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Katerina Demnerová[1*]
Martina Mackova[1]
Veronika Spevákova[1]
Katarina Beranova[1]
Lucie Kochánková[2]
Petra Lovecká[1]
Edita Ryslavá[1]
Tomas Macek[1,3]

Two approaches to biological decontamination of groundwater and soil polluted by aromatics—characterization of microbial populations

[1]Dpt. of Biochemistry and Microbiology, Faculty of Food and Biochemical Technology, Inst. of Chemical Technology Prague, Czech Republic
[2]Dpt. of Environ.Chemistry, Inst. of Chemical Technology Prague, Czech Republic
[3]Inst. of Organic Chemistry and Biochemistry, Dpt. of Natural Products, Academy of Sciences of the Czech Republic, Prague, Czech Republic

Summary. As part of the EU project MULTIBARRIERS, six new endogenous aerobic bacterial isolates able to grow in the presence of BTmX (benzene, toluene, *m*-xylene) were characterized with respect to their growth specificities. Preliminary analysis included restriction fragment length polymorphism profiles and 16S rDNA sequencing. The diversity of these strains was confirmed by denaturing gradient gel electrophoresis. Additional aerobic bacterial strains were isolated from the rhizospheres of plants grown in polychlorinated biphenyl (PCB)-contaminated soils. Pot experiments were designed to show the beneficial effect of plants on the bacterial degradation of PCBs. The effect of PCB removal from soil was evaluated and bacteria isolated from three different plant species were examined for the presence of the *bph* operon. [**Int Microbiol** 2005; 8(3):205-211]

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*Corresponding author:
K. Demnerova
Tel. +42-0220445172. Fax: +42-0220443075
E-mail: Katerina.Demnerova@vscht.cz

Introduction

Many industrial chemicals pollute the land surrounding manufacturing sites. They are also transported into the air or water, either through discharge into aquatic systems or as gaseous emissions that can be carried for considerable distances. Chemical pollutants reach the soil either directly, or as atmospheric fall-out, waste disposal, or industrial effluents. These chemicals may range from transient, low-toxicity compounds, which exert minor effects, to broad-spectrum, persistent biocides that have the potential to bioconcentrate in organisms and thus in the food chain. Urban organic wastes are generated in large quantities and, although most are disposed of in landfills, some reach the soil, particularly those of managed ecosystems. Among the main components of urban wastes are sewage biosolids, which may be heavily contaminated with pollutants such as heavy metals and pesticides. The negative effects of pollution on both the environment and human health are diverse and depend on the nature of the pollutant. Conventional technologies other than bioremediation used for in situ and ex situ remediation are typically expensive and destructive. They include solidification and stabilization, soil flushing, electrokinetics, chemical reduction/oxidation, soil washing, low-temperature thermal desorption, incineration, vitrification, pneumatic fracturing, excavation/retrieval, landfill, and disposal. Alternative methods already used for the removal of environmental pollutants

are bioremediation [1,4] and phytoremediation [3,5]. Biological systems [6–9] can be applied in situ without the need for removal and transport of the contaminated material. This approach is less expensive and less labor-intensive. In addition, it does not cause further pollution and is widely accepted by the public.

Over the last few decades, significant efforts have been devoted to studying the biodegradation of organic pollutants and the mechanisms involved [4,7,12,13]. Although various metabolic interactions have been investigated, many aspects remain to be examined to obtain a detailed overview of biodegradation in the environment and to optimize and predict in situ microbial degradation. Approaches to analyze and assess biodegradation have shifted towards the application of culture-independent methodologies to characterize natural and engineered pollutant-degrading microbial associations. An outstanding characteristic of bioremediation is that it is carried out in non-sterile open environments that contain a variety of organisms. Although bacteria, such as those capable of degrading pollutants, usually play central roles in bioremediation, other organisms (e.g. fungi and grazing protozoa, plants) also affect the process.

Our research is focused on bioremediation of different polluted media (water, groundwater, soil) with various contaminants, including benzene, toluene *m*-xylene (BTmX) and polychlorinated biphenyls (PCBs). In many cases, bioremediation involves combinations of several mechanisms. We have followed two approaches to biological decontamination [1,2,17]. In an early study, permeable barriers were constructed in contaminated groundwater systems to which degrading bacteria were added. This multibarrier system allows groundwater to flow through and degrade or remove soluble pollutants. The use of permeable, subsurface reactive barriers to groundwater pollution and contamination is becoming widespread in the United States and Canada [1]. In Europe, in 2001, an EU project was launched that aimed to develop and evaluate multifunctional permeable barriers (MULTIBARRIERS) carrying microbial biofilms for in situ treatment of groundwater containing mixed pollutants. MULTIBARRIERS combine several principles of barrier technology, and the removal of pollutants is based on both physico-chemical and biological activities. In a multibarrier system, pollutants in groundwater are treated in a trench built perpendicular to the groundwater flow and filled with a coarse material. In addition to adsorbing some compounds, the coarse material can be used to develop a biofilm comprising bacteria able to degrade toxic compounds.

Phytoremediation

Another approach recently described, involving biological systems and their consortia, is the use of plants and rhizosphere bacteria for remediation. Plants may contribute to remediation in several ways [1,5,7], including reducing off-site leaching of contaminants, aerating soil, and releasing compounds from the roots that selectively foster indigenous contaminant-degrading microorganisms [6]. Beneficial effects of vegetation have been reported in areas contaminated with metals as well as organic compounds [5,11]. Plants use solar energy, which is inexpensive and widely available; thus, evapotranspiration may be viewed as a solar driven pump-and-treat system that helps to bring contaminants to the rhizosphere and contain them on the site. Plants can also transform organic compounds that are assimilated through their roots, and the rhizosphere provides an excellent environment for the adsorption and microbial transformation of organic compounds (rhizoremediation) [10,11].

The rhizosphere could be defined as a biologically active zone of soil around plants roots that contains soil-borne microbes, including bacteria and fungi. Plants provide rhizosphere microbes with a carbon source. In turn, microbes provide nitrogen and phosphorus and other minerals through the decomposition of organic matter and by nutrient cycling; they also protect plants from parasites and pathogens. Root–microbial interactions also play key roles in several other ecosystem functions, such as the decomposition of organic matter and maintenance of soil structure [6,11] and water relationships. Rhizosphere interactions are based on complex exchanges that evolve around plant roots. Root-based interactions between plants and organisms in the rhizosphere are highly influenced by edaphic factors and involve both biological and abiotic climatic conditions, which influence the expression of a specific signal.

Plant–microbe interactions in the rhizosphere can be classified into three groups: (i) negative (pathogenic), leading to infectious diseases affecting only the plant kingdom [14]; (ii) positive (symbiotic), in which either one or both partners benefit from the interaction without harming the other; and (iii) neutral, in which none of the partners derives a direct benefit from the interaction and neither is harmed. Initial findings suggest that plants do select for taxonomic and functional groups in rhizosphere regions. Also, there is accumulating evidence that biotic interactions occurring underground play a major role in determining plant diversity above ground, by direct feedback on host growth and indirect effects on competing plants.

Before proceeding with the development of in situ phytoremediation and rhizoremediation technology, it is critical to identify plants that can survive and flourish for extended periods in contaminated soils under field conditions. In fact, the success of processes using plants may be due to the influence of the vegetation on the physical (water balance, transport processes), chemical (enzymes, redox potential, pH, complexing agents) [3], and biological (roots, microbes, mycorrhiza) factors in soil [18].

Permeable barrier technology: selecting bacteria for groundwater decontamination in a multibarrier system

The above-mentioned MULTIBARRIERS project was developed from laboratory scale up to the a pilot system in which an aquifer was simulated. The aim of the project was to test interactions among barrier materials and microorganisms in order to establish a multibarrier, and to identify microbial nutrients required for the optimal performance of the system. To this end, microbial populations were selected, the (bio)chemical/biological interactions taking place were identified, and the removal/degradation pathways of the pollutants were examined.

Culture estimation of the aquifer. The total number of culturable heterotrophic and specific pollutant-degrading microorganisms in the leachate material was determined by the conventional method of counting (Colony Forming Units, CFU) on R_2A agar plates (supplemented with 50 mg cycloheximide/l to inhibit fungus growth) after 24 h of oxic cultivation at 25°C. A total of 10^4 microorganisms per gram of moist material, which is a rather small number, was counted. However, this result comprises only culturable clones. Soil samples were previously found to contain a very large number of genetically distinct clones. Such heterogeneity of the community may be assessed by isolating total DNA from the material and subjecting it to polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE) using universal primers.

Primer selection and specificities. Several sets of primers have been designed to target different regions of the small subunit of ribosomal RNA (16S rRNA). Nine variable regions (V1–V9) can be distinguished in the rRNA genes of all living organisms. In bacteria, the rRNA gene is approximately 1520 bp long. The length of the PCR product is a limiting factor of DGGE/temperature gradient gel electrophoresis (TGGE); thus, only partial sequences, up to about 500 bp, are separated properly. Universal primers hybridize to evolutionarily conserved flanking regions. High microbial diversity and dramatic pattern changes may result from the presence or absence of only one or two microorganisms. Choosing variable regions with less interspecies diversity will therefore reduce the effect of operon diversities.

Enrichment of indigenous soil microbial associations. Batch-culture experiments were employed in this study to isolate an aerobic catabolic community able to use a mixture of the aromatic hydrocarbons benzene, toluene and *m*-xylene (BTmX), with tetrachloroethene (PCE, perchloroethylene) and trichloroethylene (TCE) either together or individually as sole carbon sources from the leachate material. The principal selection pressure was a rising concentration of pollutant (from 50 to 300 ppm of BTmX, and of PCE, TCE). The opalescence of the culture media indicated that toluene and *m*-xylene as well as the mixture were degraded easily under oxic conditions at 25°C. However, TCE and PCE were not degraded, despite their low toxicity, as was evident during the third transfer (no growth was visible in the flasks and chloride was not detected in the medium). Benzene seemed to persist under the initial conditions. After 8 weeks of cultivation, the sixth transfer was plated out on minimal medium agar plates, with toluene, *m*-xylene, and the organic mixture as sole carbon sources, and on R2A plates in order to isolate individual colonies.

Several different colonies were obtained, and a mixture of gram-positive and gram-negative rods characterized the associations. A 4-day old culture on minimal medium plates was sprayed with 100 mM pyrocatechol to detect those colonies bearing catechol 2,3-dioxygenase (reaction with the enzyme results in a visible yellowish zone of pyrocatechol cleavage within a few min). This enzyme plays a crucial role in the degradation of a wide range of pollutants [8]. Such colonies were then tooth-picked and transferred onto a fresh minimal medium plate and onto a LB plate (to check purity of the isolated strain). The plates were cultivated for one week at room temperature. The purity of isolated strains was then checked by PCR-DGGE. The pure strain was inoculated back into liquid minimal medium with TmX or the organic mixture to find out whether the isolated strain was actually the BTmX degrader. In case of growth on minimal medium, the culture was again subjected to PCR-DGGE to obtain information about the composition of the association. After 4 months of repeated isolations, pure cultures of seven strains (Table 1) with different specificity to BTmX were obtained. Gram-negative rods were accompanied by gram-negative cocci (small, barely visible colonies on MM agar plates), which actually were promoting growth on TmX and the mixture as sole carbon sources. As was later determined, the mixture of gram-negative rods and gram-negative cocci is able to cometabolize TCE in the presence of toluene. Therefore, the presence of cocci is vital for TmX, mixture degradation. After a long adaptation period, pure cultures of the strains were isolated and a mixture of two strains was obtained.

Isolation, identification, and characterization of the isolates. Six new endogenous aerobic isolates were thus obtained from landfill material from a Belgium plant site. The ability of these strains to grow on BTmX were subsequently tested within the MULTIBARRIER system project. Of the six strains, two were gram-positive, and their optimal growth temperature was 20°C. The preliminary RFLP profile of 16S rDNA revealed the diversity of the six strains, which

Table 1. Substrate specificities of isolated strains

Strain	Ben	Tol	Xyl	Mix
Ben1V	+	–	–	–
Ben 1 Ben	+	–	–	–
Ben 2	++	++	+++	+++
Xil 22	–	++	+++	++
Mix 2V	–	+++	+++	+++

was confirmed by DGGE analysis. In order to taxonomically identify the strains, their 16S rDNA genes were amplified and the PCR products were directly sequenced with internal primers 16F357 and 16F1069. A BLAST search of the nucleotide sequence revealed that the isolates belonged to three phylogenetically different groups: actinomycetes, α-proteobacteria, and γ-proteobacteria (Table 2). Since only part of their 16S rDNA genes was sequenced (roughly 50%), the strains were classed only to a particular genus only, not to a species.

Pseudomonas sp. BEN 2 seemed to have the broadest degradation potential and was able to degrade all of the studied compounds, even though the degradation of benzene was rather slow. Under the studied conditions, benzene seemed to be quite persistent. Although benzene degradation did occur, the culture medium became only slightly opalescent. By contrast, toluene and *m*-xylene were readily degraded in 3 days at 18°C. In the presence of toluene, a yellow compound was excreted into the liquid medium. The highest concentration of toluene and *m*-xylene that could be degraded was 600 ppm. Other xylene isomers (*o*- and *p*-xylenes) were tested, but *o*-xylene was not used as a growth substrate by any of the strains.

Evaluation of DGGE patterns. While individual steps in the molecular analysis of community structure have pitfalls, these were the same for all identically treated samples. For this reason, the samples could be compared with each other. In DGGE, the number and precise position of bands in a gel track are visually inspected to estimate the number and relative abundance of numerically dominant ribotypes present in samples. The DGGE profile of the aquifer sample (Fig. 1, lanes 2, 3) showed a diverse population. Only two identical bands were detected in the DNA sample obtained from the 24-h culture of the aquifer in R_2A medium, meaning that only these two clones were culturable in R_2A. In addition, R_2A medium detected two more bands that were present in the original sample at a low copy number (under the detection limit). The diversity of the DGGE pattern of the aquifer sample could be related only to the numerically dominant species and definitely not to the total number of different species in the sample.

There is no relationship between microbial community structure and degradation of BTmX in a real leachate plume, which is not surprising considering the fact that BTmX contributes less than 1% of the dissolved organic carbon in the plume; thus BTmX degraders make only a minor contribution to the total microbial community. However, under selection pressure in vitro, BTmX degraders were already selected during the third enrichment on minimal media, and they dominated the association. As in the case of PCE and TCE enrichments, during the first and second enrichment, the detected bacterial community survived most probably only due to the remains of organic nutrients from the aquifer.

To find out whether sequencing and identifying the DNA fragments (200 bp) originating from the DGGE gel (Fig. 1) was sufficient to identify the isolates, numbered bands were excised from the gel, eluted overnight, re-amplified using GC-free primers 338f-518r, and sequenced using the same

Table 2. Identities of DGGE fragments related to the bands as determined by partial sequencing of 16S rDNA

Strain	Closest relative in GenBank (accession No.)	Similarity (%)	Phylogenetic group
1	*Arthrobacter* sp. (X93356)	95	Actinobacteria
2	*Agrobacterium* sp. (AF508099)	98	α-Proteobacteria
3	*Pseudomonas* sp. (AF094745)	92	γ-Proteobacteria
12D	*Micrococcus* sp. (AF218240)	100	Actinobacteria
8	*Pseudomonas* sp. (AF534198)	99	γ-Proteobacteria
10	*Sinorhizobium* sp. (AF452129)	99	α-Proteobacteria

Escherichia coli positions 357–432 (only 75 bp) of the forward sequence were sequenced.

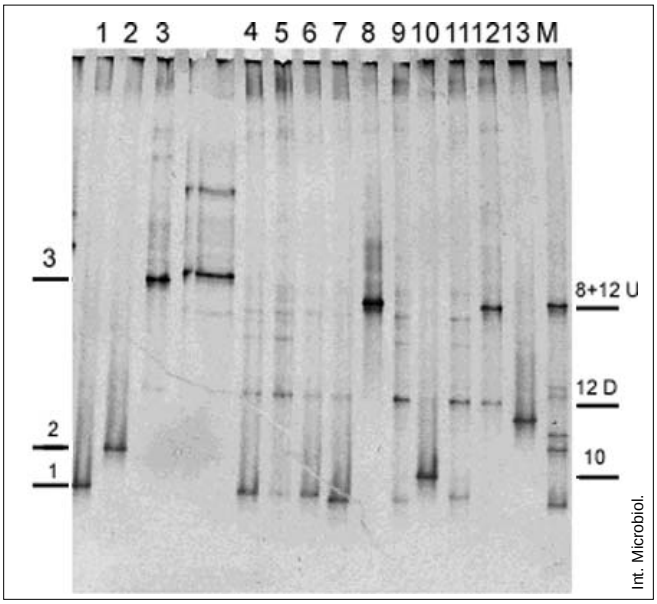

Fig. 1. DGGE profile of the isolated and purified strains. *Lane 1* BEN 1V, *lane 2* BEN 1 Z BEN, *lane 3* BEN 2, *lane 4* XYL 22, *lane 5* XYL 22V, *lane 6* XYL 22M, *lane 7* MIX 2M, *lane 8* MIX 2V, *lane 9* MIX 2 from PCE/TCE, *lane 10* MIX 31V, *lane 11* MIX 31M, *lane 12* MIX 32, *lane 13* UH133, *lane M* DGGE ladder (from top to bottom: *Pseudomonas* sp. PS20, *Comamonas testosteronii* B356, *Ralstonia eutropha* H850, *Enterobacter agglomerans*).

primers. Samples number 1 and 12D could not be determined, probably due to the DNA loops extending over the V2–V3 region of the16S rDNA. These partial sequences were then compared with the sequenced 712 bp of the 16S rDNA fragments.

PCB removal from contaminated soil by phytoremediation

The ability of different plant and bacterial species to degrade PCBs was analyzed in order to demonstrate the beneficial interactions among plants and associated bacteria as well as their positive effect on PCB degradation and removal from the soil. In parallel, differences in microbial growth between the non-vegetated and vegetated soil, particularly in the rhizoplane and rhizosphere areas, were examined.

Design of the pot experiment. Plants of three species were grown in soil that had been contaminated for a long period of time with PCBs. For pot experiments, 20-l buckets were filled with contaminated soil. Three different plant species—*Medicago sativa* (alfalfa*), Nicotiana tabacum* (tobacco) and *Solanum nigrum* (black nightshade)—were cultivated in the contaminated soil for 6 months and then the residual amount of PCBs was analyzed. Also, the total number of microorganisms, i.e., bacteria growing on biphenyl as a sole source of carbon and energy, were estimated in the rhizosphere and rhizoplane (roots) areas of the cultivated plants. Non-vegetated contaminated soil was used as a control.

PCB-analysis: field and pot experiments. Tested soil was dried overnight and sieved through a 1-mm pore size mesh, and 1 g of the soil was Soxhlet-extracted with hexane for 4 h [3]. The extract was concentrated to approximately 1 ml by nitrogen flow, purified on a Florisil column, diluted with hexane to 10 ml, and then diluted 1/100 for analysis by gas chromatography (GC). Results were calculated from the residual amounts of congener peaks present in the sample and compared to the value recommended by US Environmental Protection Agency (EPA). The total content of PCBs was expressed as the sum of recommended "indicator" congeners (Table 3) (EPA: US/EPA Methods 8089/8081).

Identification and characterization of microorganisms grown in contaminated soil. Ten-gram samples of bulk soil, rhizospheric soil, and root surface were extracted with 90 ml of medium containing 1% peptone. Flasks with samples were shaken for 2 h with glass beads, after which aliquots of the extracts were diluted with saline solution and spread on Petri dishes containing plate count agar (PCA, Oxoid) in order to estimate the total number of bacteria. Minimal medium (MM) with biphenyl as the sole carbon source was used for cultivating and counting the bacteria. Potential PCB-degraders were identified by testing indigenous bacterial strains using a rapid visible screening with dibenzofuran. The structure of dibenzofuran is similar to that of biphenyl, and during its degradation a bright, stable product was observed. Table 4 shows the numbers of colonies detected in samples extracted from the soil samples. Plants alone can accumulate and transform PCBs, but they also play a major role in supporting nutrients and increasing the bioavailability of pollutants for rhizosphere microorganisms living on the roots of the plants themselves or in their surroundings The results indicate that plants beneficially affect the growth of bacteria, even those growing on biphenyl (potential PCB degraders). The number of degraders found on the roots was at least ten times higher than the amount in non-vegetated soil. The highest numbers of degraders were present on the roots of the tobacco plant.

Table 3. Indicator congeners recommended by the US EPA

IUPAC	Chlorine substitution
28	2, 4, 4´
52	2, 2´, 4, 5´
101	2, 2´, 4, 5, 5´
138	2, 2´, 3, 4, 4´, 5´
153	2, 2´, 4, 4´, 5, 5´
180	2, 2´, 3, 4, 4´, 5, 5´

Degrading bacteria growing within the roots and in the rhizosphere represent the most important tool in the biodegradation of organic xenobiotics. Plants affect xenobiotic removal mainly by exuding compounds that are major microbial nutrients. In the case of PCB degradation, plants are also inducers of the *bph* operon [6]. Potential PCB degraders were isolated on MM containing biphenyl. This method not only gives positive results with PCB degraders, but it also reveals those bacteria able to use biphenyl as a sole source of energy. Another approach is based on the use of dibenzofuran as a substrate, but this is a rough screening technique that should be supplemented either by results based on degradation experiments or by proof of the presence of proper genes.[7].

The product of *bphA*, biphenyl-2,3-dioxygenase is a multicomponent enzyme, containing four subunits [2]. This enzyme determines the substrate specificity of PCB-degrading bacteria. The small subunit of dioxygenase, encoded by *bphA1*, is responsible for incorporation of two molecules of oxygen to one molecule of PCB and is highly homologous among different species. Studies of allelopathic interactions of microorganisms and plants in PCB metabolism [19]. By evaluation and comparison of the sequences of the known PCB degraders using the Clustal W program, the most conservative regions were found.

Primers F350, R1165, F463 a R674 were designed using program OLIGO Primer Analysis Software. Table 5 shows

Table 4. PCB removal from contaminated soil in presence of plants

PCB content (mg PCB/g soil)		Number of colonies on PCA (CFU/g soil)		Number of colonies on MM (CFU/g soil)	
Non-vegetated soil	470	$(7.6 \pm 2.6) \times 10^4$		$(6.0 \pm 1.8) \times 10^3$	
		Roots (10^5)	Rhizosphere (10^5)	Roots (10^4)	Rhizosphere (10^4)
Tobacco	358	11.4 ± 3.9	0.51 ± 1.1	16.0 ± 6.9	0.59 ± 3.0
Black nightshade	367	2.1 ± 0.9	0.74 ± 3.5	2.1 ± 0.9	0,60 ± 4.9
Alfalfa	400	4.1 ± 3.5	0.78 ± 0.5	4.4 ± 2.1	1.84 ± 8.8

PCA, Plate count agar; MM, minimal medium

the numbers of colonies growing on MM containing biphenyl that turned yellow after the addition of dibenzofuran [15]. The results obtained with dibenzofuran were confirmed by PCR with specific primers. Of the 44 colonies isolated by cultivation under these conditions and which turned yellow after addition of dibenzofuran, the presence of *bphA1* was confirmed in only eight isolates. These were isolated from the soil vegetated with black nightshade and tobacco (Table 5, Fig. 2).

Conclusions

Over the past two decades, the use of biological organisms such as plants or microbes to remove contaminants, pollutants, and other hazardous substances from soil or water (i.e., bioremediation) has increased dramatically, partly because researchers have learned more about the biological processes in soil and water. To design the most effective method of soil and water treatment and to choose the most appropriate bioremediation strategy, an elaborate study of the site is needed. Nutritional state and microbial composition are major factors that must be taken into account. The properties as well as the cultivation and degradation demands of the biological systems involved must also be evaluated. Of particular importance when considering the plant species for phytoremediation is the fact that the size, activity, and species composition of the rhizosphere community as well as the volume occupied by the rhizosphere vary appreciably with the plant species [6].

Our research activities focus on: (i) the study of two types of biological agents (bacteria, plants) that are able to degrade xenobiotics, including benzene, toluene, *m*-xylene and polychlorinated biphenyls; and (ii) the possibility to use these agents for the removal of organic pollutants from the environment (water, soil). Culturing microorganisms that are dominant at sites undergoing bioremediation is a major experimental strategy for understanding bioremediation

Table 5. Comparison of the numbers of bacterial colonies obtained by cultivation and molecular biology technique identifying

Soil from plant	Yellowish colonies after addition of dibenzofurane (CFU/g soil)	No. of colonies with *bphA1* (CFU/g soil)
Tobacco B	8	0
Tobacco 6	2	2
Tobacco A from roots	3	3
Tobacco 14 from roots	1	1
	Total 14	Total 6
Nightshade F from roots	1	1
Nightshade G from roots	1	1
Nightshade 13 from roots	4	0
	Total 6	Total 2
Alfalfa 11	3	0
Alfalfa 10 from roots	2	0
Alfalfa 11 from roots	1	0
Alfalfa 12 from roots	8	0
	Total 14	Total 0
Non-vegetated soil 1	1	0
Non-vegetated soil 2	7	0
Non-vegetated soil 3	2	0
	Total 10	Total 0
Total	44	8

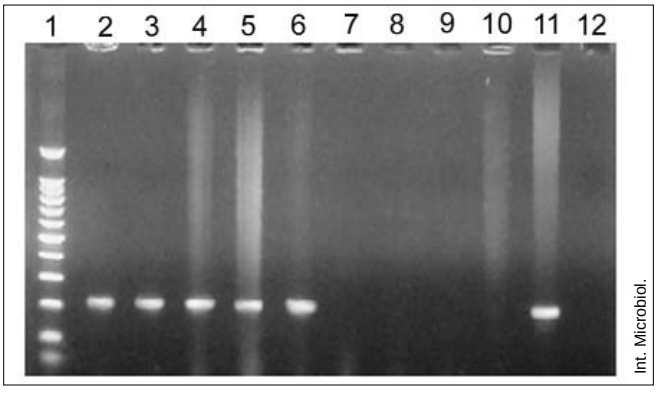

Fig. 2. Agarose electrophoresis (1.5 % gel) to detect *bphA1* (chosen isolates turned yellow after addition of dibenzofuran). Results document bands obtained after PCR with DNA primers F463 a R674 for amplification of the 211-bp sequence. *Lane 1* marker 100 bp, *lanes 2* and *3* isolates from tobacco 6, *lanes 4–6* isolates from tobacco A, *lanes 7–10* isolates from black nightshade 13, *lane 11* positive control strain JAB1, *lane 12* negative control.

processes. The contribution of the dominant species detected at the study site during active bioremediation can be more fully understood by investigating their physiologies in pure culture, provided the microbes are culturable under laboratory conditions. Another approach, using molecular biology techniques, may show the presence of degradative genes even without the need for simulation of environmental conditions, which is often difficult. We have isolated and characterized bacteria from two different polluted sources (landfill leachate and PCB-contaminated soil). In the case of landfill leachate, recovery of the entire 16S genes provided the most meaningful phylogenetic classification. For PCB-contaminated soil, PCR carried out with specific primers showed the nature of the functional genes involved in the degradation of xenobiotics. These results will be used in further studies and experiments aimed at obtaining reliable data about the diversity, abundance, and activity of microorganisms in polluted systems.

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Dos métodos de descontaminación biológica de agua freática y suelos contaminados por compuestos aromáticos. Caracterización de las poblaciones microbianas

Resumen. En el marco del proyecto europeo MULTIBARRIERS se probaron seis nuevos aislados bacterianos aeróbicos y endógenos que pueden crecer en presencia de BTmX (benzone, tolueno y *m*-xileno). Dichas cepas se caracterizaron en relación a su especificidad de crecimiento. El estudio del polimorfismo de la longitud de los fragmentos de restricción y la secuenciación del 16S rDNA fueron algunos de los análisis preliminares realizados. La electroforesis en gel de gradiente desnaturalizante confirmó la diversidad de las cepas. De la rizosfera de plantas que crecían en suelos contaminados con bifenilos policlorados (PCB) se aislaron otras cepas bacterianas aeróbicas. Se diseñaron experimentos en macetas para demostrar el efecto beneficioso de las plantas en la degradación bacteriana de los PCB. Se evaluó la eliminación de PCB del suelo y se examinaron las bacterias presentes en tres especies diferentes de plantas para buscar en ellas el operón *bph*. [**Int Microbiol** 2005; 8(3):205-211]

Palabras clave: biorremediación · sistema multibarrera · fitorremediación · xenobióticos aromáticos

Dois métodos de descontaminação biológica de água freática e solos contaminados por compostos aromáticos. Caracterização das povoações microbianas

Resumo: No marco do projeto europeu MULTIBARRIERS se provaram seis novos isolados bacterianos aeróbicos e endógenos que podem crescer na presença de BTmX (benzone, tolueno e m-xileno). Ditas cepas se caracterizaram em relação a sua especificidade de crescimento. O estudo do polimorfismo da longitude dos fragmentos de restrição e a secuenciación do 16S rDNA foram algumas das análises preliminares realizadas. A electroforesis em gel de gradiente desnaturalizante confirmou a diversidade das cepas. Da rizosfera de plantas que cresciam em solos contaminados com bifenilos policlorados (PCB) se isolaram outras cepas bacterianas aeróbicas. Se desenharam experimentos em vasos para demonstrar o efeito beneficente das plantas na degradação bacteriana dos PCB. Se avaliou a eliminação de PCB do solo e se examinaram as bactérias presentes em três espécies diferentes de plantas para buscar nelas o operón *bph*. [**Int Microbiol** 2005; 8(3):205-211]

Palavras chave: bioremediação · sistema multibarrera · fitoremediação · xenobióticos aromáticos