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ORIGINAL PAPER

Autochthonous bioaugmentation to enhance phenanthrene degradation in soil microcosms under arid conditions

L. Madueño · H. M. Alvarez · I. S. Morelli

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Abstract The aim of this work was to investigate the effect of autochthonous bioaugmentation (ABA) in phenanthrene-contaminated Patagonian soil microcosms, maintained under arid conditions, on phenanthrene elimination and soil microbial community. The polycyclic aromatic hydrocarbon (PAH)-degrading strain *Sphingobium* sp. 22B previously isolated from the Patagonian soil and selected by its resistance to drying conditions was used as inoculant. The phenanthrene concentration, dehydrogenase activity and denaturing gradient electrophoresis of 16S rRNA gene were monitored during 230 days. The results showed that when the microcosms were maintained at 20 % of soil water-holding capacity (WHC), the phenanthrene biodegradation was drastically inhibited and changes in the genetic diversity of soil microbial community were not detected, and neither the ABA nor the biostimulation managed to overcome the inhibitory effects. When the moisture was slightly increased, reached 25 % WHC, the ABA showed a significant initial stimulatory effect on phenanthrene biodegradation, demonstrating the potential of ABA in PAH bioremediation process in semiarid Patagonia.

L. Madueño · I. S. Morelli (✉)
Centro de Investigación y Desarrollo en Fermentaciones Industriales, CINDEFI, UNLP, CCT-La Plata, CONICET, Calle 50 y 115, 1900 Buenos Aires, La Plata, Argentina
e-mail: guri@biol.unlp.edu.ar

H. M. Alvarez
Centro Regional de Investigación y Desarrollo Científico Tecnológico (CRIDECIT), Facultad de Ciencias Naturales, Universidad Nacional de la Patagonia San Juan Bosco, Comodoro Rivadavia, Chubut, Argentina

I. S. Morelli
Comisión de Investigaciones Científicas de la Provincia de Buenos Aires (CIC-PBA), Buenos Aires, Argentina

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Introduction

The oil industry is the main economic activity in East Patagonia (Argentina). Following extraction, transportation and storage of crude oil, some places in this region are frequently contaminated with hydrocarbons (Peressutti et al. 2003). Aliphatic and polycyclic aromatic hydrocarbons (PAHs) are the major components of crude oil (Commendatore et al. 2012). PAH have environmental significance due to their potential toxicity to organisms.

Bioremediation is considered an appropriate technology for decontamination of polluted natural environments. The degree of bioremediation success depends on a number of factors, including the level and properties of targeted pollutants, site properties, and microbial and environmental limitations of bioremediation (Yang et al. 2009). Environmental conditions play a pivotal role in determining biological activity. These conditions fall into two general categories: those that reduce the microbial activity and those that restrict the mass transfer (mainly by diffusion) of the compound to the microorganism (Vogel 1996).

The Patagonian region is characterized by semiarid climate and fluctuating environmental conditions: low precipitations concentrated in winter, strong water deficits in spring and summer, and persistent and intense westerly winds (Paruelo et al. 1998). In arid and semiarid regions, water controls biotic processes (Prieto et al. 2011). Total soil water potential is the sum of matric, osmotic and gravitational potential. The gravitational potential is constant, whereas matric and osmotic potentials can change substantially. In non-saline soils, matric potential is the dominant

component (Chowdhury et al. 2011). As the matric potential declines, physiological stress associated with cell dehydration becomes the main factor limiting microbial activity. However, osmotic potential is a function of matric potential, because osmotic potential decreases with decreasing matric potential due to the increasing salt concentration; hence, microorganisms will also experience decreases in osmotic potential as the soil dries (Chowdhury et al. 2011). On the other hand, as dryer conditions are imposed, a lack of aqueous connectivity between soil particles can reduce the diffusion of substrates, but also restrict the mobility of microbial populations (Treves et al. 2003).

Under these extreme conditions, the reintroduction of microorganisms isolated from the polluted area could be an interesting approach for overcoming the environmental factors limiting the biodegradation (Hosokawa et al. 2009). This new concept in bioaugmentation, known as "autochthonous bioaugmentation" (ABA), has been proposed by Ueno et al. (2007) and is defined as a bioaugmentation technology that exclusively uses microorganisms indigenous to the sites (soil, sand and water) slated for decontamination (Nikolopoulou et al. 2013). In this study, we investigated the use of ABA technology as one potential strategy for the successful in situ remediation of PAH-contaminated soil in arid regions.

In a previous work, we have isolated a PAH-degrading strain, named 22B, from a PAH-contaminated soil sample collected in Pico Truncado, Patagonia, Argentina, in October 2007. The strain, which showed a phylogenetic relationship with the *Sphingomonadaceae* family, was physiologically characterized and selected by its resistance to drying conditions (Madueño et al. 2011). Also, the performance of Patagonian strain 22B and an allochthonous phenanthrene-degrading *Sphingomonas* strain, on phenanthrene-induced mineralization of contaminated Patagonian soil under arid conditions, was previously compared in a short-term study. Those results showed that only the autochthonous strain produced a significant increase in phenanthrene-induced mineralization (Madueño et al. 2011).

The aim of this work was to investigate the effects of the ABA strategy, using the strain 22B, in artificially phenanthrene-contaminated Patagonian soil microcosms maintained under semiarid conditions, on phenanthrene elimination rate and soil microbial community.

Materials and methods

Bacterial strain

Sphingobium sp. strain 22B was obtained, by direct isolation, from Patagonian contaminated soil in a previous study (Madueño et al. 2011). The strain was identified according to biochemical characteristics and 16S rRNA gene sequence analysis. The strain 22B was selected by its ability to degrade fluorene and phenantrene and for being extremely resistant to C starvation and drying conditions, showing a survival of 82 ± 2 % after 14 days of incubation at 18 % of relative humidity (Madueño et al. 2011).

For preservation, the strain was grown in R3 broth (Reasoner and Geldreich 1985) at 26 °C (100–150 rpm), and aliquots of 1 mL were stored in glycerol (20 % v/v) at −80 °C.

Soil microcosm preparation and bioaugmentation assays

The soil selected for the study was a contaminated soil from an area near Pico Truncado, Patagonia, Argentina. The soil sample was analyzed in the Laboratory of Soil Science at the University of La Plata and in the Laboratory of Oil M&S Company (Cañadón Seco, Patagonia, Argentina), showing the following physicochemical properties: pH 9.0, electrical conductivity of 0.72 dS/m (on the saturated paste extract), 2.30 % organic carbon, 3.97 % soil organic matter (SOM), 520 mg/kg total nitrogen, 6 mg/kg available phosphorus, and 7,456 mg/kg of total petroleum hydrocarbons. Phenanthrene was found in the soil lixiviate.

Soil microcosms consisted of 1 kg of sieved soil (2-mm mesh) in a plastic container with a 5 kg capacity. They were recontaminated with 2 g of phenanthrene per kilogram of dry soil. The phenanthrene was delivered in an acetone solution (150 mg/mL) and mixed into the soil manually with a spatula. Two treatments were carried out in triplicate trays: inoculated (F + 22B) and non-inoculated (F). The microcosms were incubated at 20 ± 2 °C for 250 days.

In the both treatments (F and F + 22B), three different environmental conditions were assayed, in consecutive way.

1. Natural soil (NS): At the beginning of the experiment, the microcosms were maintained to 10 ± 2 % (w/w) of water content, keeping the original C/N/P rate of the soil (100:2:0.3).
2. Fertilized soil (FS): At 86 days of treatment, the microcosms were fertilized with 7.45 g kg^{-1} dry soil of commercial fertilizer Nitrofoska® (BASF, Research Triangle Park, NC), taking the relation C/N/P to 100:5:2. The water content was maintained at 10 %.
3. Fertilized and wet soil (FWS): At 150 days of treatment, water content of the soil was increased and maintained to 15 ± 2 %.

The microcosms were mixed weekly for aeration, and the moisture content of the soil was corrected when necessary to 10 ± 2 % or 15 ± 2 % by adding distilled water.

The F + 22B microcosms were inoculated with 1.4×10^8 cfu/g of soil at 2, 86 and 150 days after phenanthrene was added to the soil. The second and third inoculations (86 and 150 days) were done immediately

after that the environmental conditions were modified. The cells were suspended in a volume of water equal to the volume necessary to adjust the moisture content of the microcosm. The inoculum was prepared as was previously described by Coppotelli et al. (2008). The same volume of sterile water was added to F microcosms to standardize the moisture content.

Chemical analysis

Soil samples (25 g) of each microcosm were collected at 0, 25, 61, 101, 137, 158, 181 and 205 days. They were mixed with 25 g of anhydrous Na_2SO_4, and hydrocarbons were extracted for 6 h with ethyl acetate (Dorwil) in a Soxhlet apparatus. The PAH concentration in the ethyl acetate extracts was analyzed by reversed phase HPLC using a Waters chromatograph with a Symmetry Waters C18 column (15 cm × 4.6 mm i.d., bead size 5 mm, pore size 100 Å) and a diode array detector. A linear gradient of 15 mM phosphoric acid in nanopure water solution and methanol (20:80 to 15:95, v:v) over 15 min and a flow rate of 1 mL/min was used. Detection was carried out at 250.9 nm (Coppotelli et al. 2010).

Microbial enumeration and biological activity

Cultivable bacteria counts were determined after 0, 2, 9, 17, 24, 31, 45, 73, 110, 148, 168, 189 and 209 days. For this purpose, a 10 g (wet weight) soil sample suspended in 100 mL of SF was homogenized for 30 min on a rotary shaker (250 rpm). Samples (0.1 mL) of dilutions were spread on plates containing R2A agar medium (Reasoner and Geldreich 1985) in order to count cultivable heterotrophic bacteria. The agar plates were incubated at 24 ± 2 °C for 10 days. The MPN of PAH-degrading bacteria was determined in 96-well microtiter plates according to Wrenn and Venosa (1996). A mixture of four PAH was used as carbon source (10 g/L phenanthrene, 1 g/L anthracene, 1 g/L fluorene and 1 g/L dibenzothiophene). The plates were incubated for 3 weeks at 24 ± 2 °C.

Dehydrogenase activity (reduction of 2,3,5-triphenyl-2*H*-tetrazoliumtrichloride, TTC, to triphenyl formazan, TPF), usually related to the cell density of viable microorganisms and their oxidative capability, was determined at 0, 4, 11, 18, 25, 34, 40, 68, 86, 115, 141, 155, 164,195 and 254 days, as described by Thalman (1968).

Genetic diversity

The total DNA was extracted and purified from 1 g soil aliquots of each microcosm after 0, 11, 62, 90, 120, 157 and 173 days using E.Z.N.A^{TM} Soil DNA Kit (Omega Biotek, Norcross, GA, USA).

PCR was realized with the set of primers for eubacteria: GC-341F (5′-CGCCCGCCGCGCCCCGCGCCCGGCCCG CCGCCCCCGCCCCCTCCTACGGGAGGCAGCAG-3′) and 907R (5′-CCGTCAATTCCTTTGAGTTT-3′) (Muyzer et al. 2004). The PCRs contained 1 μL of total community DNA, 1 U of GoTaq, the manufacturer's recommended buffer as supplied with the polymerase enzyme, 0.2 mg/mL of bovine serum albumin, 0.1 mM deoxyribonucleotide triphosphates and 1 μM of each primer in a total reaction volume of 30 μL. Amplification was performed on a Mastercycler® Eppendorff thermocycler using step-down. The PCR program included an initial denaturation step for 4 min at 94 °C, the first cycle step at 94 °C for 30 s; 62 °C for 45 s; and 1 min at 72 °C (ten cycles), followed by a step-down of 30 s at 94 °C, 40 s at 57 °C, and 72 °C for 1 min (25 cycles). The final extension was carried out at 72 °C for 10 min.

The PCR products were analyzed by agarose gel electrophoresis and purified using a QIAquick PCR Purification kit (Qiagen Inc.). Denaturing gradient gel electrophoresis (DGGE) was performed on a DGGE-2401 apparatus (C.B.S Scientific Co., Del mar, CA, USA). The purified PCR amplicons were resolved in 6 % (w/v) polyacrylamide gels (acrylamide:*N*,*N*-methylenbisacrylamide, 37.5:1). The amounts of PCR products loaded in the DGGE gels were standardized (5–7 μg per well). The gel contained a linear gradient of 40–70 % denaturant [100 % denaturant corresponds to 7 mol/L urea and 40 % (v/v) formamide]. Electrophoresis was performed in TAE buffer (pH = 8.1, 40 mM Tris, 20 mM acetic acid, 1 mM Na_2EDTA) at a temperature of 60 °C. A prerun at 50 V for 30 min was followed by the main run at a constant voltage of 100 V for 16 h. The post-electrophoresis gel was stained for 30 min with SYBR Gold and documented with a Chemi-doc gel documentation system (Bio-Rad, Hercules, CA, USA) and analyzed using a GelComparII software package (Applied Mathematics, Kortrijk, Belgium). Similarity matrixes of the banding patterns were made with the Dice equation, and the dendrogram was calculated by the unweighted pair group method with arithmetic mean (UPGMA) (Sokal and Michener 1958).

Denaturing gradient gel electrophoresis banding data were used to estimate the Shannon index of general diversity ($H' = -\Sigma(ni/N)\log(ni/N)$). For this analysis, each band was treated as an individual operational taxonomic unit (OTU). The relative surface intensity of each band, expressed as peak height in the densitometric curve (ni), and the sum of all peak heights in the curve of a given sample (*N*) were used as estimates of species abundance (Fromin et al. 2002).

Statistics

The mean and standard deviation of triplicate independent experiments were calculated. The mean values were

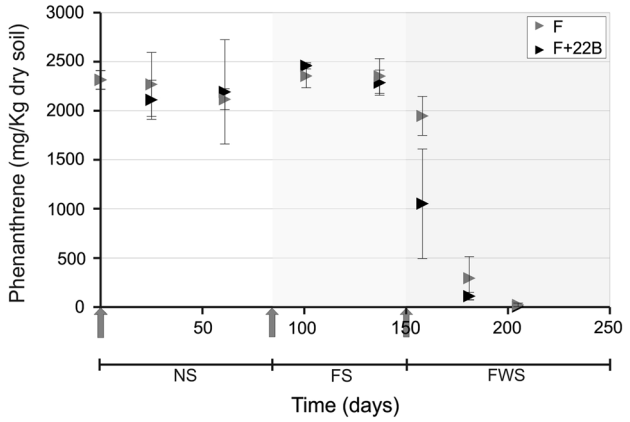

Fig. 1 Phenanthrene concentration in F and F + 22B microcosms under NS, FS and FWS conditions during 210 days. Mean values and standard deviations of three measurements

compared by parametric one-way ANOVA test at the level of $P \leq 0.05$. All statistical analyses were performed using the SigmaPlot/SigmaStat software program (SPSS Inc., Chicago, IL, USA).

Results and discussion

Chemical analysis

The concentration of phenanthrene during the treatment in F and F + 22B microcosms is shown in Fig. 1. During the first 85 days of treatment, when the microcosms were under NS conditions, and even after that the microcosms were fertilized (between 86 and 149 days), elimination of phenanthrene was not observed, neither in the non-inoculated microcosms, nor in the inoculated microcosms (Fig. 1). After the water content of the microcosms was increased, at 149 days, a rapid elimination of phenanthrene was observed in the both treatments; however, at 161 days, the concentration of phenanthrene in the F + 22B microcosms was significantly lower ($P < 0.05$) than that in the F microcosms. After 200 days of treatment, such the inoculated microcosms as non-inoculated, reached a phenanthrene concentration below the cleanup standards for soil (50 mg/kg Argentinian National Law 24051).

Clearly, the typical arid conditions of Patagonian soil would be the principal factor that limits the phenanthrene biodegradation, and the inoculation of a phenanthrene-degrading strain resistant to drying conditions did not manage to overcome its inhibitory effects. As the matric potential decreases, the water films around aggregates become thinner and disconnected; water availability decreases because the water is held more tightly to the aggregate surfaces (Ilstedt et al. 2000). As a result, substrate and nutrient diffusion are restricted and microbes become substrate limited. Holman and Tsang (1995) determined that a water content of 50–70 % of field capacity was optimum for biodegradation of aromatic hydrocarbons to proceed at maximum rate; also Viñas et al. (2005) found that in a heavily creosote-contaminated soil, the higher percentage of hydrocarbon degradation was obtained with a 40–60 % of soil water-holding capacity (WHC), while with 20 % WHC, only slight degradation was observed. In our case, values near 20 % WHC (10 % of water content) drastically inhibited the phenanthrene biodegradation; when the moisture was increased, reached a 25 % WHC (15 % of water content), the soil biodegradation activity was recovered.

In spite of the long permanence of phenanthrene in the soil microcosms (149 days), the bioavailability of phenanthrene was not affected in an irreversible way, allowing a rapid and total (98 %) phenanthrene biodegradation after water content was increased. In soil, only a small fraction of the organic pollutants is bioavailable, while a large fraction is sequestrated to be unavailable and undegradable due to the strong interaction with the SOM or diffusion into nanoscale pores (Barriuso et al. 2008). Ma et al. (2012) showed that the proportion of the desorbed phenanthrene decreased, from an initial of 82, to 65 % after 150 days of soil aging. However, the PAH sequestration is strongly affected by SOM, and the Patagonian soils are typically poor in organic matter.

Enumeration of cultivable bacterial populations

Neither the addition of phenanthrene into the soil nor the inoculation with the strain 22B produced an increase in the density of heterotrophic bacteria in the F and F + 22B microcosms, under NS and FS conditions, compared with the unamended soil (time 0) (Fig. 2). The water addition (after 149 days of incubation) produced an important increase (approximately two orders of magnitude) in the number of cultivable heterotrophic bacteria in both, inoculated and non-inoculated, microcosms. But, despite the F + 22B microcosms were inoculated three times, one of these at the same time of the water addition, the increase of the cultivable heterotrophic bacteria was not significantly different than F microcosms ($P > 0.05$).

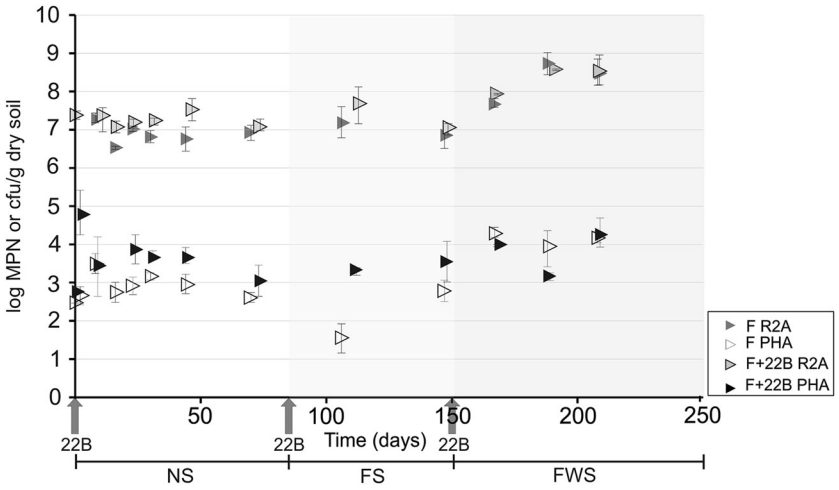

Fig. 2 Concentration of heterotrophic cultivable bacteria (log cfu g^{-1} of dry soil) and PAH-degrading cultivable bacteria (log MPN g^{-1} of dry soil) in F and F + 22B microcosms under NS, FS and FWS conditions during 210 days of treatment. Mean values and standard deviations of three measurements

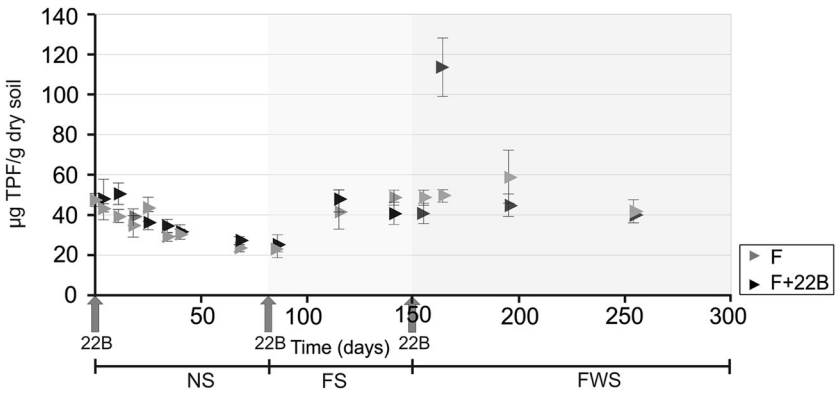

Fig. 3 Dehydrogenase activity (µg of TPF per g of dry soil) of F and F + 22B microcosms under NS, FS and FWS conditions during 250 days. Mean values and standard deviations of three measurements

The addition of phenanthrene and the first inoculation of the strain 22B produced a significant increase (near three orders of magnitude) in the number of PAH-degrading bacteria in F + 22B microcosms, but with a posterior rapid decline. After 9 days of incubation, the number of PAH-degrading bacteria in F + 22B microcosms reached an order between 10^3 and 10^4 MPN/mL that was kept more or less constant in spite of the two later inoculations, the fertilizer addition and the increase in the water content (Fig. 2). On the other hand, the F microcosms showed an increase of PAH-degrading bacteria after 8 days of phenanthrene incorporation. Afterward, the PAH-degrading bacteria populations in F microcosms declined to levels significantly minor than F + 22B microcosms. When soil humidity was increased to 15 %, the curves were inverted and the number of PAH-degrading bacteria in the F microcosms overcomes the values found in F + 22B microcosms.

Dehydrogenase activity

Figure 3 shows the dehydrogenase activity determined by analyzing the reduction of TTC to TPF in F and F + 22B microcosms. Dehydrogenase activity was determined to evaluate the impact of the inoculation on quantitative changes of the whole soil microbial community and as an indicator of microbial oxidative activity in the soil. Since metabolic pathways of PAH degradation involve several dehydrogenases, dehydrogenase activity could be proposed as indicator of PHA oxidation (Del Panno et al. 2005).

In both microcosms and under NS conditions, the phenanthrene incorporation did not cause a stimulatory

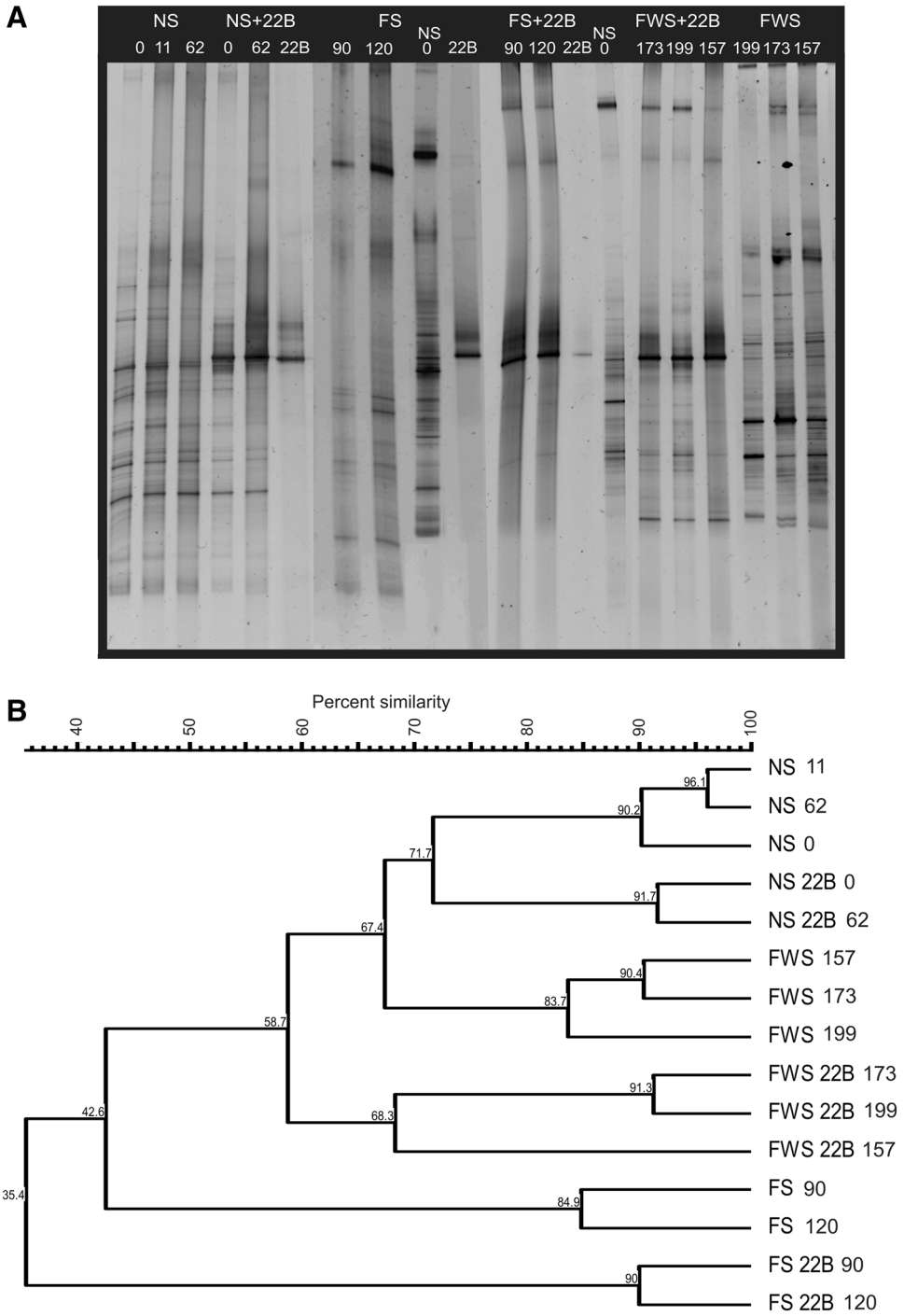

Fig. 4 **a** PCR–DGGE analysis of bacterial populations of F and F + 22B microcosms under NS, FS and FWS conditions during 199 days. **b** Dendrogram of the clusters based on DGGE patterns and calculations derived using Dice correlation and UPGMA. The differences between profiles are indicated by percentage similarity. 22B: *Sphingobium* sp. 22B

effect on the dehydrogenase activity. In contrast, a continuous reduction of dehydrogenase activity was observed during the NS conditions time (85 days) in inoculated and non-inoculated microcosms.

The fertilization of the microcosms caused a stimulatory effect on the dehydrogenase activity in F and F + 22B microcosms, but this effect was not correlated with phenanthrene degradation activity (Fig. 1).

After the humidity was adjusted to 15 % and a third inoculation was done, the F + 22B microcosms showed a significant increase in dehydrogenase activity, which was not observed in F microcosms under the same humidity

condition; however, both microcosms showed a rapid phenanthrene biodegradation under FWS conditions (Fig. 1). These results are in agreement with those of other authors who observed that the level of microbial activity is not necessarily a reliable indicator of the potential for degradation of recalcitrant molecules by microbes (Grosser et al. 1991).

Genetic diversity

The effect of phenanthrene addition, cells inoculation and the different environmental conditions on the structure and dynamics of the soil bacterial community in F and F + 22B microcosms were analyzed by DGGE (Fig. 4a). Analysis of the DGGE patterns by UPGMA is shown in Fig. 4b; also the DGGE banding data were used to estimate the H′ diversity index (Table 1). Three replicates per treatment and per sampling time were analyzed for DNA extraction, and there was no difference in DGGE profiles between them; it is for that reason that only one set of results is presented.

The DGGE profiles of F microcosms under NS conditions showed that the incorporation of phenanthrene did not produce detectable changes in the genetic diversity of soil microbial community (Fig. 4a; Table 1), and the DGGE patterns of F microcosms after 11 and 62 days showed a 90.2 % of similarity with the profile of the original Patagonian soil (F microcosms at the beginning of the experiment). The inoculation of the strain 22B caused few changes in the DGGE profiles of the F + 22B microcosms; the most important change was the appearance of an intense band that showed the same position in the DGGE gel that the strain 22B (Fig. 4a) and that caused the apparent decrease in the H′ diversity index of this microcosm (Table 1). This band was excised, reamplified and cloned, and its partial 16S rRNA gene sequence revealed a phylogenetic relationship (99 %) with the *Sphingobium* sp. strain 22B. However, and in disagreement with other bioaugmentation studies that used similar inoculation strategies (Coppotelli et al. 2008; Ibarrolaza et al. 2011), under this arid conditions the inoculation did not produce drastic changes in the structure of soil bacterial community of F + 22B microcosms (Fig. 4a), and their DGGE profiles clustered with those of F microcosms (71.7 % of similarity) (Fig. 4b). Accordingly, both microcosms showed similar behavior regarding to phenanthrene elimination (Fig. 1), cultivable bacterial enumeration (Fig. 2) and dehydrogenase activity (Fig. 3). These results suggest that under low matric potential, the limitation in the phenanthrene diffusion and the spatial isolation of microbial populations, produced by the lack of aqueous connectivity between soil particles (Treves et al. 2003), might prevent that the phenanthrene operates as pressure of selection on the structure of the soil bacterial community, as well as may inhibit the activity of the inoculated strain and its competition with the autochthonous soil bacteria.

The biostimulation with N and P (FS) produced drastic changes in the composition of the soil bacterial community of F and F + 22B microcosms (Fig. 4a, b), despite the absence of phenanthrene removal (Fig. 1) and changes in the number of cultivable PAH degraders and heterotrophic bacteria (Fig. 2). In both microcosms, the fertilization produced a decrease in the H′ diversity index (Table 1) that was going along with a slight increase in the dehydrogenase activity (Fig. 3). The impact on the soil bacterial community produced by biostimulation strategy might be attributed to the increase of the salinity caused by the addition of nutrient. Under low matric potential, the incorporation of salts would have a marked influence on osmotic potential. Microbial community composition can be affected by salinity because microbial genotypes differ in their tolerance to osmotic stress (Wichern et al. 2006).

The increase of the soil matric potential through the water incorporation caused a new succession process in the soil bacterial community of both microcosms (Fig. 4a, b). In consequence, the DGGE profiles of F and F + 22B microcosms under FWS conditions were joined to a cluster that shares more than 58 % of similarity with the profiles under NS conditions, and markedly different from the microcosms under FS conditions (Fig. 4b). Also the water addition produced an increase of the H′ diversity index of F and F + 22B microcosms, in comparison with that under FS conditions (Table 1). These results suggest that the changes in the DGGE profiles caused by the water addition

Table 1 Genetic diversity of soil bacterial community of microcosms F and F + 22B under NS, FS and FWS conditions

Environmental conditions	Time of incubation (days)	Shannon diversity index (H′)	
		F	F + 22B
NS	0	5.20	–
	11	5.29	4.60
	62	5.16	4.46
FS	90	3.97	3.42
	120	4.07	3.15
FWS	157	5.14	4.12
	173	5.18	4.52
	199	5.04	4.42

could be partially attributed to a reduction of the osmotic stress generated by the nutrient addition.

The DGGE profiles of F + 22B microcosms under FWS conditions formed a specific subcluster (Fig. 4b), independently of the incubation time; in the other subcluster, the profiles of the F microcosms after water addition formed a separated group from the profiles of F and F + 22B microcosms under NS conditions. The difference in the DGGE profiles of F and F + 22B microcosms under FWS conditions was also clearly visible in the gel (Fig. 4a). These results suggested that the increase in soil matric potential enhanced the phenanthrene bioavailability, evidenced by the rapid phenanthrene elimination (Fig. 1), and promoted a significant increase in the number of cultivable heterotrophic bacteria (Fig. 2). At the same time, the increase in water content allowed the selective shifts in the structure of soil bacterial community promoted by the addition of phenanthrene (in F microcosms) and also the inoculation of strain 22B (in F + 22B microcosms). In consequence, the F and F + 22B microcosms showed phenanthrene-degrading communities noticeably different at the level of genetic structure (Fig. 4a) and dehydrogenase activity (Fig. 3).

One interesting aspect of this study was the successful use of DGGE profile analysis to evaluate the effect of inoculant strains on the dynamics of the soil bacterial community, despite the recognized limitations usually showed by the DNA extraction and PCR amplification of environmental samples (De Araujo and Schneider 2008) and the specific limitations of DGGE electrophoresis (like the comigration of different DNA sequences). Despite the high abundance of the inoculant cells in the soil, which may cause disturbances of DGGE profiles (Gomes et al. 2005; Coppotelli et al. 2008), the DGGE method used in this study was able to show clear differences between the profiles of soil bacterial communities under no competitive conditions (F + 22B microcosms under NS conditions) and when the inoculation produced a selective shift in the structure of soil bacterial community (F + 22B microcosms under FWS conditions) (Fig. 4).

Although bioremediation is a promising technology to clean up hydrocarbon-contaminated sites, its potential on semiarid or arid soils has not been explored yet (Tibbett et al. 2011). Tibbett et al. (2011) showed that in tropical arid environments, soil moisture may be the rate-limiting abiotic parameter governing alkanes biodegradation. In addition, the response to moisture amendment clearly demonstrated the importance of maintaining soil moisture at appropriate levels in any bioremediation scheme in arid environments. In concordance, the results of our study showed that the typical dry conditions of Patagonian soil (below 20 % WHC) limits drastically the PAH biodegradation process, which may explain the frequent occurrence of chronically polluted environments in this region. Moreover, our results also showed that a slight increase of humidity of soil promoted rapid phenanthrene elimination (Fig. 1).

A bioremediation process in a semiarid region like Patagonia may be economically and environmentally expensive, considering the scarcity of water usually found in such places. For this reason, the addition of water to promote elimination of pollutants by microbial activity and the maintenance of soil humidity must be carefully managed. In this context, the inoculation of a dry-tolerant bacterial strain with the ability to enhance and accelerate bioremediation processes in polluted soil is of interest in the region. Thus, bioaugmentation with autochthonous degrading strains like 22B may shorten the duration of the treatment and reduce the water volume needed for the bioremediation process, to some extent. This effect may result in a reduction of the managing costs of the treatment and a valuable saving of water. We envisage the application of 22B bioaugmentation on limited extensions of polluted soil, such as the treatment of biopiles or mud pits, making the cell inoculation together with the addition of water to the soil. Taken together, the in situ inoculation of *Sphingobium* sp. strain 22B to polluted soil, which is well adapted to the fluctuating conditions of the environment in the region, may be a promising tool for shortening the time of treatment and reducing operative costs during PAH elimination in Patagonian contaminated soil.

Conclusion

To our knowledge, this is the first study on the efficiency of ABA strategy for enhancing PAH degradation under semiarid conditions in Patagonia. Results obtained in this study contribute to a better understanding of factors that limit PAH biodegradation in semiarid soils in the region. We found that the inoculation of the strain *Sphingobium* sp. 22B, selected by its high resistance to drying conditions, promoted an initial stimulatory effect on phenanthrene biodegradation when the soil water content was slightly increased, demonstrating the potential of ABA as an effective and economic strategy to enhance PAH bioremediation process in semiarid Patagonian soils.

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