J Biochem Tech (2010) 2(5):S58-S59
ISSN: 0974-2328

Study of microorganisms degrading PCB in vegetated contaminated soil

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Received: 25 October 2010 / Received in revised form: 13 August 2011, Accepted: 25 August 2011, Published: 25 October 2011

Abstract

Removal of PCBs from contaminated soil is one of the challenges of environmental microbiology. In our study, we aimed to isolate, characterize and identify microorganisms from contaminated soil and to find out the plant effect on microbial diversity in the environment. Microorganisms were isolated by two ways, direct extraction and isolation after cultivation with biphenyl as a sole source of carbon. Isolated bacteria were biochemically characterized and the composition of ribosomal proteins in bacterial cells was determined by mass spectrometry MALDI-TOF. Bacteria with required properties were chosen and the *bphA* gene was amplified and detected. Bacteria with detected *bphA* gene were then identified by 16S rRNA sequence analyses.

Keywords: PCB, microbial diversity, *bphA* gene, 16S rRNA, MALDI-TOF.

Introduction

Polychlorinated biphenyls are organic compounds that were used in industry in the last century and during this time, large amounts of these compounds were released to the environment. Later on their toxicity was discovered and they use was stopped (Demnerova et al. 2005). Removing PCBs from contaminated soil using biological systems is inexpensive, easy and environmentally friendly process. Bacteria degrade PCBs by enzymes encoded by genes that are included in the biphenyl opern. The first gene of the biphenyl operon is the *bphA* gene encoding biphenyl dioxygenase (Abramowicz 1990). The amplification and detection of this gene is one of the way how to verify the degradative potential of bacteria.

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Materials and Methods

Samples were prepared from long term contaminated soil with four paralels vegetated by *Nicotiana tabacum* (tobacco) and four by *Solanum nigrum* (nightshade). Different bacterial species were inoculated to vegetated soil. The control sample was non-vegetated and with no bacteria added. Samples were kept in greenhouse for three months and then cultivable bacteria were isolated, characterized and identified. Bacteria were isolated by two ways, by direct extraction and after long term enrichment cultivation with biphenyl as a sole source of carbon and energy. Obtained isolates were characterized by biochemical tests, and the composition of ribosomal proteins was determined by mass spectrometry MALDI-TOF. The plant effect on microbial diversity was determined.

Results and Discussion

38 isolates were obtained after isolation by direct extraction from contaminated soil and 18 after cultivation with biphenyl. These isolates were biochemically and analytically characterized. Chosen isolates were analyzed by molecular-biological methods, *bphA* gene detection and 16SrDNA sequencing were performed in DNA of colonies growing on minimalm medium with biphenyl.

The experiments showed that plants significantly affect the microbial diversity in the rhizosphere. It was documented, that in tobacco rhizosphere different bacterial species than in nightshade rhizosphere were identified. More bacterial species able to degrade PCBs were detected in tobacco rhizosphere. These data correlate with findings published by Mackova et al. 2009, when tobacco was shown as the plant strongly supporting PCB degraders in studies carried out during ten years with different plant species. In our experiment only one bacterial species was isolated from both rhizospheres. The bacteria of the genus *Pseudomonas* were detected in all samples including the control sample.

Methods used for bacterial characterization and identification were compared. The fastest and the easiest analytical method is MS MALDI-TOF, but the proper and expensive instrument and specific database are needed. The 16S rRNA gene sequence analysis is still the most accurate method for bacterial identification and for studying microbial diversity in the environment.

Conclusion

Comparison of the methods for the characterization and identification of bacteria is one of the aim of environmental microbiology. In our study we cultivated two plant species in PCB contaminated soil and after several months of growth we compared microbial diversity in rhizosphere of both plants after straight extraction and enriched cultivation with several passages with biphenyl. After stright extraction 38 isolates were identified, 11 of them possesed *bphA* gene, enrichment isolation gave 18 isolates with 8 containing *bphA* gene. Described microorganisms obtained from these experiments can be used for bioremediation. Spesies identified were classified as genera *Pseudomonas*, *Achromobacter* and *Ochrobactrum*. After enrichment isolation *Pseudomonas mendocina* and *Pseudomonas alcaliphila* were identified. Other species were isolated after straight extraction from the soil. In rhizosphere of tobacco 7 bacterial species were identified in nightshade rhizospere only 4 species were detected. Analysis of PCB removal showed that the lowest concentration of PCB was measured in soil vegetated by tobacco with augmented bacteria of genus *Pseudomonas*. In this case almost 53% of original PCB concentration were removed.

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Acknowledgement

This work was supported by the grants GACR 525/09/1058 a MSM 6046137305, MSMT ME 09024.

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