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Tolerance, growth and degradation of phenanthrene and benzo[*a*]pyrene by *Rhizobium tropici* CIAT 899 in liquid culture medium

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ABSTRACT

Polycyclic aromatic hydrocarbons (PAH) are ubiquitous pollutants that are toxic and recalcitrant to degradation by bacteria. This research evaluated the toxicity of different concentrations [10, 20, 40, 60, 80 and 100 μg mL^{-1}] of phenanthrene (PHE) or benzo[*a*]pyrene (BaP) on the growth of *Rhizobium tropici* CIAT899 under *in vitro* conditions as well as the potential degradation of PHE and BaP by this bacterium. At 24 h, a 40% decrease in *Rhizobium* growth was observed when exposed to 40 μg mL^{-1} of either PHE or BaP. Furthermore, bacterial growth was completely inhibited by PHE or BaP applied in 80 and 100 μg mL^{-1}. After 96 h, the growth of *R. tropici* at 40 μg PHE mL^{-1} or 60 μg BaP mL^{-1} was similar to those treatments without PAH. To evaluate *R. tropici* degrading capabilities, supernatants of cultures with 40 μg PHE mL^{-1} or 60 μg BaP mL^{-1} were analyzed by gas chromatography coupled to mass spectrophotometer (GC–MS). *R. tropici* was able to degrade either PHE or BaP diminishing its concentration in 20% and 25% during the first 24 h, degradation obtained at 120 h was 50% and 45% for PHE or BaP, respectively. This research shows for the first time that *R. tropici* CIAT 899 grows in liquid culture medium contaminated with PAH, and moreover is able to growth and to degrade either PHE or BaP.

1. Introduction

Polycyclic aromatic hydrocarbons (PAH) consist of two or more fused rings of benzene whose principal source is from byproducts of fossil fuels and from pyrogenic origin (Cerniglia, 1992; Seo et al., 2009). The PAH have a wide distribution in most ecosystems; besides they are recalcitrant to degradation and very persistent in the environment. Due to their toxicity, genotoxicity and carcinogenesis effects or properties, the United States Environmental Protection Agency (USEPA) has classified them as principal pollutants in the environment (Kanaly and Harayama, 2000; USEPA, 2004; ATSDR, 2005).

Contamination with PAH causes the inhibition of soil microorganisms (Eom et al., 2007). Nevertheless, some microbial groups may adapt and grow in presence of these organic compounds (Wickle, 2000; Peixoto et al., 2011). For instance, aerobic bacteria can tolerate PAH (Habe and Omori, 2003; Kanaly and Harayama, 2000; Zhang and Bennett, 2005; Si-Zhong et al., 2009), and are common in sites contaminated by PAH or toxic compounds like pesticides, and heavy metals. Tolerant bacteria may utilize PAHs as source of carbon and energy for their growth and reproduction; furthermore, bacterial metabolism releases enzymes such as dioxygenases, peroxidases and catalases, by which PAH are oxidized and consequently, transformed or degraded to less toxic compounds (Harvey et al., 2002; Siciliano et al., 2002).

Microbial mediated PAH degradation has been studied in free living microorganisms, and it is frequently reported for those PAH with low molecular weight that are characterized by having two or three aromatic rings and being less toxic, for example, phenanthrene (PHE) (Chauhan et al., 2008). Bacteria that degrade this kind of organic compounds are *Pseudomonas, Sphingomonas*, or *Xanthomonas* (Kanaly and Harayama, 2000). In contrast, *Burkholderia*, *Mycobacterium*, or *Rhodococcus* are able to degrade PAH with high molecular weight (Seo et al., 2009; Santos et al., 2008; Xu-Xiang et al., 2006). Typically, PAH with high molecular weight are more recalcitrant because they consisted on four or more fused rings of benzene, making them less available for their degradation (Atlas and Bartha, 2002). An example of this kind of compounds is benzo[*a*]pyrene (BaP) which has five fused benzene rings with genotoxic and carcinogenic properties, and is resistant to microbial

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attack (Pothuluri and Cerniglia, 1994). These PAH with high molecular weight may also be degraded by co-metabolism, where PAH-transformation depends on the availability of an alternate source of carbon (such as glucose, peptone, *etc.*) that is used as a main substrate (Johnsen and Karlson, 2005; Zhong et al., 2010).

PAH-biodegradation has been mainly studied in free living bacteria; although there are few cases of biodegradation studied in symbiotic bacteria. In this regard, symbiotic bacteria belonging to the *Rhizobium* genus are well known for establishing a mutualistic symbiosis with legumes, and for their contribution on the biological fixation of atmospheric nitrogen (Oldroyd and Downie, 2008).

The advantage of using rhizobia in PAH-bioremediation processes is related with their ability to fix nitrogen which is limiting during bioremediation since deposition of petroleum hydrocarbons significantly increases the accumulation of organic carbon in soil, and generates a very high C/N unbalance (Alexander, 1999; Dashti et al., 2010; López-Martínez et al., 2005; Moreto et al., 2005; Radwan et al., 2007).

Rhizobium tropici CIAT 899 has been reported as tolerant to heavy metals and to antibiotics such as chloramphenicol, spectinomycin, carbenicillin and streptomycin (Martínez-Romero et al., 1991). Other members of *Rhizobium* or *Mesorhizobium* are able to tolerate and to degrade toxic substances such as dibenzothiophene (Frassinetti et al., 1998), 2,4-dinitrotoluene (Dutta et al., 2003), and some PAH like PHE and acenaphthylene (Poonthrigpun et al., 2006). Our aim was to evaluate the tolerance of *R. tropici* CIAT 899 to PHE and BaP, and to determine the degradation of both PAH by this bacterium grown under *in vitro* conditions.

2. Materials and methods

R. tropici CIAT 899 strain was obtained from Peter Graham from International Center for Tropical Agriculture (CIAT), Colombia. The strain was isolated from nodules of *Phaseolus vulgaris* collected from Carmen del Viboral, Colombia (Martínez-Romero et al., 1991; Weisburg et al., 1991; Rincón-Rosales et al., 2009). Its 16S rRNA gene was obtained by PCR with fD1 and rD1 primers and sequenced by Sanger (Martínez-Romero et al., 1991). The whole genome sequence of this bacterial strain is now available (Ormeño-Orrillo et al., unpublished data). Strain CIAT 899 was grown in yeast extract mannitol medium with agar and Congo red (YEMACR) incubated at 28 °C for three days (Microbiological Incubator, Thermo Scientific®). Bacterial growth was assessed every 24 h. Once bacteria were reactivated a test of purity was performed on the basis of the litmus milk procedure (Vincent, 1970). Then, bacteria were transferred to inclined tubes that contained yeast extract mannitol agar (YEMA), for its conservation and later use. Stock solutions were prepared for each PAH: phenanthrene (PHE; molecular weight 178.23; Sigma–Aldrich®) and benzo[*a*]pyrene (BaP; molecular weight 252.31; Sigma–Aldrich®) using acetone as solvent. Concentrations of each stock solution were 400 μg mL^{-1} for PHE, and 600 μg mL^{-1} for BaP, and these stocks were used to obtain the following concentrations: 10, 20, 40, 60, 80 and 100 μg mL^{-1}. Solutions were maintained in amber vials to prevent photo-oxidation of hydrocarbons, and maintained at 4 °C to avoid solvent volatilization.

2.1. Growth of Rhizobium tropici CIAT 899 in liquid medium contaminated with increased concentrations of phenanthrene and/or benzo[a]pyrene.

Yeast extract mannitol (YEM) liquid medium was utilized, but added with 50% of its original carbon source. The composition of this medium was as follows (g L^{-1}): 0.5 K_2HPO_4, 0.2 $MgSO_4 \cdot 7H_2O$, 0.1 NaCl, 5.0 mannitol, 0.75 yeast extract, and pH 7.0.

To evaluate the toxic effects of both PAH on bacterial growth, *R. tropici* was exposed to YEM medium contaminated with either PHE or BaP at the following concentrations: 10, 20, 40, 60, 80 and 100 μg mL^{-1}, with three replicates. For both assays, two treatments with *Rhizobium* without PAH were also set. Bacterial growth was estimated by optical density (O.D.) at 600 nm, during 24 h. This evaluation time was determined from previous data in which *R. tropici* CIAT 899 under uncontaminated medium shows a dormant phase (lag phase) of three hours, and its maximum cellular division (log phase) starts after the third hour and finishes at 18 h, afterwards the stationary phase occurs (data non shown).

The experiment was completely randomized with seven concentrations of each PAH (PHE or BaP), and three replicates. The experiment for each PAH was repeated twice. Data were analyzed using an analysis of variance, and the mean comparison test (Tukey, $\alpha = 0.05$) using the SAS statistical program (SAS Institute, 2000).

2.2. Growth responses of Rhizobium tropici CIAT 899 and its contribution on the degradation of PHE or BaP

Single concentrations of each PAH were chosen (40 μg PHE mL^{-1} or 60 μg BaP mL^{-1}) based on the results from the previous section for evaluating the growth responses of *R. tropici* and its contribution on degrading either during 120 h. The modified YEM medium was used in the following treatments: (a) inoculated medium with *Rhizobium*, (b) contaminated medium with 40 μg PHE mL^{-1} + *Rhizobium*; (c) contaminated medium with 60 μg BaP mL^{-1} + *Rhizobium*; (d) contaminated medium with 40 μg PHE mL^{-1}, and (e) contaminated medium with 60 μg BaP mL^{-1}. The latter two treatments were set as abiotic controls by which PHE or BaP degradation was estimated. For those treatments inoculated with *Rhizobium* an aliquot of 500 μL of a bacterial suspension taken in logarithmic growth was inoculated (10^7 UFC mL^{-1}). All treatments were incubated under reciprocal shaking at 180 rpm (Incubator MAQX4000, Thermo Scientific®) at 28 °C for four days.

Rhizobium growth was evaluated through the estimation of colonies forming units (CFU) at 24, 48, 72 and 96 h. Bacterial CFU were counted and, transformed to logarithmic units (Log CFU) for statistical analyses (Vincent, 1970). In addition, bacterial biomass from each treatment was determined by centrifugation at 726 × *g* for 20 min, and the bacterial pellet was dried at 60 °C for 72 h to record its dry biomass.

The extraction from the liquid medium and the abundance analysis of PHE or BaP was performed with liquid–liquid extraction. The culture medium (20 mL) was poured in a separation funnel in which 10 mL of hexane HPLC grade (J.T. Baker®) were added. The funnels were manually shaken for 10 min for treatments contaminated with PHE, and for 20 min in the case of BaP, to completely extract each PAH. Funnels were kept at rest until two phases were clearly observed, and from the upper phase (dissolvent with the PAH) aliquots of 1 mL were taken and transferred to 1.5 mL dark vials.

The PAH extracted from each treatment were quantified in a gas chromatograph (Agilent Technologies® Model 6890 N) using a DB-5 column (5%-phenylmethylpolysilosane) 60 meters long, 0.25 mm in diameter and a film thickness of 0.25. The starting temperature was 70 °C, which was maintained for 5 min, and risen to 280 °C in 10 min, using a heating ramp of 40 °C min^{-1}. The carrier gas was helium, which had a flow speed of 1 mL min^{-1}. The injector's temperature was 250 °C, and the injection was splitless. Once the chromatogram was obtained, each of the chromatographic peaks was identified by means of mass spectrometry, using a mass detector (Agilent Technologies® Mod. 5975 inert XL). Mass spectra were obtained by ionization by electronic impact at 70 eV, and for identification, the mass spectra were compared for each compound, with a database (HP Chemstation-NIST 05 Mass Spectral Search

Program, version 2.0d). Data were compared to the corresponding standards: PHE (98% purity; $C_{14}H_{10}$; MW 178.23; bp 340 °C/760 mm Hg; mp98 °C; Sigma–Aldrich®), and BaP (≥96% purity; $C_{20}H_{12}$; MW 252.31; bp 495 °C/760 mm Hg; mp 177–180 °C; Sigma–Aldrich®). These two standards were analyzed under the same GC–MS conditions, and used for estimating the final concentration in each treatment.

A 2×2 factorial experiment was set in which treatments consisted on flasks inoculated or not with *R. tropici*, and with or without PAH. Each type of PAH was separately analyzed and treatments had four replicates each. Bacterial growth was determined at 24, 48, 72, and 96 h, and degradation of each PAH was evaluated at 24, 48, 72, 96, and 120 h. The experiment was repeated twice. Data were analyzed using an analysis of variance, and the mean comparison test (Tukey, $\alpha = 0.05$) using the SAS statistical program (SAS Institute, 2000).

3. Results and discussion

3.1. Growth of Rhizobium tropici CIAT 899 in liquid medium contaminated with increased concentrations of phenanthrene and/or benzo[a]pyrene

Fig. 1 shows a significant decrease (>40%) on the growth of *R. tropici* with the application of 40 μg mL^{-1} of PHE or BaP with respect to the control. Moreover, bacterial growth was significantly inhibited by both PAH at concentrations greater than 60 μg mL^{-1}; but at 80 and 100 μg mL^{-1} the bacterial growth was completely inhibited (Fig. 1).

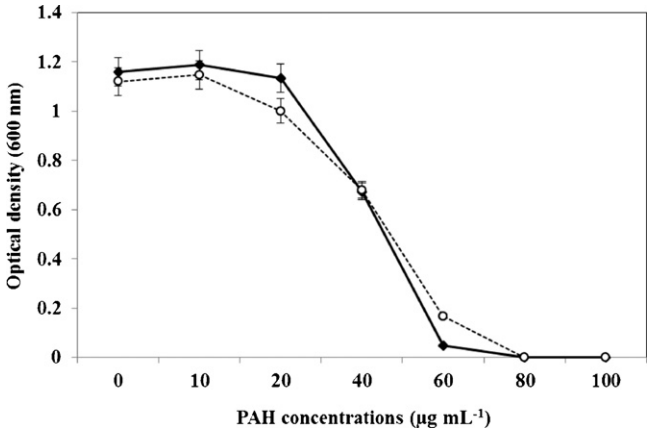

Fig. 1. Growth of *Rhizobium tropici* CIAT 899 measured as optical density (600 nm) in liquid culture medium contaminated with concentrations of two polycyclic aromatic hydrocarbons (PAH): (♦) phenanthrene and (○) benzo[*a*]pyrene, at 24 h. Mean ±Standard error. $n = 3$.

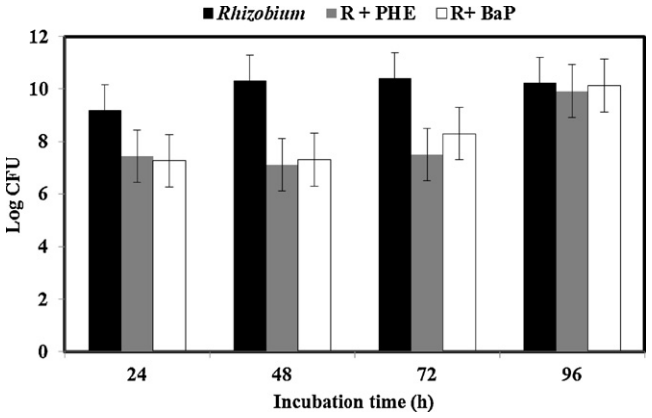

Fig. 2. Growth (logarithmic units, Log CFU) of *Rhizobium tropici* CIAT 899 in liquid culture medium contaminated with phenanthrene [40 μg PHE mL^{-1}] or benzo[*a*]pyrene [60 μg BaP mL^{-1}]. ■ *Rhizobium*, ▩ *Rhizobium* + PHE; □, *Rhizobium* + BaP. Mean ±Standard error. $n = 4$.

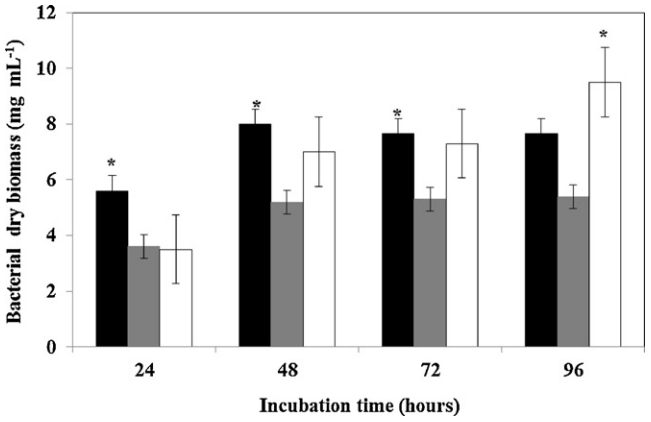

Fig. 3. Dry biomass of *Rhizobium tropici* CIAT 899 obtained in liquid culture medium contaminated with phenanthrene [40 μg PHE mL^{-1}] or benzo[*a*]pyrene [60 μg BaP mL^{-1}]. ■ *Rhizobium* (Control); ▩ *Rhizobium* + PHE; □ *Rhizobium* + BaP. Mean ±Standard error. $n = 4$. Asterisks indicate significant differences (Tukey, $\alpha = 0.05$) among treatments at the same sampling time.

There are no reports to compare the negative effects of PAH on the growth of free living rhizobia; however, some toxic compounds may affect their growth and tolerance. For instance, the application of pentachlorophenol (>180 mg kg^{-1}) resulted in growth inhibition of *R. leguminosarum* (Chaudri et al., 1996). Xenobiotic compounds such as chloride biphenyls also have shown negative effects on four *Rhizobium*/*Bradyrhizobium* strains, but these inhibitory effects

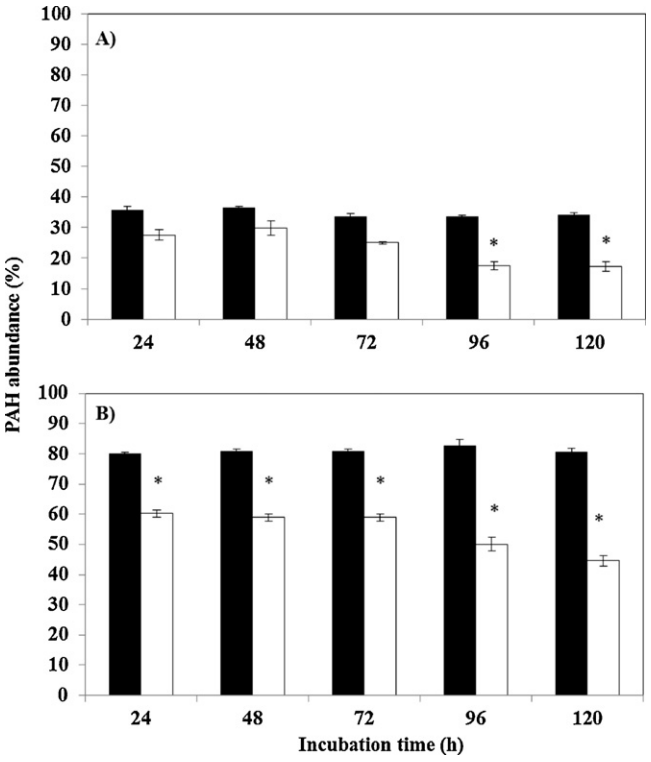

Fig. 4. Abundance expressed as percentage of two polycyclic aromatic hydrocarbons (PAH) in liquid culture media without (■) or with (□) the inoculation of *Rhizobium tropici* CIAT 899. (A) Abundance of phenanthrene (40 μg PHE mL^{-1}), and (B) Abundance of benzo[*a*]pyrene (60 μg BaP mL^{-1}). Mean ±Standard error. $n = 4$. Asterisks indicate significant differences (Tukey, $\alpha = 0.05$) among treatments at the same sampling times.

were mitigated when an alternate carbon source was applied to the culture medium (Damaj and Ahmad, 1996).

It was expected that BaP with five aromatic rings had greater toxic effects on the bacterial growth than PHE, also because BaP is one of the most potentially toxic hydrocarbons with genotoxic, teratogenic, and carcinogenic properties (Van Hamme et al., 2003). Nevertheless, *R. tropici* grew and tolerated the presence of PHE or BaP at concentrations lower than 60 μg mL^{-1}. This bacterial response is comparable to other free living bacteria such as *Rhodococus, Nocardia, Mycobacterium, Gardona* and *Bacillus subtilis* (Juhasz and Naidu, 2000; Hunter et al., 2005; Eom et al., 2007).

3.2. Growth responses of Rhizobium tropici CIAT 899, and its contribution on the degradation of PHE or BaP

Fig. 2 shows that the population of *R. tropici* varied in the presence of both PAH. At 24 and 48 h the Log CFU were significantly reduced in the presence of PHE or BaP (20 and 27%, respectively) when compared to the control, and this behavior remained at 72 h, indicating that bacteria were still in the process of adaptation to the contaminated medium. However, at 96 h the population of *Rhizobium* was similar to the control (Fig. 2). In our experimental conditions, yeast addition in the culture media may help the bacterial growth in spite of the presence of each PAH which caused a growth delay at initial stages (Fig. 2). This agrees with the finding of Keum et al. (2008) who demonstrated that the addition of alternate carbon sources such as glucose or pyruvate enhanced the growth of *Sinorhizobium* sp C4 in presence of PHE (500 mg in 1.5 L). It seems that *R. tropici* needs more time for adapting to PHE or BaP, and this adaptation occurs after 72 h of PAH-exposure. This growth response has been described by Kaushish et al. (2009) by observing that *B. subtilis* BMT4 (MTCC9447) had 12 h of latency when exposed to 50 μg BaP mL^{-1}, and after that time its growth was exponential. Nevertheless, this behavior may depend on the bacterial genotype or on the type and combination of PAH in the culture medium (Zhong et al., 2010).

The tolerance and growth of *R. tropici* observed at 96 h at PHE or BaP contamination is also attributed to the fact that these bacteria are using both organic compounds as a carbon source and energy. In this regard, Seo et al. (2009) mentioned that the growth of *Burkholderia* is related to the availability of PHE in the culture medium, and also indicated that the bacterial growth decreased after five days, time in which PHE was totally used as carbon source and energy as also described by Atlas and Bartha (2002).

Fig. 3 shows the dry biomass production by *R. tropici* in the presence of PHE or BaP when compared to the treatment without

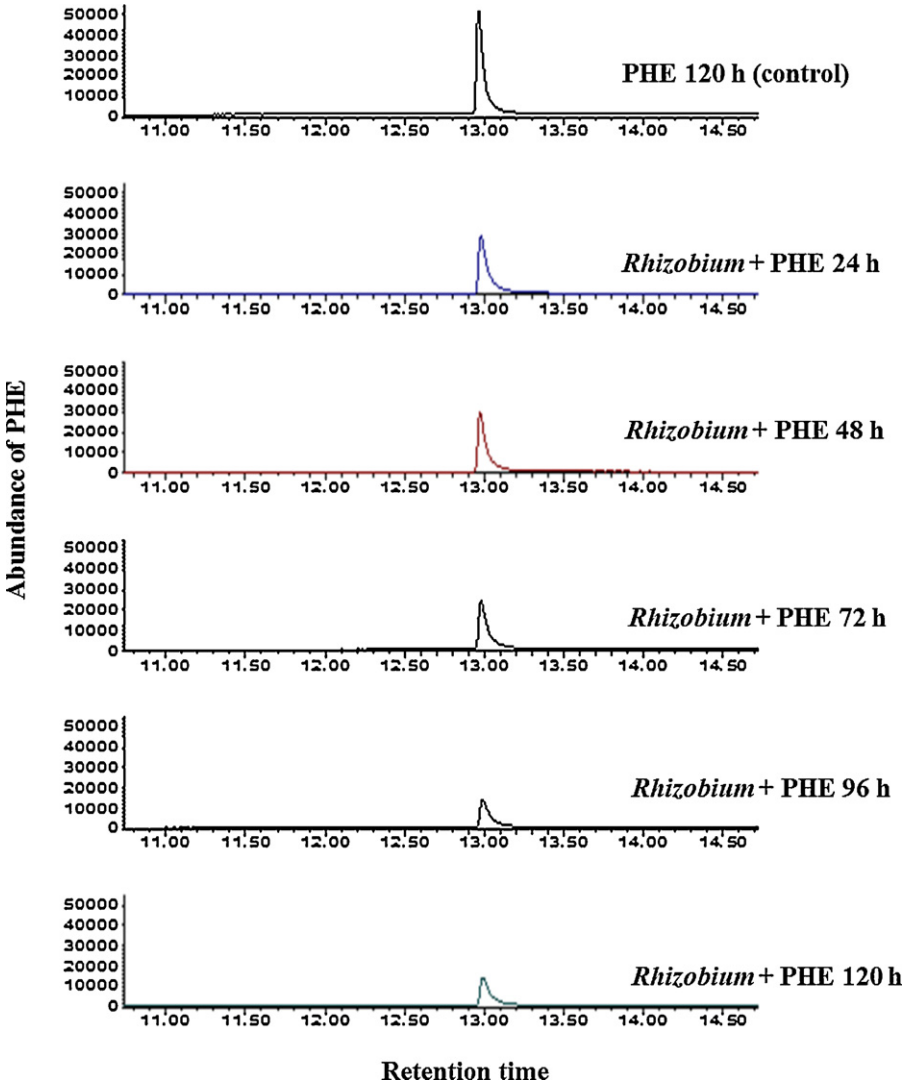

Fig. 5. GC–MS chromatograms that show the abundance peaks of phenanthrene (40 μg PHE mL^{-1}) from liquid culture medium inoculated with *Rhizobium tropici* CIAT 899 at five sampling times.

PAH (control). At 24 h, bacterial dry biomass (BDB) was significantly reduced (36%) due to PAH contamination, although no significant differences were observed between both contaminants at their corresponding concentrations (Fig. 3). After 48 h, the BDB increased in presence of both PAH, especially with BaP in which the BDB had an increase of 60% at 96 h (Fig. 3) when compared to that BDB achieved at 24 h; however, the BDB in presence of PHE kept constant from 48 h to 96 h (Fig. 3). In contrast, BDB in the control was similar from 48 h to 96 h, but significantly greater than that obtained in presence of PHE (Fig. 3).

The *Rhizobium* population observed in Fig. 2 is in agreement with the BDB accumulated in all sampling times (Fig. 3). Dhote et al. (2010) demonstrated that the growth of a bacterial consortium in presence of chrysene was directly proportional to bacterial biomass, and they also pointed out that these two parameters may indicate the utilization of chrysene as carbon source by bacteria. The difference of the BDB obtained between the PHE and the BaP at 48 h indicates that PHE exerts more toxic effects to *Rhizobium* (Fig. 3). In this regard, Traczewska (2000) mentioned that some intermediates from low molecular weight PAH such as anthracene and PHE have strong toxic and mutagenic properties, and may accumulate in cells when an incomplete degradation occurs.

The latest suggests that if *R. tropici* is degrading PHE, its intermediates may become more toxic than the original compound, thus causing a growth inhibition or even the death of bacterial cells. Kazunga and Aitken (2000) indicated that during pyrene degradation there are some metabolites that can be mineralized by the same bacteria or by other microorganisms; however, for PHE there are some metabolites (*cis*-4,5-dihidro-4,5-dihydroxipyrene and pyrene-4,5-dione, for example) that may significantly inhibit its further degradation.

The constant BDB observed by *R. tropici* at 48 h to 96 h in treatments without PAH can be attributed to the carbon consumption in the culture medium, which may result in a limitation for continuous bacterial growth and reproduction (Fig. 3).

Regardless sampling time, Fig. 4 shows a significant decrease in the abundance of PHE (Fig. 4A) or BaP (Fig. 4B) due to inoculation of *R. tropici*. The decrease for both PAH was concordant to the bacterial growth (Figs. 2 and 3). The abundance of PHE or BaP at 120 h decreased significantly in 50% and 45%, respectively, when compared to those treatments in which each PAH was solely applied, in which the PAH abundance was constant along the experimentation (Figs. 4 and 7).

These results show for the first time that *R. tropici* significantly contributes on the degradation of either PHE or BaP. There are only

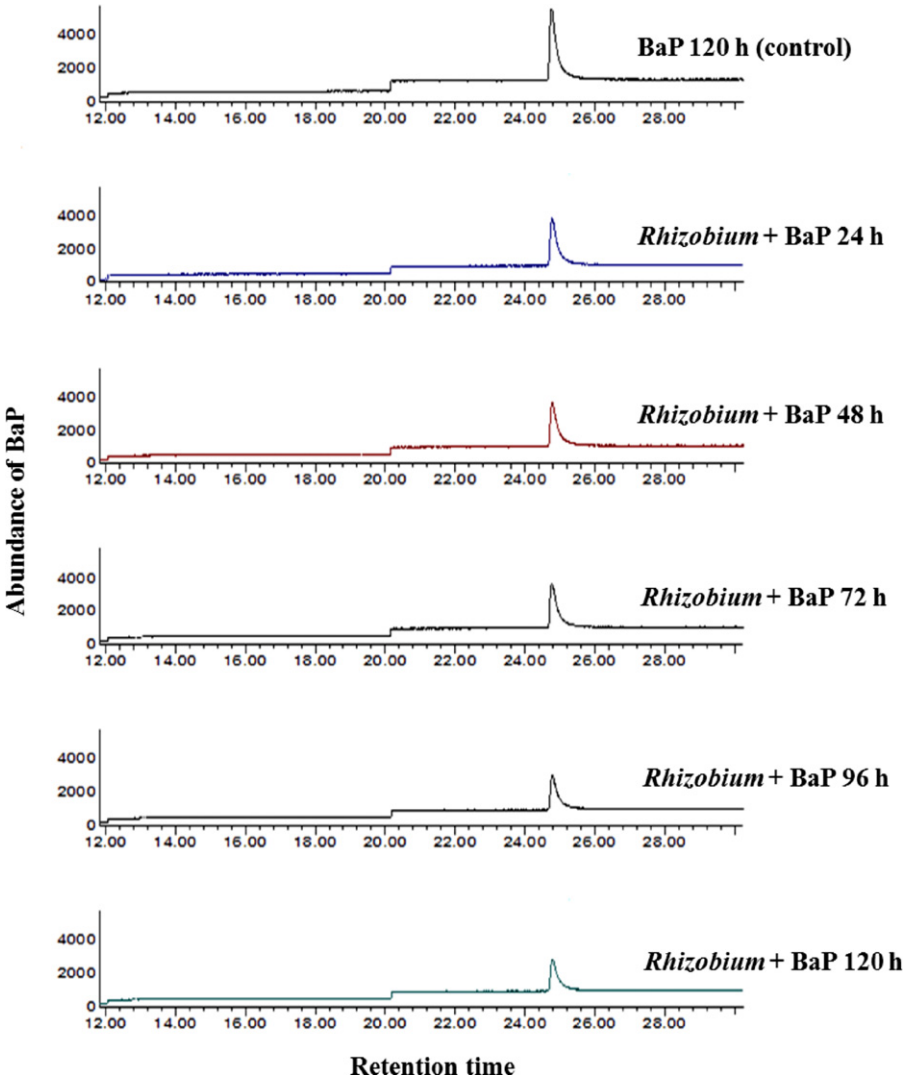

Fig. 6. GC–MS chromatograms that show the abundance peaks of benzo[*a*]pyrene (60 μg BaP mL^{-1}) from liquid culture medium inoculated with *Rhizobium tropici* CIAT 899 at five sampling times.

two reports about the contribution of rhizobia on PAH degradation. *Sinorhizobium* sp. C4 (bacteria isolated from an industrially contaminated site with PAH) is able to take PHE as a source of carbon and energy, thus contributing on its degradation (Keum et al., 2006). Poonthrigpun et al. (2006) demonstrated that *Rhizobium* sp. CU-A1 (isolated from a petroleum-contaminated soil) may completely degrade acenaphthylene (600 mg L^{-1}). Nevertheless, there are some studies in which rhizobia (*Rhizobium* or *Sinorhizobium*) may degrade other toxic substances such as dibenzothiophene (sulfured aromatic hydrocarbon) or 2,4-dinitrotoluene by using either wild type or genetically modified bacteria (Frassinetti et al., 1998; Dutta et al., 2003).

Either PHE or BaP degradation by *R. tropici* CIAT899 may be due to direct activity of the bacteria on the chemical compound or to co-metabolism since the culture medium was added with mannitol and yeast extract for sustaining the bacterial growth and the degradation of both PAH. The beneficial effects of alternate sources of carbon like as yeast extract, acetate, glucose, and pyruvate on the degradation of PAH such as PHE or BaP by other bacterial genera has been also described in several studies (Ye et al., 1996; Yuan et al., 2000; Teng et al., 2010). The enrichment of culture media with an alternate carbon source also allows the induction of oxidative enzymes needed for degrading BaP (Rentz et al., 2005). Furthermore, *R. tropici* CIAT 899 was able to degrade both PAH and this ability is comparable to bacteria like *Rhodococus, Nocardia, Mycobacterium, Gardona* and *B. subtilis* that use this compound as only source of carbon and energy (Juhasz and Naidu, 2000; Hunter et al., 2005).

Figs. 5 and 6 show a series of chromatograms in which it is clearly observed the degradation of each PAH by *R. tropici* CIAT899 at five sampling times (from 24 to 120 h). *R. tropici* CIAT 899 degraded PHE in a comparable extent to that reported for other bacteria (Yuan et al., 2000; Jing et al., 2007; Seo et al., 2007; Leneva et al., 2009), when exposed to lower or similar concentrations of PHE than those utilized in the present experiment. Nevertheless, some species of *Rhizobium* has been also demonstrated to have null effects on the degradation of PHE (Jing et al., 2007).

In regards to BaP, this study shows for the first time that *R. tropici* CIAT 899 can degrade this recalcitrant PAH under *in vitro* systems, and this degradation started at the first 24 h (25%), reaching a maximum degradation (45%) at 120 h (Figs. 4B and 7).The extent of the BaP degradation obtained by *R. tropici* (27.2%) is greater than that reported for *B. subtilis* BMT4 (21.7%) at 72 h (Kaushish et al., 2009). Our maximum degradation of BaP (45%) was achieved during a short incubation period of 120 h (5 days); however, there are reports in which the confrontation of *B. subitilis* with this PAH resulted in a degradation of 84.6% for an incubation period of 28 days (Kaushish et al., 2009).

These findings support the hypothesis that rhizobia may contribute on the degradation of PAH; however, it has been demonstrated that the formation of root nodules by rhizobia is significantly reduced due to PAH or petroleum hydrocarbons (Adam and Duncan, 2003; López-Ortiz et al., 2012). Moreover, further efforts are needed for elucidating the benefits of these bacterial symbionts when legumes are part of phytoremediation processes.

The ecological relevance of this symbiosis at contaminated environments may be related with the incorporation of nitrogen in contaminated systems and consequently, on the improvement for detoxifying or degrading PAH at contaminated soils since a biostimulation process is occurring for the microbial communities in the rhizosphere. Rhizobia are successful rhizospheric bacteria. Therein a "cometabolism" strategy may have evolved to profit from plant exudates and from soil derived substances. It is worth considering that *Rhizobium* may simultaneously use different carbon sources (Romanov and Martínez-Romero, 1994). Bacterial degradation of soil substances would benefit not only the bacteria but also the plant. Interestingly, flavonoids found in root exudates stimulate bacterial gene expression responsible for chlorobiphenyl degradation (Pham et al., 2012). Soil is the depository of plant or insect decay products that contain lignin, cellulose, chitins or waxes or phenolic compounds. Structurally, some of the plant or insect derived molecules may resemble PAH or other human produced contaminants (Vela et al., 2002). Rhizospheric bacteria that degrade such natural substances could be "pre-adapted" to degrade others that mimic them. Thus, if *R. tropici* may have the catabolic machinery to degrade those or related substances, it could be recruited when this strain is exposed to BaP. Here we show for the first time that *R. tropici* CIAT 899 not only tolerates but also degrades BaP in cometabolism with mannitol most probably. *Rhizobium* strains have been used extensively as inoculants in agriculture for over a hundred years. The application of rhizobia for phytoremediation has been more limited (López-Ortiz et al., 2012) but it could be advantageous since these bacteria are safe and capable of fixing nitrogen, especially when nitrogen is a limiting nutrient at contaminated sites.

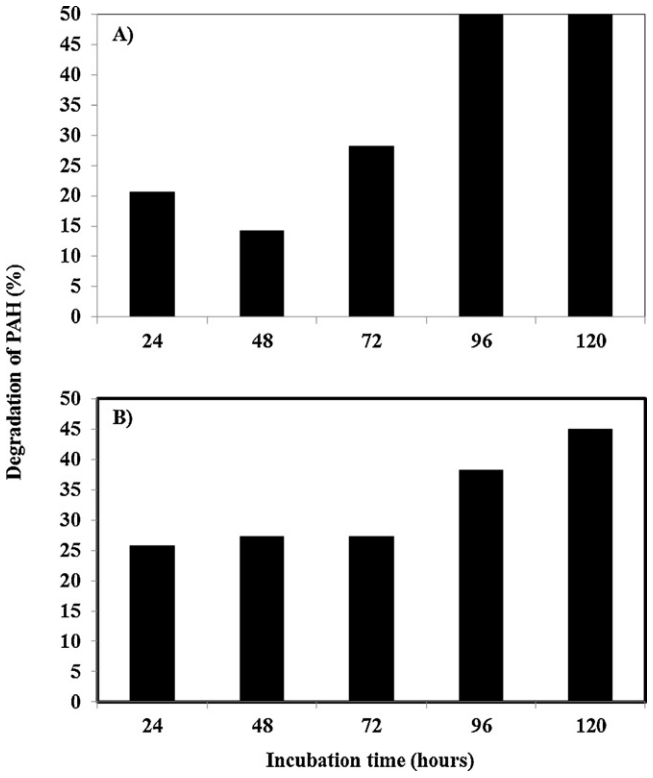

Fig. 7. Degradation (expressed as percentage) of (A) phenanthrene (40 μg PHE mL^{-1}) and (B) benzo[*a*]pyrene (60 μg BaP mL^{-1}) in liquid culture medium inoculated with *Rhizobium tropici* CIAT 899 at five sampling times.

4. Conclusions

The application of either PHE or BaP at 80 and 100 μg mL^{-1} in the culture medium inhibited the growth of *R. tropici* CIAT 899 during the first 24 h. However, this bacterium showed good tolerance to either 40 μg PHE mL^{-1} or 60 μg BaP mL^{-1}, in which its growth and BDB were similar to that obtained by *Rhizobium* without PAH. In spite of its less complex chemical structure, PHE had more toxic effects on the growth of *R. tropici* CIAT 899 than BaP. This study shows for the first time that *R. tropici* CIAT 899 is able to tolerate and grow under PHE or BaP contaminated liquid media; and furthermore, *R. tropici* contributed on degrading 50% of PHE, and 45% of BaP, after 120 h.

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