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T-RFLP analysis of bacterial communities in cyclodextrin-amended bioreactors developed for biodegradation of polychlorinated biphenyls

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Abstract

In this study, T-RFLP analysis was used to determine the structure and spatial distribution of the indigenous bacterial community of an actual-site PCB-contaminated soil treated in aerobic packed-bed loop reactors (PBLRs) in the absence or in the presence of a mixture of randomly methylated β-cyclodextrins (RAMEB) at 0.5 or 1% w/w. RAMEB was found to significantly enhance the aerobic bioremediation of soil with effects that increased proportionally with the concentration at which it was applied. At the end of treatment (180 days), T-RFLP analysis of the soil samples collected from the top and bottom regions of the PBLRs showed a series of 50 single T-RFs. Remarkably, the number of T-RFs was significantly lower (13–22) in samples collected from different sections of the RAMEB-amended bioreactors with respect to equivalent samples collected from the RAMEB-free reactor. Cluster analysis based on the presence or the absence of T-RFs peaks revealed high similarity, inside each reactor, between the top and bottom parts of its soil bed. Soil samples collected at the top and bottom regions of the two bioreactors amended with RAMEB, clustered together while the equivalent samples of the bioreactor without RAMEB formed a separate cluster which was distantly related to the soil samples obtained from the parallel amended bioreactor. Notably, T-RFLP analyses combined with extensive sequencing of 16S rDNA allowed us to tentatively allocate a series of bacterial species corresponding to specific peaks of the T-RFLP profiles and to determine their phylogenetic affiliation.

Keywords: Bioreactor; Cyclodextrins; PCB degradation; *Proteobacteria*; T-RFLP analysis

1. Introduction

Polychlorinated biphenyls (PCBs) are toxic organic compounds of great ecological concern present in large amounts throughout the environment, and in particular in soils and sediments [2,18]. However, several bacterial species contain various aerobic and anaerobic pathways by which PCBs can be degraded depending upon the specific PCB and the physical conditions present in the environment [2]. Specifically, PCBs occurring in soils can be partially biodegraded by aerobic consortia of PCB-cometabolizing bacteria and chlorobenzoic acid (CBA)-mineralizing bacteria [17].

The low bioavailability of PCBs is the main factor which negatively affects PCB biodegradation in contaminated soil; indeed, PCBs tend to adsorb strongly onto soil organic matter, thus becoming poorly available in the soil water-phase in which the PCB- and CBA-degrading microorganisms are mainly located [30]. Soil supplementation with oxygen, biphenyl, and exogenous specialized bacteria, along with treatments providing a high degree of soil mixing and heterogeneity, can improve the efficiency of the PCB biodegrading process [7,12,31]. The bioavailability of PCBs in soils may also be enhanced by supplementing them with

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a suitable surfactant [40]. Synthetic surfactants have been tested for this purpose with no consistent results [10,22,40]. In addition, they often have proven to be recalcitrant toxic compounds in the amended matrices [10,40].

During the past decade, several studies have demonstrated that cyclodextrins (CDs) can be advantageously employed to markedly increase PCB bioavailability and aerobic biodegradation in contaminated soils [8,9,11]. Recently, an industrially produced mixture of randomly methylated β-cyclodextrins (RAMEB) has been successfully applied to improve the aerobic biodegradation of PCBs in different pristine soils spiked with a PCB-containing transformer oil [9] and in the aerobic treatment of aged-contaminated soils [8]. In the latter study, RAMEB significantly intensified the aerobic bioremediation of two different historically heavily contaminated soils under solid-phase and saturated fixed-phase conditions, by enhancing the occurrence of both PCBs and specialized bacteria in the soil water phase. RAMEB effects increased by increasing the CD amount from 0.5 to 1.0% w/w and when they were applied to the packed-bed loop reactors, where degrees of homogeneity and mass transfer rates higher than those achieved in the corresponding solid-phase reactors probably occurred [8].

In previous studies, no detailed analysis of microbial communities occurring in the reactors during aerobic PCB biodegradation was performed. Information on the microbial population catalyzing the biochemical process is generally of great relevance for developing a new bioremediation strategy (like the RAMEB-based one), especially when it is based on the use of innovative bioreactor technology (i.e., the packed-bed loop reactor) and directed to very complex matrixes, such as actual-site contaminated soils and sediments. Molecular screening techniques such as T-RFLP or fingerprint analyses provide an alternative approach compared to more traditional methods for species isolation and identification based on both enrichment and selective media [1,39]. These molecular techniques are based upon the extraction of DNA along with comparative sequence analysis of 16S rRNA [15,26]. Recently, T-RFLP (terminal restriction fragment length polymorphism) analysis was used to describe bacterial communities in soil, marine, and fresh water-environments using the 16S rRNA gene or different functional genes [25,27,33,35]. T-RFLP is based on detection of a single restriction fragment in each sequence, directly amplified from the environmental sample DNA, and is capable of surveying dominant members comprising at least 1% of the total community [6]. Furthermore, the T-RFLP fingerprint method in association with cloning and sequencing analysis enables assessment of the spatial heterogeneity of microbial communities in natural or artificial environments [32].

In the present study, we used T-RFLP analysis to study the structure and spatial distribution of bacterial soil communities occurring in a packed-bed loop reactor (PBLR) recently employed by our research group to treat a PCB-contaminated soil (named S1) in the presence or absence of RAMEB [8]. Our main objective was to evaluate the possible influence of RAMEB on the composition of bacterial communities involved in PCB degradation and how this effect was exerted at different depths of the packed bed and on the recycled mobile phase of each bioreactor. We concluded that significant modification of bacterial communities inhabiting the bioreactors amended with RAMEB took place. In addition, sequence analysis of 16S rRNA clone libraries allowed us to identify a few of the main bacterial genera present in the microbial community of the bioreactors following a long period of PCB degradation activity.

2. Materials and methods

2.1. Soil and mobile phase sample analyses

Soil and water samples were collected from three packed-bed loop reactors previously employed to assess the role of RAMEB in aerobic bioremediation of a contaminated soil, named S1 [8]. S1 was a sandy soil contaminated by about 890 mg/kg of medium-high chlorinated PCBs and significant quantities of a few toxic heavy metals. S1 contained large amounts of aerobic heterotrophic cultivable bacteria ($3.0 \times 10^8 \pm 2.0 \times 10^8$ CFU/g) and aerobic bacteria capable of growing on a mixture of 2-, 3- and 4-chlorobenzoic acids ($6.0 \times 10^4 \pm 1.0 \times 10^4$ CFU/g). S1 also contained a bacterial population growing on biphenyl at concentrations lower than 10^2 CFU/ml [8]. S1 was amended and treated in three packed-bed loop reactors, namely: one without RAMEB, a second with RAMEB at 0.5% w/w, and a third with RAMEB at 1.0% (w/w) as previously described [8]. At the end of the treatment (180 days), the reactors were opened and triplicate samples of about 50 g of saturated soil were collected at about 3 and 20 cm of the height (from the bottom) of each reactor along with triplicate samples (50 ml each) of the mobile phase recycled in each reactor. A portion of 20 g of each soil sample was then subjected to extraction with a mixture of hexane:acetone (1:1) using a pressurized fluid extraction system (Dionex Corp., Sunnyvale, CA) [8]. Qualitative and quantitative analyses of PCBs extracted from the different soil aliquots were performed using a gas chromatograph (GC, HP-5890 Series II), equipped with an HP-5 capillary column (30 m $\times$ 0.25 mm) and an electron captures detector (ECD; Hewlett–Packard Co., Palo Alto, CA) according to the procedures described by Fava et al. [11]. In particular, the % of PCB removal were calculated by comparing the average area values (calculated using data deriving from two successive GC analyses of each single sample) of each selected GC peak ascribed to a PCB (or a group of PCBs) detected in the organic extracts of the soil before and at the end of the treatment.

The other portion of each soil sample was subjected to DNA extraction as described below. Each mobile-phase sample was separated into 4 different portions: one (25 ml) was subjected to two successive batch extractions with