Isolation and characterization of indigenous soil bacteria for bioaugmentation of PAH contaminated soil of semiarid Patagonia, Argentina

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ABSTRACT

The aim of this work was to isolate PAH degrading bacteria from contaminated Patagonia soil with the ability to tolerate the usual environmental stresses (oligotrophic and dryness conditions). Two approaches were utilized to obtain PAH-degrading bacteria from the Patagonian soil. With a traditional enrichment approach, only the PAH-degrading strain 36 was isolated. Using a direct isolation approach, three PAH-degrading strains (1A, 22A and 22B) were isolated. The phylogenetic analysis revealed that all isolates belonged to the Sphingomonas genus. The PAH degrading activity and the resistance to stress conditions of the strains were determined and compared with those of the exogenous PAH-degrading Sphingomonas paucimobilis 20006FA. The strains 1A, 22A and 36 were phylogenetically closely related between them and with the strain 20006FA. The strain 22B, that showed a different phylogenetic position, was more resistant to C-starvation and drying conditions than other Patagonian strains. The effect of the inoculation of these strains on phenanthrene-induced mineralization and elimination was studied in Patagonian soil artificially contaminated, at different environmental conditions. The results suggest that strain 22B is the most suitable strain for bioaugmentation in PAH-contaminated soils of Central Patagonia, due to its adaptation to the usual environmental conditions. Our results show the importance of a detailed physiological characterization of isolates for autochthonous bioaugmentation strategies success.

1. Introduction

The contamination of soil with aromatic compounds is of particular environmental concern as they exhibit carcinogenic and mutagenic properties (Mrozik and Piotrowska-Seget 2010). Some of the main sources of these toxic substances are the spills or leaks during the extraction, transport and storage (pipelines, storage tanks, etc.) of crude oil as well as the activity of oil refineries and petrochemical industries (Berthe-Corti et al. 2007). The oil industry is the main economic activity in East Patagonia (Argentina), as consequence some places in this region are frequently contaminated with PAH.

Bioremediation is considered a relatively cost-effective and environmentally friendly technology for decontamination of PAH polluted soil. The successful application of bioremediation depends on the presence of appropriate hydrocarbon-degrading microorganisms and the environmental conditions in situ. In the Patagonian region, the conditions most likely to limit hydrocarbon degradation include cold and fluctuating temperatures, low moisture contents, low nutrient levels and alkaline pH (Haro et al. 2007).

The bioaugmentation (introduction of specific competent microorganisms) has been considered a valuable tool for increasing the rate and extent of biodegradation of pollutants (Coppotelli et al. 2008). However, there are few issues in environmental biotechnology that generate more controversy than the use of bioaugmentation strategies in bioremediation (Thompson et al. 2005).

Many studies have shown that biotic and abiotic environmental factors influence the effectiveness of bioaugmentation (Mrozik and Piotrowska-Seget 2010). Therefore, it would be more practical when using bioaugmentation to use autochthonous microorganisms (Hosakawa et al. 2009). Autochthonous bioaugmentation (ABA) is defined as a bioaugmentation technology that uses microorganisms indigenous to the site (soil, sand and water) to be decontaminated (Ueno et al. 2007). Despite of the ABA technique is not a new concept (Vecchioli et al. 1990; Weber and Corseuil 1994),
only a limited number of reports have been published on ABA strategy compared with other types of bioaugmentation treatments (Hosakawa et al. 2009).

The aim of this work was to isolate PAH degrading-bacteria from Patagonia soil samples chronically contaminated with PAH, and to study their physiological properties which could suggest the presence of mechanisms of adaptation to the typical local environmental conditions. The ability of the strains to enhance the degradation of phenanthrene in Patagonian soil artificially re-contaminated was also determined.

2. Materials and methods

2.1. Patagonian soil characterization

The soil selected for this study was a chronically contaminated soil from an area near Pico Truncado, Santa Cruz, Argentina. The soil was analyzed in the Laboratory of Oil m&s S.A. (Cañadón Seco, Santa Cruz, Argentina) and showed the following physicochemical properties: pH of 8.6, 2.30% organic carbon, 3.97% soil organic matter, 520 mg kg\(^{-1}\) total nitrogen, 6 mg kg\(^{-1}\) available phosphorus, 1592 mg kg\(^{-1}\) hydrocarbons. Fluorene and phenanthrene were found in the soil extract.

2.2. Isolation of PAH-degrading bacteria

Two different approaches were utilized to obtain PAH-degrading bacteria from the Patagonian soil. The first approach was performed by an enrichment technique; 100 µl of the soil suspension were inoculated in liquid mineral medium (LMM) (Coppotelli et al. 2010) supplemented with 2000 mg l\(^{-1}\) of fluorene as sole carbon and energy source. Three successive batch cultures were performed. The cultures were incubated at 24 °C, 150 rpm, for 7 days. The establishment of a fluorene degrading consortium was evidenced by the appearance of a brownish color. The predominant bacteria of the consortium from the last batch culture were isolated in R3A plates (Reasoner and Geldreich 1985).

In the second approach, a direct isolation technique was performed from a soil suspension (1/10 in 0.85% of NaCl) using the agarose overlay technique (Bogart and Hemmingsen 1992) with fluorene as sole carbon and energy source. The plates were incubated during 10 days at 24 °C. The colonies showing fluorene solubilization and/or color changes were selected and isolated in R3A plates.

The ability of the isolates to degrade different PAH (fluorene, phenanthrene, anthracene and pyrene) was tested in solid mineral medium (SMM) (Vecchioli et al. 1990) using the agarose overlay technique. The isolates showing the capacity of degrading three or more PAH were selected.

2.3. Phenotypic characterization

Morphological features and biochemical characteristics of the isolates were studied. The colonies (shape, size, colour, contour, etc.) were studied on R3A plates after 5 days of incubation at 24 °C. Biochemical characterization included: Gram staining, KOH, motility, sugar fermentation (manitol, glucose, xylose), nitrate reduction and gelatinase and catalase activity (Gerhard et al. 1981).

2.4. Genotypic characterization (16S rRNA gene sequencing and analysis)

Genomic DNA of the isolates were extracted with boiling method (Sambrook et al. 1989), 1 ml of each overnight cultures grown in broth R3 were centrifuged and resuspended in 1 ml of distilled H\(_2\)O. The suspensions were boiled for 10 min and the supernatants were collected after spinning for 2 min in a micro-centrifuge. Soluble DNA was recovered from supernatants.

The amplification of the complete 16S ribosomal ribonucleic acid (rRNA) gene was obtained by using 27 forward primer (5’-AGAGTTTGATCCTGTCAG-3’) and 1492 reverse primer (5’TACGGGCTACCTTGACTT-3’) in a reaction volume of 30 µl of GoTaq DNA Polymerase (Promega, Madison, WI) was used. The polymerase chain reaction (PCR) program consisted of initial denaturation step at 95 °C for 2 min, followed by 30 cycles of 95 °C for 1 min, 60 °C for 30 s, 72 °C for 35 s, and the final extension step was carried out at 72 °C for 5 min on a Eppendorf® Mastercycler® thermocycler (Eppendorf, Hamburg, Germany). The amplicons were purified using a PCR purification kit (Qiagen) and were sequenced by sequencing service, Macrogen, Korea. Nucleotide sequences were compared to those in the National Center for Biotechnology Information GenBank database by using BLAST program. Phylogenetic tree was performed using the Molecular Evolutionary Genetics Analysis package (MEGA version 4.0). The sequences were aligned with the clustalW function and Neighbor-Joining phylogenetic tree was constructed with the Jukes–Cantor algorithm. The robustness of the phylogeny was tested by bootstrap analysis with 500 iterations.

2.5. PAH degradation in liquid culture

Liquid cultures were performed in LMM with 26 mg l\(^{-1}\) of phenanthrene or fluorene as sole carbon and energy source. The batch cultures were incubated at 24 °C for 72 h on a rotary shaker at 150 rpm. The remnant PAH was extracted with ethyl acetate using exhaustive extraction method (Coppotelli et al. 2010). The PAH concentration in the ethyl acetate extracts was analyzed by reversed-phase high-pressure liquid chromatography (HPLC) using a Waters® chromatograph with a Symmetry Waters® C18 column (15 cm × 4.6 mm i.d., bead size 5 µm, 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, vol vol\(^{-1}\)) 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).

2.6. C-starvation resistance testing

To measure the survival of the strains in total absence of carbon source (CS), the strains were grown in R3 medium for 24 h on a rotary shaker at 150 rpm, two washing steps with 0.85% NaCl solution (FS) were performed and 1 × 10⁷ cfu/ml were inoculated in 5 ml of LMM in total absence of CS; the strains were cultivated at 24 °C during 85 days. Periodically the number of cfu/ml was determined by counting on R3A medium.

2.7. N-starvation resistance testing

To investigate survival of cells under nitrogen starvation conditions, the isolates were grown in R3 medium for 24 h on a rotary shaker at 150 rpm, two washing steps with FS were performed and 1 × 10⁷ cfu/ml were inoculated in 20 ml of LMM with 0.5% of glucose in total absence of nitrogen source (NS); the strains were cultivated at 24 °C during 70 days. Periodically the OD\(_{600}\) of the cultures and the number of cfu/ml on R3A were determined.

The occurrence of lipids granules were screened during N-starvation conditions. 1 ml of culture was centrifuged and the pellet was resuspended in 30 µl of FS, 20 µl were carefully and uniformly deposited on a glass slide and thoroughly air dried. Cells
were stained with Sudan Black B (Burdon 1946) and observed by optical microscopy.

2.8. Water stress resistance test

2.8.1. Dehydration

The survival of the strains under water stress was investigated by the calculation of the survival rate on drops of a cells suspension (OD600 = 4). Drops were spotted onto Petri plates and incubated during 14 days at 24 °C and allowed to dry at a relative humidity of 17–18%. The cells from each drop were separated separately, and the viable count (cfu) was determined. The survival rate was calculated as log (cfu after drying)/log (cfu before drying) × 100 (Alvarez et al. 2004).

2.8.2. Salinity

The response of strains to different salinity concentrations was determined studying the growth of the strains in R3 broth with 0.5, 1.5, 2.5 and 5% NaCl. Determinations of OD600 were performed each one hour during 18 h in a Beckman Du® 640 spectrophotometer.

2.9. Biometers systems

The effects of the inoculation of the isolates on phenanthrene-induced mineralization in three different environmental conditions of Patagonia soil were assayed. Natural Patagonia soil (NPS): 100/2.2/0.26 of C/N/P ratio and 10% (w/w) of water content, Fertilized Patagonia Soil (FPS): 100/5/2 of C/N/P ratio and 10% (w/w) of water content, and Wet Patagonia soil (WPS): 100/2.2/0.26 of C/N/P ratio and 15% (w/w) of water content. All the systems were contaminated with 2000 mg kg⁻¹ and inoculated with 1.17 × 10⁶ cfu/g dry soil of the strains. In each condition, contaminated but non inoculated system was used as control. Three replicates of each system were placed into the biometer flasks (50 g of dry soil). The flasks were incubated at 24 ± 2 °C. The CO2 production during the course of 30–35 days of treatment was determined. The CO2 produced was trapped in 10 ml of 0.4–0.6 M KOH. Periodically the KOH was replaced by fresh KOH solution. The removed KOH was titrated to the phenolphthalein endpoint with standard HCl (Bartha and Pramer 1965).

After the incubation period the residual phenanthrene concentration was determined. A soil sample (25 g) was mixed with anhydrous sodium sulfate (25 g) and hydrocarbons were extracted for 6 h with ethyl acetate in a Soxhlet apparatus. The phenanthrene concentration in the ethyl acetate extracts was determined by HPLC.

2.10. Statistics

All experiments in this study were performed in triplicates. The mean and standard deviation of triplicate independent experiments were calculated. The mean values were compared by parametric one way ANOVA test. All statistical analysis were performed using the SigmaPlot/SigmaStat software program (SPSS Inc., Chicago, Illinois, USA).

3. Results and discussion

3.1. Isolation and characterization of PAH-degrading bacteria

For the isolation of PAH degrading bacteria two strategies were used. Three isolates (1A, 22A y 22B) were obtained directly from a soil suspension; whereas one strain (36) was obtained from successive batch cultures with fluorene as sole carbon and energy source. All the strains showed clearing zones of PAH solubilization and/or colour on plates of SMM supplemented with fluorene, phenanthrene, anthracene or pyrene.

All the isolated strains were Gram-negative rods, motile in the hanging drop, catalase positive, urease and gelatinase negative and not able to reduce NO₃⁻.

Submission of a segment of 1400 bp of the 16S rRNA gene to GenBank (HQ603685, HQ603686, HQ603687 and HQ625564) indicated that the isolates belonged to Sphingomonas genus (Fig. 1a). To determine the phylogenetic position of the strains, the 16S rDNA was aligned with the corresponding sequences of the known PAH-degrading strains within the genus Sphingomonas (Fig. 1b). This genus is frequently isolated from contaminated soils as one of the main culturable PAH degraders (Uyttebroek et al. 2007).

The phylogenetic tree (Fig. 1b) showed that the Patagonian strain 1A, 22A and 36 are closely related between them and they were part of a subcluster that additionally harboring PAH-degrading strain belonging to Sphingomonas and Sphingobium genus. The strain 22B formed a separate subcluster, together with the dioxin-degrading strain Sphingomonas wittichii RW1 (Nam et al. 2005) and the hexachlorocyclohexane-degrading strain Sphingo- monas japonicum UT26 (Nagata et al. 2006).

With the aim of comparing PAH degrading activity and the resistance to different stressing conditions of these Patagonian isolates with that of the exogenous Sphingomonas strain, the PAH degrading strain Sphingomonas paucimobilis 20006FA (Coppotelli et al. 2010), isolated from a La Plata soil microcosm contaminated with phenanthrene and phylogenetically closely related with the Patagonian strains, was incorporated to our studies.

The capacity of the isolates to degrade different PAH was analyzed in liquid cultures with LMM and 26 mg l⁻¹ of fluorene or phenanthrene as unique source of carbon and energy. It was observed that, in batch cultures, all the strains were able to grow using phenanthrene as the sole carbon and energy source (Fig. 2a), reaching a degradation of around 90% of the phenanthrene supplied, after 3 days of incubation (Table 1).

A different behavior was observed in the case of fluorene. Despite of all the Patagonian strains, isolated from a soil with evidences of fluorene contamination (see M&M), showed a percentage of fluorene elimination significantly different from the abiotic control, the strains 1A and 36 showed a percentage of fluorene biodegradation significantly higher (P < 0.01) than the strains 22A and 22B (Table 1). However none of these strains were able to grow using fluorene as the sole carbon and energy source (Fig. 2b). This result suggest that, similar to other bacteria strains (Baboshin et al. 2008), the Patagonian Sphingomonas sp. strains oxidize fluorene by co-metabolism. The strain S. paucimobilis 20006FA, isolated of a phenanthrene contaminated soil microcosm, did not show evidence of fluorene degradation, after 3 days of incubation (Table 1).

In order to evaluate the survival of the strains to C-starvation, they were stored in LMM in total absence of CS, for 85 days. Fig. 3 shows the cultivable cell density during the C-starvation process. Two of the isolates from the Patagonia soil (1A and 36) and the strain S. paucimobilis 20006FA displayed a pattern of response to starvation with an important reduction in cultivable cells, reaching at the end of starvation period a cultivability between 15 to 1 percent, in relation to the initial values of cfu/ml.

The strains 22A and 22B displayed a different pattern of response to starvation, with an initial decrease in cultivable cell number followed by an increase to a constant value. After 85 days of starvation, survival of the strain 22A and 22B showed cultivability of 83.5 and 146.6% respectively.

The survival of the strains to N-starvation was determined in LMM with glucose as CS and in total absence of NS. The cultures were stored and monitored for around 70 days. During the
N-starvation the culturable cell density (Fig. 4a) and the OD<sub>600</sub> (Fig. 4b) of the cultures were determined. As shown in Fig. 4a, all the strains displayed an initial decrease in culturable cells number. After 22 days of N-starvation the reductions in cfu/ml were between 1.14 and 1.17 log; afterwards the culturable cell density remained constant throughout the whole experiment.

The Fig. 4b shows that the OD measures did not follow the trend of the cells counts. The OD of all cultures exhibited a rise on day six by a factor between 2.7 and 7.4, followed by a rapid decline. These results suggest the augmentation of the cell size, which could be caused by the accumulation of intracellular storage compounds. All strains were positive for presence of lipid granules detected by Sudan Black B stain (data not shown).

To study the dehydration tolerance of the strains, cells were grown in R3 medium and tested for their survival under drying conditions (18% of relative humidity). As shown in Table 2, clear differences in the survival under drying conditions were found between the strains. The strain 22B was extremely resistant to this challenge, showing a percentage of survival after 14 days of incubation at 18% of relative humidity, significantly higher (P < 0.01) than the other strains, including the strain of Staphylococcus aureus used as resistant control of drying resistance. The strain 22A presented an intermediate behavior, being significantly more sensitive to drying than the strain 22B, but more resistant than the clearly sensible strains 1A, 36, and S. paucimobilis 20006FA.

The response of strains to different salinity concentrations was determined. All the strains showed similar behaviors, an important decrease in the specific growth rate and maximum OD<sub>600</sub> was observed in R3 broth batch cultures with 1.25 and 2.50% w/v of NaCl, compared with the respective cultures in R3 broth supplemented with 0.5% of NaCl (Table 3). None of the strain was able to grow in presence of 5% of NaCl.

With the traditional enrichment approach only one strain with PAH-degrading capacity was isolated, the strain Sphingomonas sp. 36. This strain showed phenanthrene and fluorene degrading activity (Table 1). However, the Sphingomonas sp. strain 36 was very sensitive to drying conditions (Table 2), the one of the principal abiotic factors that clearly limits the biodegradation activity of microbial community in the Patagonian soil (Fig. 5). The traditional enrichment approach resulted in the selection of strains that express the required degradation ability, but the procedure is unlikely to have any influence on other traits that are also required for strains to be competitive and effective in the target environment (Thompson et al. 2005).

Using the direct isolation approach three PAH-degrading Sphingomonas sp. strains (1A, 22A and 22B) were isolated, and they might be considered as members of the predominant culturable soil bacteria. The strains 1A and 22A are phylogenetically closely related between them and with the Sphingomonas sp. strain 36 and S. paucimobilis 20006FA (Fig. 1b). However, the strain 22A showed...
a different physiological response, being significantly more resistant to C-starvation (Fig. 3) and drying conditions (Table 2) than other related strains. The other PAH-degrading strain isolated using the direct approach, the strain 22B, showed a different phylogenetic position than the Patagonian strains 1A, 22A and 36, and the strain S. paucimobilis 20006FA. The strain 22B displayed a similar pattern of response to C-starvation than the strain 22A, but it turned out to be the strain that showed the highest resistance to drying conditions (Table 2). This would be demonstrating the functional redundancy of the soil microorganism related with the PAH catabolism that exhibit different physiological traits. In the soil several factors mitigate the severity of the competition, and the competitors often coexist, supporting the homeostasis of the habitat front of the fluctuating environmental conditions (Atlas and Bartha 1998).

3.2. Effects of strain inoculation on phenanthrene-induced mineralization and phenanthrene elimination

The effects of C/N/P ratio and water content on phenanthrene-induced mineralization and phenanthrene elimination of a Patagonia soil contaminated with phenanthrene and individually inoculated with the strains 1A, 22A, 22B and S. paucimobilis 20006FA were studied (Fig. 5). The inoculation with the strain 36 was not conducted because it displayed a similar behavior than the strain 1A in terms of phenanthrene and fluorene degrading activity (Table 1), survival on C- and N-starvation (Figs. 3 and 4) and water stress (Tables 2 and 3).

Fig. 5a shows that in NPS (Natural Patagonia Soil, typically under starvation and drying conditions) the contamination with phenanthrene did not produce a substantial increase in the CO2 production of the control systems (NPS-C) during the whole experiment time (30 days). And by this time the phenanthrene elimination reached only to 17.59 ± 1.45% of the phenanthrene supplied.

Whereas the inoculation of contaminated NPS with the strain 1A, 22A and S. paucimobilis 20006FA did not produce differences in the CO2 production, in comparison with the control system, the strain 22B produced a significant increase in the phenanthrene-induced mineralization (Fig. 5a). After 30 days of incubation the NPS-22B system reached a production of 23.01 ± 4.05 μmoles of CO2 g⁻¹ of dry soil, that was significantly higher (P < 0.05) than the values achieved by the NPS-C, NPS-1A, NPS-22A and NPS-Sp systems (0.26 ± 0.98–3.25 ± 2.38 μmoles of CO2 g⁻¹ of dry soil). However, in this condition and after 30 days of incubation, all the inoculated systems showed percentages of phenanthrene elimination (12.14 ± 0.79–21.16 ± 3.65%) not significantly different (P < 0.05) between them and from the control system (NPS-C).

The results of FPS systems (Fig. 5b) showed that the incorporation of N and P to the Patagonia soil caused a stimulatory effect on phenanthrene-induced mineralization of the FPS-C, FPS-1A, FPS-22A and FPS-Sp systems, reaching the CO2 production level of system inoculated with the strain 22B. The fertilization did not cause significant differences (P > 0.05) in the total CO2 production between FPS-22B (23.01 ± 4.05 μmoles of CO2 g⁻¹ of dry soil) and FPS-22B (25.47 ± 3.39 μmoles of CO2 g⁻¹ of dry soil) systems.

An important stimulatory effect on phenanthrene-induced mineralization (Fig. 5c) and phenanthrene elimination (Table 4) was observed in all systems, including the WPS-C system, when the water content was increased to 15% w/w. In this conditions the total CO2 production and the percentage of phenanthrene elimination, after 30 days of incubations, of all WPS systems were significantly higher (P < 0.05) than the values obtained by the corresponding NPS and FPS systems.

When the water stress was partially corrected, the total CO2 production of the WPS-Sp (88.80 ± 5.85 μmoles of CO2 g⁻¹ of dry soil), WPS-1A (62.79 ± 4.07 μmoles of CO2 g⁻¹ of dry soil) and WPS-22B (63.15 ± 2.46 μmoles of CO2 g⁻¹ of dry soil) systems was significantly higher (P < 0.05) than the WPS-C and WPS-22A systems. In agreement with that results the WPS-1A, WPS-22B and

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**Table 1**

Percentage of phenanthrene and fluorene elimination by PAH-degrading bacteria isolated from Patagonia soil (1A, 22A, 22B and 36) and S. paucimobilis 20006FA.

<table>
<thead>
<tr>
<th>Strains</th>
<th>% of PAH elimination after 3 days of incubation</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Phenanthrene</td>
</tr>
<tr>
<td>1A</td>
<td>94.00 ± 6.22&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>22A</td>
<td>98.00 ± 1.79&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>22B</td>
<td>96.20 ± 2.77&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>36</td>
<td>97.90 ± 1.52&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>S. paucimobilis 20006FA</td>
<td>85.64 ± 8.86&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Abiotic Control</td>
<td>9.10 ± 0.50&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values, gives as means of triplicate independent experiments (standard deviation), followed by the same letter are not significantly different (P < 0.01).

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**Fig. 2.** Growth of PAH-degrading Patagonian strains 1A, 22A, 22B and 36 and the strain S. paucimobilis 20006FA (Sp) in A: phenanthrene, B: fluorene. Values represent the averages of triplicate determinations.

**Fig. 3.** Survival of PAH-degrading Patagonian strains 1A, 22A, 22B and 36 and the strain S. paucimobilis 20006FA (Sp) under C-Starvation. Results are means of three independent experiments. Bars represent standard deviations.
WPS-Sp systems showed percentage of phenanthrene elimination significantly higher \((P < 0.05)\) than WPS-C and WPS-22A systems (Table 4).

With this simple approach we could determine that:

i) Clearly is the water stress, and not the N and P starvation conditions, one of the environmental factors that controls de activity of the introduced phenanthrene-degrading bacteria and the soil microbial community, in the Patagonian soil. Ronen et al. (2000) found that the biodegradation of tribromophenol (TBP) proceeded to a small extent in contaminated soil with only 10% of water content and that the soil moisture influenced the survival of the inoculated TBP-degrading bacteria. In the same sense, Mashreghi and Prosser (2006) demonstrated that the soil water potential affects the survival and degradative activity of Pseudomonas stutzeri in soil amended with phenanthrene. Similar results were observed with the strain S. paucimobilis 20006FA, in a previous work we demonstrated the capacity of this strain to establish itself in phenanthrene-contaminated soil with a 20 ± 2% of moisture, causing a significant increase in the CO2 production rate of the soil microflora (Coppotelli et al. 2008). However the inoculation with the strain S. paucimobilis 20006FA did not produce stimulatory effect neither on phenanthrene-induced mineralization (Fig. 5a and b) nor in phenanthrene degradation, in soil systems with 10% of moisture. When the water stress was partially corrected (15% of moisture) this strain showed the highest values of CO2 production in the Patagonian soil system (Fig. 5c) and significant phenanthrene elimination was also found (Table 4).

ii) The effect of inoculation on CO2 production activity (as indication of soil microbial activity) was not correlated with phenanthrene elimination activity. The inoculation of Patagonian strain 22B produced a significant increase of phenanthrene-induced mineralization of NPS systems (Fig. 5a), but without a corresponding increase in phenanthrene elimination. Similar effect was observed in FPS systems, the incorporation of N and P caused a stimulatory effect on phenanthrene-induced mineralization that was not correlated with an increase in the phenanthrene elimination. In the same way, in WPS systems, whereas the inoculation with the strain S. paucimobilis 20006FA produced the highest stimulatory effect on phenanthrene induced mineralization, the WPS-22B showed the highest phenanthrene degrading activity.

Soil drying causes adverse physiological effects associated with cell dehydration but also produces diffusional limitation of the substrate supply, and for xerotolerant microbial populations, the substrate diffusion may represent the primary limiting factor at lower water potentials (Stark and Firestone 1995).

iii) In concordance with the physiological characterization of the Patagonian strain 22B, that had showed the highest resistance to drying conditions, it was the unique strain that produced a significant increase on phenanthrene-induced mineralization when was inoculated in the NPS systems (Fig. 5a). Also, when the water stress was partially corrected (15% w/w), the system inoculated with de Patagonian strain 22B showed the highest percentage of phenanthrene elimination (Table 4).

Table 2

Percentage of survival of PAH-degrading bacteria isolated from Patagonia soil (1A, 22A, 22B and 36) and S. paucimobilis 20006FA, after 14 days of incubation under water stress conditions. S. aureus was used as water stress resistance control.

<table>
<thead>
<tr>
<th>Strain</th>
<th>% of survival under water stress</th>
<th>Time (Days)</th>
<th>14 days of water stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>38.5 ± 3.5</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>22A</td>
<td>72.6 ± 1.3</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>22B</td>
<td>82.2 ± 2.3</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>36</td>
<td>49.5 ± 1.2</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>S. paucimobilis 20006FA</td>
<td>45.5 ± 1.0</td>
<td>14</td>
<td>14</td>
</tr>
<tr>
<td>S. aureus</td>
<td>81.5 ± 1.17</td>
<td>14</td>
<td>14</td>
</tr>
</tbody>
</table>

Values, gives as means of triplicate independent experiments (standard deviation), followed by the same letter are not significantly different \((P < 0.01)\).

Table 3

Effect of NaCl concentration on growth kinetic of PAH-degrading bacteria isolated from Patagonia soil (1A, 22A, 22B and 36) and S. paucimobilis 20006FA grown in R2 medium batch culture.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Concentration of NaCl (w/v)</th>
<th>0.50%</th>
<th>1.25%</th>
<th>2.50%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>μ (h⁻¹) Max. OD₆₀₀</td>
<td>Max. OD₆₀₀</td>
<td>μ (h⁻¹) Max. OD₆₀₀</td>
<td>μ (h⁻¹) Max. OD₆₀₀</td>
</tr>
<tr>
<td>1A</td>
<td>0.44 ± 0.17</td>
<td>0.23 ± 0.27</td>
<td>0.17 ± 0.12</td>
<td>0.10 ± 0.13</td>
</tr>
<tr>
<td>22A</td>
<td>0.38 ± 0.20</td>
<td>0.23 ± 0.27</td>
<td>0.17 ± 0.12</td>
<td>0.10 ± 0.13</td>
</tr>
<tr>
<td>22B</td>
<td>0.41 ± 0.17</td>
<td>0.23 ± 0.27</td>
<td>0.17 ± 0.12</td>
<td>0.10 ± 0.13</td>
</tr>
<tr>
<td>36</td>
<td>0.49 ± 0.17</td>
<td>0.28 ± 0.31</td>
<td>0.20 ± 0.25</td>
<td>0.20 ± 0.25</td>
</tr>
<tr>
<td>S. paucimobilis 20006FA</td>
<td>0.52 ± 0.20</td>
<td>0.28 ± 0.31</td>
<td>0.20 ± 0.25</td>
<td>0.20 ± 0.25</td>
</tr>
</tbody>
</table>

μ: specific growth rate.
The results of un-inoculated systems are also showed (control). Results are means of three independent experiments. Bars represent standard deviations.

Table 4

<table>
<thead>
<tr>
<th>Systems</th>
<th>% of phenanthrene elimination after 30 days of incubation</th>
</tr>
</thead>
<tbody>
<tr>
<td>WPS-C</td>
<td>32.48 ± 7.22a</td>
</tr>
<tr>
<td>WPS-1A</td>
<td>56.85 ± 10.71b</td>
</tr>
<tr>
<td>WPS-22A</td>
<td>36.59 ± 5.19b</td>
</tr>
<tr>
<td>WPS-22B</td>
<td>72.98 ± 11.13b</td>
</tr>
<tr>
<td>WPS-Sp</td>
<td>51.28 ± 13.00b</td>
</tr>
</tbody>
</table>

Values, gives as means of triplicate independent experiments (standard deviation), followed by the same letter are not significantly different (P < 0.05).

The results suggest the presence of mechanisms of adaptation to the typical environmental local conditions in the Patagonian strain 22B, and it might be the most suitable strain for being used as a bacterial inoculum in PAH-contaminated soils of Central Patagonia.

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References


