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Characterization of cultivable heterotrophic bacterial communities in Cr-polluted and unpolluted soils using Biolog and ARDRA approaches

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Abstract

The bacterial communities of two soils with different chromium levels were characterized by Biolog carbon substrate utilization patterns and amplified 16S ribosomal DNA restriction analysis (ARDRA). For each bacterial community sample, cell suspensions containing 10,000 or 100 colony-forming units (CFU) were inoculated in each well of Biolog-GN microplates. The number of carbon compounds utilized by the bacterial community consisting of 100 CFU from unpolluted soil was significantly lower than that detected for the bacterial community consisting of 10,000 CFU. The size of inoculum did not substantially influence the percentage of carbon sources utilized by the Cr-polluted soil bacterial community. ARDRA approach was applied to about 100 bacterial isolates for each soil sample. A similar number of clusters for Gram-negative bacteria were found in both soils, but there were differences in percentages of isolates belonging to each group and specific genomic groups were found in each soil. *Pseudomonas* was the dominant taxon in both soils. Comparing the ARDRA clusters obtained from Gram-positive isolates it was evident that the culturable bacterial communities of Cr-polluted and unpolluted soils were dominated by the genus *Arthrobacter* and the genus *Bacillus*, respectively.

Keywords: Chromium; Biolog; ARDRA; *Arthrobacter*; *Bacillus*; *Pseudomonas*

1. Introduction

Many agricultural and industrial activities have led to soil pollution with chromium. If chromium is an essential microelement to plant, animal and human life, elevated concentrations of chromium are toxic. In contrast with other heavy metals the oxidation state of chromium is important for exerting its toxic effect. While the trivalent form is considered not very harmful, the hexavalent state is highly toxic to all living forms and it has shown to have mutagenic and carcinogenic properties. Chromium can be present mainly as Cr(III) or Cr(VI) in soil. These two different oxidation states can inter-convert and, generally, the

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reduction of Cr(VI) to Cr(III) is favored, but high concentrations of Cr(VI) may overcome the reducing capability of the environment and thus Cr(VI) persists as pollutant (Cervantes et al., 2001); moreover, a part of Cr(III) can be transformed to Cr(VI) in Bartlett positive soils (Bartlett, 1997). Therefore, the potential risk of oxidation of Cr(III) to Cr(VI) in soil suggests that the effect of chromium-pollution in soil needs further scientific attention. In particular, since the microbial community can be altered by heavy metals without resulting changes in the overall performance of the soil system, it is necessary to deepen the knowledge about chromium effect on the structure and composition of soil microbial community (Brookes et al., 1997; Konopka et al., 1999). Recent studies have shown that a high chronic concentration of total chromium in soil affected both oxygenic phototrophic microorganisms and cultivable heterotrophic bacteria, and the percentage of Cr(VI)-tolerant bacteria was heavily dependent on soil total chromium content (Viti and Giovannetti, 2001). Results obtained using soil microcosms indicated that chromium decreased microbial activity and led to soil organic carbon accumulation (Shi et al., 2002). A better understanding of the microbial community response to chromium stress not only will permit to deepen the knowledge upon the significance and impact of chromium in soil, but could contribute to the implementation of a bioremediation strategy for chromium-polluted environments.

The objective of this study was to investigate the impact of long-term chromium exposure on the cultivable heterotrophic bacterial community of soil and to determine if high concentrations of chromium could select specific bacterial populations. To this end the cultivable heterotrophic bacterial communities were characterized by analysis of the physiological profile and genotypic structure, in a soil chronically polluted with a high concentration of chromium and in a comparable unpolluted soil.

2. Materials and methods

2.1. Soils

Soils were sampled in two sites in Ivano-Frakovsk (UKR). Soil 1 was taken from an area inside a leather tannery and soil C outside the perimeter of the same leather tannery. Both soils were grass-covered.

Five soil cores (15–20 cm depth × 6 cm diameter) were take from each soil. Cores, after removing plant material, stones and visible soil fauna, were mixed in plastic bags and were stored at 4 °C in sterile containers until further use. Soil samples were sieved (2 mm) before use.

The main chemical–physical characteristics of soils, determined in a previous paper by Viti and Giovannetti (2001), are given in Table 1.

2.2. Biolog carbon substrates utilization patterns

From each soil, the bacterial cells were extracted as described by Hitzl et al. (1997). The inoculum density was estimated by the plate count method and the inoculum was adjusted in order to inoculate 10,000 colony-forming units (CFU) or 100 CFU in each Biolog-GN well. Three Biolog-GN microplates were used for each inoculum type. The Biolog microplates were incubated at 25 °C for 208 h. Changes in color intensity were measured several times after the first appearance of color, using a DV990BV4 computer-assisted microplate reader (GDV, Italy) at 590 nm. The optical density values in each well were corrected for the background value of the control wells. A well of a Biolog microplate was assessed as positive if the absorbance at each time point was $\geq$0.400 units (Verschuere et al., 1997).

2.3. Bacterial strains, growth conditions and DNA extraction

About 100 strains of each soil were randomly selected among isolates obtained in a previous work (Viti and Giovannetti, 2001). The selection was carried out on the base of the ratio of Gram-negative/Gram-positive isolates found in unpolluted soil C, which showed the lowest number of Gram-positive bacteria (Viti and Giovannetti, 2001). Strains were cultivated on Luria Agar medium (5 g L^{-1} tryptone, 2.5 g L^{-1} yeast extract, 2.5 g L^{-1} NaCl and 0.5 g L^{-1} D-glucose) at 25 °C. Type strains (^{T}) and reference strains (*Ralstonia eutropha* DSM 531^T, *Pseudomonas putida* DSM 291^T, *P. fluorescens* DSM 50090^T, *Raoultella planticola* DSM 3069^T, *Rhizobium rhizogenes* DSM 30148^T, *Curtobacterium flaccumfa-*