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Comparison of microbial numbers in soils by using various culture media and temperatures

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Summary

The influence of different media and incubation temperatures on the quantification of microbial populations in sorghum, eucalyptus and forest soils was evaluated. Microbial growth was compared by using complex (tryptone soybean agar, TSA, casein-starch, CS, and Martin) and saline (Thorton, M3, Czapeck) media and incubation temperatures of 25 and 30°C. Higher numbers of total bacterial and fungal colony-forming units (CFU) were observed in sorghum soils, and of spore-forming and Gram-negative bacteria in forest soils than other soils. Actinomycetes counts were highest in forest soil when using CS medium at 30°C and in sorghum soil at 25°C in M3 medium. Microorganism counts were dependent on the media and incubation temperatures. The counts at temperatures of 30°C were significantly higher than at 25°C. Microbial quantification was best when using TSA medium for total and spore-forming bacteria, Thorton for Gram-negative bacteria, M3 for actinomycetes, and Martin for fungi.
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Introduction

Bacteria and fungi, among soil organisms, actively participate in organic matter decomposition liberating chemical nutrients and furthering plant growth. Microorganism numbers vary in and between different soil types and conditions, with bacteria being the most numerous. Bacterial counts in different soils ranged from 4×10^6 to $2 \times 10^9\,g^{-1}$ dry soil (Whitman et al., 1998).

Growth of microbial populations and their action on soils are dependent on the interaction between plant species and soil (Grayston et al., 1998). According to Marschner et al. (2001), bacterial community composition results from the interaction between soil type, plant species and its rhizosphere localization. In a comparison of counts in corn, soybean and bare soils, fungal but not bacterial populations were influenced by the soil type. Nevertheless, both groups of microorganisms

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were affected by plant type (Buyer et al., 2002). Similar reports have shown that the size and structure of microbial populations are affected by soil type and plant species (Wieland et al., 2001; Pinto and Nahas, 2002). However, a study made on three soils in England, showed that the primary determinant of composition in bacterial communities was the soil type (Girvan et al., 2003).

Soil microorganisms have been isolated and quantified using several selective and non-selective culture media (Sorheim et al., 1989; Buyer 1995; Tabacchioni et al., 2000) that have some advantages over non-culturable methods, allowing for taxonomic, genetic and functional studies in the isolated microorganisms.

Different growth rates are often seen in microbial populations from different soils, which have different nutrient requisites that are not supplied by the media (Kennedy and Gewin, 1997). Thus, many media containing different nutritional requirements have been suggested for the estimation of bacterial diversity (Balestra and Misaghi, 1997), which include saline media prepared using a chemically defined mixture of salts and a carbon source, as well as complex media, rich in polypeptides, amino acids and vitamins or minerals (Seeley et al., 1991).

Soil or rhizosphere organisms include total, spore-forming and Gram-negative bacteria, actinomycetes and fungi, but only a small percentage (1%) are culturable, even when using of a set of media (Bakken, 1997). The results relating to group distributions and densities are thus affected by the choice of the media (Buyer, 1995). Furthermore, different incubation temperatures have been reported for the optimal growth of bacteria and fungi. Considering the soil type, culture media, and the time and temperature of incubation, the counting results may be different between authors (Devliegher and Verstraete, 1995; Buyer and Kaufman 1996; Cattelan et al., 1998; Grayston et al., 1998; Scott and Knudsen, 1999; Taylor et al., 2002).

The aim of this study was to compare the growth of different groups of microorganisms using complex and saline culture media at two incubation temperatures, 25 and 30 °C, and to evaluate factors which allow maximal microbial counts in soils with different plants.

Materials and methods

Soils

Sub-samples (20) from an Oxisol were randomly collected from the superficial layers (1–20 cm) of forest (tropical rain forest), sorghum and eucalyptus soils and pooled to form composite sample from each soil. These samples were homogenized and spread in trays to be cleaned of extraneous materials (pieces of root, leaves, small stems, etc.) followed by drying and storing in plastic containers. When used for microbial counts, the samples were sifted with 2-mm mesh sieves, hydrated to $\frac{3}{4}$ of the field capacity and incubated for 5 days. This was a standard procedure whenever a new group of organisms was studied.

Microbial counts

Ten grams of each soil sample were added to 95 mL of 0.1% (w/v) solution of sodium pyrophosphate. After homogenization for 30 min, this solution was decimally diluted (10^{-1} to 10^{-7}) and aliquots of the resulting solutions plated on appropriate culture media. After incubation at 25 or 30 °C, for up to 10 days, the colony forming units (CFU) were counted.

Culture media

The media selected were among the most frequently cited in the literature, such as the complex media: Tryptone Soya Agar (TSA, Oxoid, Basingstoke, Hampshire, England), Martin (1950) and casein-starch (Kuster and Williams, 1964) and the saline media: Thorton (Sorheim et al., 1989), M3 (Rowbotham and Cross, 1977) and Czapeck (Acea and Carballas, 1990). TSA and Thorton media were utilized for the counting of total, Gram-negative and aerobic spore-forming bacteria. To count Gram-negative bacteria, 5 μg mL^{-1} crystal violet and 100 μg mL^{-1} cycloheximide were added to these media. Spore-forming bacteria were counted after being placed in serial dilution tubes at 80 °C for 10 min and then cooled to room temperature prior to the inoculation of the media. Martin and Czapeck media were used for fungal growth and casein-starch and M3 for actinomycetes growth after the addition of 50 μg mL^{-1} nystatin, 50 μg mL^{-1} cycloheximide, 5 μg mL^{-1} polymixin B sulfate and 1 μg mL^{-1} sodium penicillin (Williams and Davies, 1965).

Statistical analysis

The SAS statistical package (SAS Institute, Cary, NC, USA) was used for data analysis. When a significant *F* value was detected, Tukey's estimates of honest significant differences (HSD) were calculated from the ANOVA analysis. The level of

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