria to be studied as a suspension in the water. Other items (e.g. malt extract to mimic root exudates, xenobiotic compounds, competing organisms, etc.) can also be added at this stage. As the bacteria to be studied are added to the water prior to mixing this ensures that their distribution is as even as possible throughout the soil matrix. The ratios of the different components can also be adjusted to resemble any soil type of interest.

Microcosms typically consisted of 50 g of dry component mix hydrated with 15 ml water in a plugged 250 ml conical flask. Bacterial population densities were monitored by using standard plating procedures utilizing selective media where necessary. Using this approach microbial counts were below the limits of detection (i.e. <150 CFU (g wet soil)$^{-1}$) in uninoculated artificial soil at time 0 and remained below 3×10^3 CFU (g wet soil)$^{-1}$ for at least 20 days. Initial experiments demonstrated that an inoculum of *Pseudomonas fluorescens* SBW25 (Bailey et al., 1995) at 5×10^5 CFU (g wet soil)$^{-1}$ quickly colonised the artificial soil, leading to population sizes of approximately 2×10^8 SBW25 CFU (g wet soil)$^{-1}$ (Fig. 1A). A population size such as this is comparable to total microbial densities found in natural soils (Prosser, 1997) or maximum rhizosphere populations of SBW25 achieved in glass-house grown plants (Thompson et al., 1995). Furthermore, this population was maintained for at least 20 days. The population size achieved was independent of the original inoculum size, but could be increased by the addition of low levels of labile nutrients in the form of malt extract (results not shown). Thus, it appears that 'naturalized' bacterial populations can be established under aseptic conditions in these microcosms. In contrast, inoculation of the soil with an isolate of *Bacillus thuringiensis* (Bt27a) resulted in populations of only 4×10^6 CFU (g wet soil)$^{-1}$ (Fig. 1B). When SBW25 and Bt27a were added together, the pseudomonad out-competed Bt27a, resulting in a 14-fold smaller *B. thuringiensis* population than when it was added to the microcosms alone (Fig. 1B). Irrespective of the presence of competing cells the Bt27a populations also persisted at the same levels for the duration of the experiment (20 days). The population size or persistence of SBW25 was not significantly altered by competition with BT27a. This demonstrates that the artificial soil microcosms can be used to study competition and other interactions between different microbes. Furthermore, population dynamics are highly reproducible, both between replicate microcosms set up at the same time and between repeated experiments set up completely independently (coefficient of variation $<5\%$).

It is envisaged that there are number of different uses for this system, such as studying competition between co-habiting microbes (as described above), the effects of xenobiotics, and the extent of horizontal gene flow. The impacts of fundamental parameters such as temperature and water tension could also be addressed using a system similar to the one described here. The microbial communities can be as simple or as complex as required; each species can be grown independently and added at predetermined levels. Preliminary assessments have also shown that the nematode, *Caenorhabditis elegans*, can persist for at least a week in these microcosms, opening the way for investigations of multitrophic interactions.

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