African Journal of Biotechnology Vol. 11(12), pp. 2977-2984, 9 February, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.2623
ISSN 1684–5315 © 2011 Academic Journals

*Full Length Research Paper*

# Endophytic bacteria with potential for bioremediation of petroleum hydrocarbons and derivatives

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Accepted 5 September, 2011

**Endophytic microorganisms live inside plants and show no apparent damage for the host. They often assist in plants' survival and facilitate their growth, or they can metabolize organic contaminants. This study aimed to isolate and identify the endophytic bacteria of plants present in impacted areas, as well as to test their ability in petroleum and their derivatives' degradation. Plant samples were collected in an asphalt mud impacted area, and then they were superficially disinfected. After maceration and fragmentation, they were incubated at 30°C for about 72 h, when growth of microorganisms was observed in culture media. The verification of petroleum and derivatives degradation capacity was performed in ELISA plates, thereby exposing the bacteria to a solution of minimal medium, the dye DCPIP solution (2,6-dicloroindofenol sodium salt) and the petroleum or derivative tested (burning oil, lubricating oil, diesel oil and gasoline). A positive reading for degradation was observed by discoloration of DCPIP. Among nine bacteria tested, three showed degradative activity in different fractions of petroleum, diesel oil and gasoline, and others showed different profiles. Some isolates were identified from the sequencing of 16S DNA, after which they were diagnosed as *Bacillus cereus, Staphylococcus pasteuri* and *Pseudomonas* sp. The endophytic bacteria isolated from Cerrado plants confirmed their potential for application in bioremediation processes.**

**Key words:** Endophytic microorganisms, petroleum, bioremediation, Cerrado.

## INTRODUCTION

Endophytic microorganisms live a part or all of their life cycles within plants. Unlike phytopathogens, they do not cause any apparent harm to their hosts (Azevedo, 1999). Initially defined as asymptomatic, their importance has grown since the verification of certain properties that contribute to the survival of vegetables, such as greater resistance to biological and abiotic stress (Hallmann et al., 1997), increasing vegetable ability to resist pathogens, herbivores and other vegetables (Wei et al., 1996; Sturz et al., 1998; Elliot et al., 2000).

These organisms receive nutrients and protection from the host plant while producing alkaloids, enzymes, antibiotics and other substances that protect and assist the plant under stress condition (Azevedo et al., 2000). Since these microorganisms are intimately linked to vegetables, they are considered a rich yet little known biotechnological source with applications in medicine, in the pharmaco-chemical industry and in agriculture (Strobel and Daisy, 2003).

Bioremediation exploits the metabolic properties of microorganisms to degrade contaminating agents. This ability may be natural or acquired through the acquisition of codifying genes for specific functions (Ilyina et al., 2003). Although, microorganisms are the main agents utilized in this way, plants are also commonly used (Newman and Reynolds, 2005). Vegetables can recruit bacteria with specific genotypes for the degradation of

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toxic agents on or within their roots. This selection may be specific for certain contaminants, where these bacteria presumably become protectors against the phytotoxic effects of the contaminants (Siciliano et al., 2001). The microbiota present, not only within the vegetable, a characterization of the endophytic community, but also influences the result of the decontamination process in the rhizosphere region. Hydrocarbon degradation is, undoubtedly, the most important bioremediation process and, although not yet totally clear, is directly linked to the recruiting ability of microorganisms and provides the conditions for these to perform their degrading activity (Muratova et al., 2003).

The importance of hydrocarbon remediation is due mainly to the environmental impacts caused by petroleum and its derivatives. Petroleum is a fuel that originated from vast fossil depositions with a variable composition of organic compounds, mainly hydrocarbons (GESAMP, 1993). Hydrocarbon chains present light fractions forming gases and heavy fractions forming oil. The distribution of these chains is differentiated according to the place of origin (Ramos, 2006). The source of the environmental impact caused by the oil industry is in the processes involved in the discovery of new oil wells, oil refinement and transportation, where roughly 10% of the product's use is lost in spills with catastrophic consequences to coastlines (Van Hamme et al., 2003). A large amount of crude oil is also lost in storage causing highly contaminating sludge. Soil contamination by hydrocarbons, as well as underground and surface water, seriously affects the ecosystem, especially through the accumulation within animals and plants resulting in death and mutation (Ilyina et al*.,* 2003).

The activity of microorganisms in the cycle and remediation of hydrocarbons in the environment is undeniable. They are often found in contaminated environments (Labud et al., 2007), although, their action depends on the contaminant present and its availability as a carbon source. A number of bacteria have, therefore, been described as responsible for petroleum and its derivatives bioremediation processes, among these are *Pseudomonas*, *Mycobacterium* (Solano-Serena et al., 2000), *Acinetobacter* sp. (Gallego et al., 2001) and *Serratia marcescens* (Wongsa et al., 2004). Most of the bacteria involved in these bioremediation processes present aerobic metabolisms, some of which are capable of degrading both crude oil and more refined fractions.

The main purpose of this study was the isolation of endophytic bacteria present in vegetables growing in locations impacted by asphalt paving waste, their identification and the determination of their degrading capacity for petroleum and its derivatives, with a view to investigating new forms of hydrocarbon bioremediation.

## MATERIALS AND METHODS

### Sample isolation

Grass samples, predominant in the asphalt mud waste treatment site of the municipal paving company in Goiânia, Goiás – Brazil (Departamento de Estradas e Rodagem da Companhia Municipal de Pavimentação DERMU COMPAV-GO), were collected, placed in previously sterilized flasks and taken to the Laboratório de Microbiologia Ambiental e Biotecnologia (LAMAB) at the Instituto de Patologia Tropical e Saúde Pública in the Universidade Federal de Goiás (IPTSP-UFG) for microorganism isolation. The grass roots were utilized to isolate the microorganisms since they had been in direct contact with the contaminated environment, as well as being one of the main microorganism entrances (Azevedo et al*.,* 2000; Oliveira et al., 2003). The roots were washed in water and soap and then left to dry on an absorbent paper. Once they get dried, they were weighed (1.0 g) and superficially disinfected according to the methodology described by Araújo et al. (2002). The control of the root surface disinfection was performed by inoculating three 1.0 mL aliquots of the last wash water in tubes containing nutrient broth or BHI broth and incubated at 30°C for 72 h. The absence of turbidity in the tubes was considered a positive response regarding the efficiency of the disinfection process. For confirmation, after the incubation period, samples taken from the tubes were inoculated onto Petri dishes containing Nutrient Agar and incubated at 30°C for 48 h. The disinfection efficiency was verified by the non development of colonies.

The isolation of the endophytic microorganisms was performed by the fragmentation technique (Araújo et al., 2002). For this technique, the previously disinfected and weighed samples were cut into 1 cm fragments using a sterilized scalpel. The fragments were then inoculated onto Petri dishes containing the following culture mediums: Agar nutrient (NA), TSA (Tryptone Soy Agar) and King, and then incubated at 30°C for 15 days. After the growth of the microorganisms in culture, they were isolated and purified by depletion. The inoculum transfer procedure by plating was repeated five times to obtain pure colonies. Once purified, the bacteria were kept in NA and stored under refrigeration at 4°C. The isolates were also preserved in 50% glycerol and stored in a freezer at -20°C. A gram coloring morpho-tinctorial identification was performed for the initial classification of the isolated microorganisms.

### Rapid determination of the degrading capacity of petroleum hydrocarbons and derivatives

The degrading capacity of petroleum and its derivatives was determined by adapting the methodology described by Peixoto and Vieira (2005). The isolated bacteria were inoculated in tubes containing 5 mL of minimal medium (g/L: NaCl 5.0, $K_2HPO_4$ 1.0, $NH_4H_2PO_4$ 1.0, $(NH_4)_2SO_4$ 1.0, $MgSO_4$. $7H_2O$ 0.2, $KNO_3$ 3.0) with 10 g/L of glucose and 1.0 g/L of yeast extract, after which they were grown for 72 h in a shaker at 30°C and at 120 rpm. The inoculum was prepared by removing an aliquot of 5.0 ml which was then centrifuged at 10.000 rpm for 5 min at 10°C, resuspended in a phosphate buffer pH 7.0 and centrifuged once again. This procedure was performed twice yet again for the removal of culture medium residue. After the last centrifugation, the concentration of microorganisms was standardized to tube no. 3 of the McFarland Scale in minimal medium without glucose and yeast extract.

The microorganism suspensions were inoculated onto 96 well acrylic plates (ELISA type), previously treated with ultraviolet (UV) light for 30 min. The test was set by adding 20 µl of the suspension with the microorganisms to 168 µl of minimal medium, 12 µl of DCPIP (Meloan and Pomeranz, 1973) and 2 µl of petroleum or the following derivatives: lubricating oils (20W40, 20W50, 5W50 and 15W40), B diesel oil (biodiesel at 3%), C gasoline (25% ethanol) and burnt oil. In addition to the test mixture containing the different isolated microorganisms, the experiment had a positive control produced by mixing 148 µl of minimal medium with 12 µl of DCPIP solution, 20 µl of 10% glucose solution and 20 µl of the tested microorganism suspension; and a negative control produced by

mixing 168 µl of minimal medium with 20 µl of the microorganism suspension and 12 µl of DCPIP solution. The plates were incubated at 30°C with readings performed after 24, 48 and 72 h of incubation.

#### Chromatography determination of gasoline degradation

Chromatography is widely used to quantify and characterize hydrocarbon composition. A number of studies mention the technique as a confirmation test for hydrocarbon degradation by microorganisms (Cunha and Leite, 2000; Ramos, 2006; Mariano et al., 2007).

The confirmation of the hydrocarbon degrading capacity of the isolated bacteria was therefore performed by studying the gasoline degradation by gas chromatography. The analysis of the gasoline degradation by the isolated bacteria, whose capacity had been previously determined by the acrylic plate test, followed the method proposed by Cunha and Leite (2000), with modifications (Peixoto and Vieira, 2005). The bacteria were grown in test tubes containing 5 ml of minimal medium with 10.0 g/L of glucose and 1.0 g/L of yeast extract at 30°C for 24 h. A 500 µL aliquot of this growth was inoculated in tubes with 5 ml of minimal medium and 5% gasoline (v/v). Three series were made for each bacterium, each one with three test tubes. One tube was removed from each of the series after the incubation time of 0, 48 and 72 h. To maintain aerobic growth, 200 µl of hydrogen peroxide were added to the test mediums. A control tube was used as negative standard containing minimal medium and gasoline, but without the bacteria inoculum, and kept under the same incubating conditions as the tubes being tested. After the different incubation periods, the material was centrifuged and the overfloats were stocked in sealed tubes for analysis of their products.

The analysis of the biodegradation products was performed according to the methodology proposed by Bonfim (2006), using an Agilent HP6890 gas chromatograph equipped with an FID detector and a split/splitless injector. The chromatography conditions were of injector and detector at 270°C, split injection mode, split ratio 1:20, injection volume of 1 µl for the liquid phase and 100 µl for the vapor phase. With an initial temperature of 30°C, the oven was heated at a rate of 40°C / min, until it reached 110°C, after which it was heated at 15°C / min. until it reached 200°C with a total analysis time of 8 min. Hydrogen was used as the carrier gas. The FID operated with a synthetic air flow of 400 ml/min and hydrogen flow of 40 ml/min. However, a 20 m DB-1 column for Fast-HRGC was utilized (0.10 mm internal diameter and 0.4 µm stationary phase thickness).

#### Total DNA extraction and amplification to the coding region for 16S rRNA

The DNA extraction was carried out utilizing the isolates grown in nutrient Agar medium (NA) for 24 h, following the protocol proposed by Van Soolingen et al. (1994) with modifications. In this protocol, two to three cultures were removed from the plate and resuspended in tubes containing 400 µl TE buffer with 8 µl of lysozyme solution (20 µg/ml) and then incubated in a double boiler for 1 h and 30 min at 37°C. Following this, 70 µl of SDS at 10% and 12 µl of proteinase K solution were added to the tube and again incubated at 56°C for 10 min. After this incubation, 100 µl of NaCl 5 mol/L followed by 80 µl of CTAB/NaCl solution were added. The tube was shaken gently until the solution became milky and was then incubated again for 10 min at 65°C, after which 650 µL of chlorophyll were added to the tube which was then shaken for 30 s and then centrifuged for 5 min at 12.000 g. The overfloat containing the DNA in suspension was transferred to another tube, precipitated with 400 µl of isopropanol, homogenized and then incubated at -20°C for 24 h. Subsequently, the tube was centrifuged at 16.000 g for 20 min, after which the overfloat was discarded and washing with 70% ethanol was performed. After drying, the extracted DNA was resuspended in 50 µl of MiliQ water. The confirmation of the extraction process was carried out in 1% agarose gel using a 5 µl aliquot of the extracted material and 1 µl of the sample buffer. The remaining extracted material was stored in a freezer at -20°C.

The DNA coding region for the 16SrRNA gene was amplified by the polimerase chain reaction (PCR), with the initiating oligonucleotides 27f (5'-AGAGTTTGATCCTGGCTCAG-3') and 1541r (5'-AAGGAGGTGATCCAGCC-3') (Weisburg et al., 1991) modified. The PCR amplifications reached a final volume of 50 µL, containing 35.5 µL of water, 5 µL of buffer 10X for the Taq polimerase enzyme (CenBiot), 1.5 µl of magnesium chloride ($MgCl_2$) (50 mM) (CenBiot), 1 µl of each initiating oligonucleotide solution (10 µM) (Biosource), 4 µl of dNTP solution (2.5 mM) (Ludwig Biotec), 1 µl of Taq polimerase (5U) (CenBiot) and 1 µl of extracted DNA. The PCR reaction parameters include firstly 3 min of denaturing at 94°C, followed by 30 cycles with denaturing at 94°C for 1 min, annelation at 55°C for 30 s, extension at 72°C for 30 s and final extension at 72°C for 10 min (Garcia, 2006). A 5 µl aliquot of the PCR product was analyzed in 1% agarose gel with 1 µL of the sample buffer (RBC), using the molecular marker 1 kb Sharp DNA Marker (RBC) for the DNA size pattern.

#### PCR product purification and sequencing

For the PCR product purification, an Omega Bio-Tek E.Z.N.A. Cycle – Pure Kit was used. The sequencing was carried out at the Centro de Estudos do Genoma Humano at the Universidade de São Paulo (USP), using the MegaBACE 1000 sequencer and DYEnamic ET Dye Terminator Kit (with Thermo Sequenase™ II DNA Polimerase) code US81090. The sequences were analyzed by the Sequence Analyzer using the Base Caller Cimarron 3.12. For the analysis of the products obtained by sequencing, the sequences received were compared with the sequences present in the GenBank NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi) data bank. The Chromas 2.33 program was used to edit the sequences which were compared using the data bank.

## RESULTS AND DISCUSSION

The initial isolation consisted of 23 endophytic bacteria present in gramineous plants collected from the site impacted by asphalt mud. Only 6 samples were adequate for testing. The large number of samples lost was due to the difficulty in reproducing, in the laboratory, the real environmental conditions where these microorganisms live, which also encumbered the estimate of the real endophytic microbial diversity (Rozsak and Colwell, 1987; Pace, 1997). After sequencing the 16S DNA region of the isolates, these were identified (Table 1).

Among the isolates tested in this study, the bacteria identified as belonging to the genus *Pseudomonas* presented the best test results regarding the degradation of petroleum and its derivatives, presenting positive results for at least one of the compounds tested. The incubation time for detection of the degrading activity also varied from 24 to 72 h. The only isolate tested that did not present positive results was *Staphylococcus pasteuri.* The plate degradation test results are represented in Tables 2 to 4. The final results for 72 h of incubation (Table 4) suggest that the bacteria analyzed present a

**Table 1.** Identification of isolates for sequence comparison with GenBank data bank.

| Isolate | BLAST result | Access number | Identity (%) |
|---|---|---|---|
| AB4 | *Bacillus cereus* | U500VRPU016 | 100 |
| AB5 | *Pseudomonas* sp. | 70U1MD1901S | 99 |
| AB7.1 | *Staphylococcus pasteuri* | U50SZM0U016 | 99 |
| K2 | *Pseudomonas* sp. | 7GYN114Y01S | 99 |
| K3 | *Staphylococcus pasteuri* | 7GX5YJY8014 | 99 |
| KB2 | *Pseudomonas* sp. | U548U88U016 | 87 |

**Table 2.** Degradation profile of bacteria incubated at 30°C and 24 h plate growth.

| MO | C(+) | BO | 20W40 | 20W50 | 5W50 | 15W40 | C(-) | P37* | P38* | P40* | D | G |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AB4 | + | - | - | - | - | - | - | - | - | - | + | - |
| AB5 | + | - | - | - | - | - | - | + | + | + | + | + |
| AB7.1 | + | - | - | - | - | - | - | - | + | - | - | - |
| KB2 | + | - | - | - | - | - | - | - | + | + | - | + |
| K2 | + | - | - | - | - | - | - | + | + | + | + | + |
| K3 | + | - | - | - | - | - | - | - | - | - | - | - |

MO = Microorganism; C(+) = positive control; BO = burnt oil; 20W40, 20W50, 5W50 and 15W40 = lubricating oil; C(-) = negative control; P37, P38 and P40 = petroleum fractions; D =diesel oil; G = gasoline; + = positive degradation; - = no degradation. *Types of petroleum: P37 (ºAPI 37.7), P38 (ºAPI 19.6) and P40 (ºAPI 18.6). ºAPI = (141.5/g) – 131.5, where "g" is the relative density of the petroleum at 15.6°C (Source: PETROBRAS).

**Table 3.** Degradation profile of bacteria incubated at 30°C and 48 h plate growth.

| MO | C(+) | BO | 20W40 | 20W50 | 5W50 | 15W40 | C(-) | P37* | P38* | P40* | D | G |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AB4 | + | - | - | - | X | - | - | - | - | - | + | - |
| AB5 | + | - | - | - | X | - | - | + | + | + | + | + |
| AB7.1 | + | - | - | - | X | - | - | - | + | - | - | - |
| KB2 | + | - | - | - | X | X | - | + | + | + | - | + |
| K2 | + | - | - | - | - | - | - | + | + | + | + | + |
| K3 | + | - | - | - | - | - | - | - | - | - | - | - |

MO = Microorganism; C(+) = positive control; BO = burnt oil; 20W40, 20W50, 5W50 and 15W40 = lubricating oil; C(-) = negative control; P37, P38 and P40 = petroleum fraction; D =diesel oil; G = gasoline; + = positive degradation; - = no degradation; X = partial degradation. *Types of petroleum: P37 (ºAPI 37.7), P38 (ºAPI 19.6) and P40 (ºAPI 18.6). ºAPI = (141.5/g) – 131.5, where "g" is the relative density of the petroleum at 15.6°C (Source: PETROBRAS).

**Table 4.** Degradation profile of bacteria incubated at 30°C and 72 h plate growth.

| MO | C(+) | BO | 20W40 | 20W50 | 5W50 | 15W40 | C(-) | P37* | P38* | P40* | D | G |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| AB4 | + | - | - | - | + | - | - | - | - | - | + | - |
| AB5 | + | - | - | - | + | - | - | + | + | + | + | + |
| AB7.1 | + | - | - | - | + | - | - | - | + | - | - | - |
| KB2 | + | - | - | - | + | + | - | + | + | + | + | + |
| K2 | + | - | X | X | X | - | - | + | + | + | + | + |
| K3 | + | - | - | - | X | - | - | - | - | - | - | - |

MO = Microorganism; C(+) = positive control; BO = burnt oil; 20W40, 20W50, 5W50 and 15W40 = lubricating oil; C(-) = negative control; P37, P38 and P40 = petroleum fractions; D = diesel; G = gasoline; + = positive degradation; - = no degradation; X = partial degradation. *Types of petroleum: P37 (ºAPI 37.7), P38 (ºAPI 19.6) and P40 (ºAPI 18.6). ºAPI = (141.5/g) – 131.5, where "g" is the relative density of the petroleum at 15.6°C (Source: PETROBRAS).

different degradation profile in comparison with the results of the 24 h growth (Table 1). These results may be due to the complexity of the composition of the petroleum derivatives analyzed.

Wongsa et al. (2004) suggested in their experiments, when testing the *in vitro* degradation of petroleum derivatives, that the isolates tested needed from 1 to 2 weeks and that *Pseudomonas aeruginosa* presented the best results for the degradation activity. Considering that the DCPIP is a fast mini scale plate test, the 24 and 72 h incubation readings presented positive results, suggesting their real potential as a fast test for the selection of degrading microorganisms for different compounds. Peixoto and Vieira (2005) suggest that the plate test, using the DCPIP is a low cost screening method for microorganisms with simple degrading capacity, which can be widely standardized and used for detecting degradation in other interesting compounds.

In general, the bacteria tested did not present positive results for the degradation of lubricating oils, except for the 5W50 lubricating oil. Seabra (2008) considers that lubricating oils, due to the presence of acenaphthenes present a lower degradation rate when compared with other petroleum derivatives. According to the data described in the literature and the results obtained in this study, bacteria of *Pseudomonas* genus are known for their bioremediation potential, particularly in hydrocarbons (Evans et al., 2004; Andreoni and Gianfreda, 2007; Vetrova et al., 2007). Bacteria of the *Bacillus* genus are recognized as great enzyme producers (Heck et al., 2002; Carrim, 2005; Anto et al., 2006), and may also present bioremediation potential. Cubitto et al. (2004) describe a sample of *Bacillus subtilis* as a biosurfactant producer contributing to the bioremediation process by the autochthonous microbiota in sites contaminated by petroleum.

The chromatograms obtained with the control for the presence of gasoline are characteristic of this fuel, according to the analysis by Bonfim (2006). Once the controls were compared, the chromatograms of the samples that underwent bacterial degradation presented a variety of degradation profiles, particularly for light hydrocarbons (Figure 1). According to the analysis of the chromatograms exposed for the K2, AB5 and KB2 samples, the degradation of light hydrocarbons ($C_4$-$C_7$) occurred after 48 h of incubation (Figure 1). The chromatographic analyses confirm the efficiency of the microorganisms isolated in gasoline biodegradation processes, as well as the efficiency of the test using the DCPIP.

For all the isolates, the vapor phase was considered a better comparison parameter than the liquid phase. In the liquid phase, the presence of gasoline was not detected in the control or in the test tubes with the samples, unlike the vapor phase where the chromatogram presented characteristics of this compound when compared to analyses described in the literature (Bonfim, 2006).

The gasoline marketed in Brazil presents ethanol as its oxygenating compound, which may facilitate its solubility in aqueous compounds. This probably occurred in the test carried out as the culture medium was used to present an aqueous composition and, therefore, did not allow the detection of gasoline in some of the liquid samples. According to Österreicher-Cunha et al. (2009), the presence of ethanol with the gasoline may affect infiltration, distribution and final degradation of the gasoline compounds in cases of soil contamination, due to its high solubility in aqueous compounds with the BTEX (benzene, toluene, ethylbenzene and xylenes) compounds, retarding its degradation. The 48 h incubation time was considered ideal for the chromatography analysis as both the control and sample analyses were considered satisfactory. In the control and sample analyses after 72 h of incubation, the presence of gasoline was not detected in the vapor phase, possibly because this incubation time was sufficient for most of this fuel to continue its degradation, while a part of it was solubilized in the test medium.

The tests performed showed that the isolated microorganisms can be used for the bioremediation of petroleum hydrocarbons and the derivatives tested. Yet, for *in situ* use, further studies are required for better understanding of their metabolic products. Furthermore, their efficiency will be directly influenced by the environmental conditions to which they are submitted, as well as the availability of nutrients and contaminants.

In spite of the knowledge regarding the biodegradation process in hydrocarbons, this is not yet fully understood and may not be totally effective in the absence of other factors necessary for cellular metabolism. Even so, it is common knowledge that the endophytic microorganisms isolated must be considered when using hydrocarbon bioremediation mechanisms, due to the potential they presented in the assays carried out.

## Conclusions

In general, the bibliographical analysis suggests that part of the information on endophytic microorganisms considers fungi, while little is known regarding bacteria and their potential. The real role of the endophytic community is not yet understood and its diversity is poorly characterized, so it is estimated that much still remains to be studied and discovered regarding this group of microorganisms.

This study contributes toward the initial elucidation of this endophytic diversity by isolating bacteria found in plants present in environments contaminated by asphalt mud residues, demonstrating that even in this nutritionally unfavorable environment, there is the presence of microbial growth.

Although, the degradation of most lubricants was not detected, the analyses carried out definitely determined

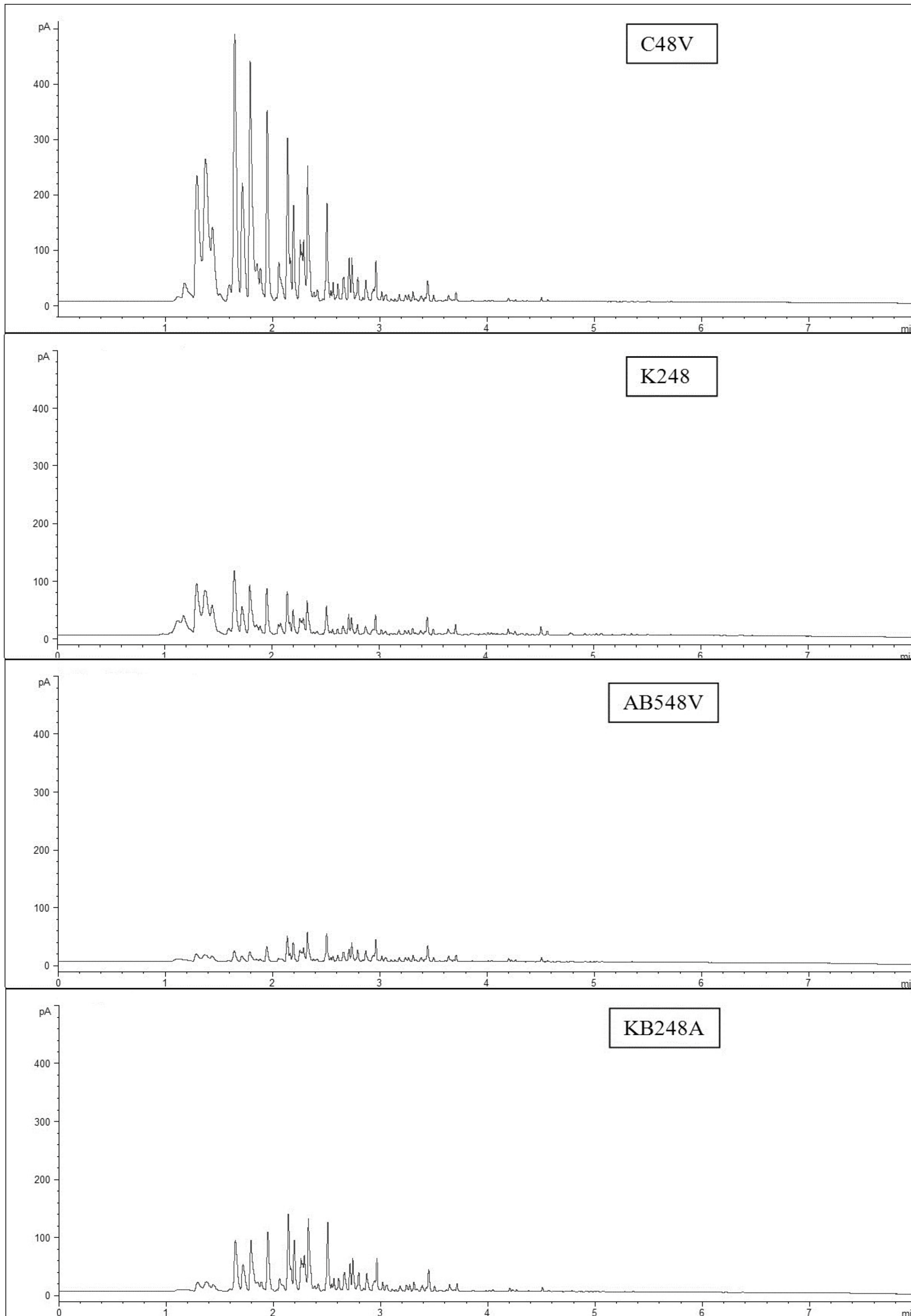


**Figure 1**. Chromatograms referring to gasoline profiles (C48V) and gasoline degradation by K2, AB5 and KB2 isolates after 48 h of incubation.

that the endophytic bacteria isolated showed a potential for use in bioremediation processes of petroleum hydrocarbons, as well as some of its derivatives, such as gasoline and diesel oil, even though *in situ* tests were still necessary to standardize their utilization conditions. For these hydrocarbons, the degradation period of 48 to 72 h was seen to be sufficient for the bioremediation of most of the components present in the samples.

## ACKNOWLEDGEMENTS

The authors would like to thank FINEP for their financial aid (ANAPETRO-CTPETRO-FINEP-MCT Project, convention 65.00.0235.00) and CAPES for the grant they gave to Natalia Carvalhaes de Oliveira.

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