Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 4, Issue, 01, pp.025-029, January, 2012

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

BIODEGRADATION OF HYDROCARBONS AND CULTIVABLE BACTERIAL DIVERSITY IN RADA TILLY COAST LINE, PATAGONIA, ARGENTINA

Adrián J. Acuña*, Graciela N. Pucci, Natalia L. Tonín, Cecilia Tiedemann, Oscar H. Pucci

Universidad Nacional de la Patagonia San Juan Bosco, Centro de Estudios e Investigación en Microbiología Aplicada (CEIMA), Facultad de Ciencias Naturales, Ruta Provincial No 1 4km. Comodoro Rivadavia (CP 9000), Chubut, Argentina

ARTICLE INFO

Article History:
Received 06th October, 2011
Received in revised form
12th November, 2011
Accepted 19th December, 2011
Published online 31th January, 2012

Key words:
Hydrocarbons,
Biodegradation,
Seawater,
Marine sediment.

ABSTRACT

Rada Tilly city is located between Comodoro Rivadavia and Caleta Olivia cities. Both cities have oil industry activity and oil tankers sail in front of the beach on their way to the loading buoy. The aim of the work was to study the bacterial communities and their capacity to use hydrocarbons. We took samples from three different points in every season of the year, both sediment and seawater samples. Oligotrophic and hydrocarbon degrading bacteria counts were done, and the mineralization of diesel, kerosene, gasoline, oil and lubricant oil were carried out during 50 days. Bacterial communities were grown for 10 days on oligotrophic liquid media and media containing phenanthrene and hexadecane as contaminants and analyzed by methyl fatty acids (FAMEs) profiling. In addition, bacterial isolates obtained were identified by FAMEs. The results showed that the cultivable bacterial communities were similar in spring, summer and autumn and this was made up of *Pseudoalteromonas*, *Rhodobacter*, *Aeromonas*, *Ochrobactrum*, *Photobacterium*, *Brevundimonas* and *Vibrio*. In winter season, the bacterial communities changed and were made up of *Micrococcus*, *Staphylococcus*, *Bacillus*, *Arthrobacter* and *Paenibacillus*. The hydrocarbon mineralization was higher in sediment samples than in seawater samples. The best results were observed in summer. The best values were obtained from oil, diesel oil, kerosene and lubricant oil.

Copy Right, IJCR, 2012, Academic Journals. All rights reserved.

INTRODUCTION

San Jorge Gulf is located between the south of Chubut province and the north of Santa Cruz province in Argentine. The main activity is oil extraction and the oil products are transported by tankers. The Gulf can be exposed to oil spill, in fact, in December 26, 2007 in Caleta Cordova beach, 100 cubic meters of oil impacted on the coast (Iantanos *et al.,* 2008). Micro spill is a situation that may go unnoticed because it can be degraded by local bacterial communities (Head *et al.,* 2006). Previous studies on the coast of two cities of the Gulf, Comodoro Rivadavia and Caleta Olivia, showed bacterial communities in seawater and intertidal sediment with capacity to use oil and distilled hydrocarbons (Pucci *et al.,* 2009a, b). Many bacterial strains have been isolated from coastal and oceanic environments; these bacteria, including the genera *Pseudomonas*, *Vibrio*, and *Flavobacterium*, have been considered to be representative of some marine bacteria (Harayama *et al.,* 2004). Diverse petroleum degrading bacteria inhabit marine environments. They have often been isolated as degraders of alkanes, of aromatic compounds as toluene, naphtalene and phenanthrene. Several marine bacteria capable of degrading petroleum hydrocarbons have been isolated (Gerdes *et al.,* 2005; Head *et al.,* 2006; Hadlund *et al.,* 1999). Many "non- professional" hydrocarbonoclastic bacteria have been isolated: for example *Vibrio*, *Pseudoalteromonas* and *Halomonas* have been isolated as marine bacteria capable of degrading polycyclic aromatic hydrocarbons (PAHs) (Jiajun *et al.,* 2010). Rada Tilly (RT) is a summer village located between Comodoro Rivadavia and Caleta Olivia cities, both are oil industrial cities and with hydrocarbon loading buoys. Rada Tilly receives the visit of tourists only during the summer season, but its location means that it is exposed to the possibility of pollution by oil traces from a spill or microspills. It is important to know how the bacterial community that lives on the beach of Rada Tilly is compose, and whether these organisms possess the ability to carry forward the process of biodegradation of hydrocarbons (Head *et al.,* 2006). The main objectives of this work are (i) to study the structure of the culturable bacterial population according to the method of fatty acid methyl esters (FAMEs), and (ii) to study the hydrocarbon biodegradation potential of the bacterial community present in the intertidal sediments and in seawater of three coastline points during 4 seasons.

Corresponding author: ajcuna@unpata.edu.ar

MATERIALS AND METHODS

Sampling

Sediments and seawater samples were obtained during 1 year from three sites in Rada Tilly beach, site 1 (S45° 56.720

WO67° 33.154), site 2 (S45° 56.058 WO 67° 33.187) and site 3 (S45° 54.993 WO67° 32.554) (Fig. 1). The temperature at the sediment surface was around 14ºC. We took sediment and seawater samples in every season of year. The samples were designated by prefixing an S before the number of sample for sediment and SW for seawater. We used Sp, Su, A and W to indicate whether the sample corresponds to spring, summer, autumn or winter, respectively.

Bacterial count

The number of colony-forming units (CFU) was determined by plating 0.1 mL of undiluted serial dilutions of sediment and seawater samples. The media were: Oligotrophic bacteria (BBR) (Tripteine 0.5 g, yeast extract 0.5 g, K_2HPO_4 1 g; $(NH_4)_2SO_4$ 2 g; agar-agar 15 g, sterile marine water 700 mL, sterile water 300 mL, pH 7,2) (Pucci *et al.,* 2009a) and MM-PG: 20mL of MM (NaCl 5 g, K_2PO_4H 0.5 g, $NH_4PO_4H_2$ 0.5 g, $(NH_4)_2SO_4$ 1 g, $MgSO_4$ 0.2 g, KNO_3 3 g, $FeSO_4$ 0.05 g, agar-agar 15 g, sterile marine water 700 mL, sterile water 300 mL); were distributed in a Petri plate and after solidification, 30 µL of a mixture 1:1 of petroleum-diesel oil were spread on the surface (Pucci *et al.,* 2009b). The plates were incubated at 28ºC over 28 days.

Hydrocarbon mineralization

Basal respiration was measured by monitoring CO_2 evolution using NaOH to capture it. Fifty milliliters of seawater or 5 g of sediment with 45 mL of sterile seawater, were poured in a brown bottle with 50 µL of HDB medium (K_2HPO_4 100 g, $(NH_4)_2PO_4$ 200 g, distilled water 1000 mL, pH 7) and 0.1% of gasoline, kerosene, diesel oil, lubricant oil or crude oil and microcosms without hydrocarbons and nutrients were used as controls for the calculation of the hydrocarbon mineralization. The NaOH was titrated by HCl 0.1 N (Pucci *et al.,* 2009b). The microcosms were incubated at 28ºC over 50 days.

Communities FAMEs analysis

Ten grams of sediments plus 90 mL of sterile seawater or 100 mL of seawater samples with 1 mL of HDB medium were incubated with 0.1% phenanthrene and 0.1% hexadecane separately and 10 g of sediments plus 90 mL of sterile seawater or 100 mL of seawater samples with 1 mL of BBR medium concentrate a hundred time. After 10 days of incubation at 28ºC, the samples were centrifuged at 4000 r.p.m. for 30 minutes. The FAMEs were extracted and analyzed as described by MIDI (MIDI Newark, Del., USA).
The MIDI microbial identification system (Microbial ID, Inc, Newar, NJ) was applied to separate fatty acid methyl ester using a gas chromatograph (HP 6890) equipped with a split/splitless injector, a flame ionization detector, a capillary column Ultra 2 (25m, 0.2mm, 0.33µm); an automatic sampler; an integrator; and a program which identifies the fatty acids (Microbial ID 6.0 version). The injector and detector temperatures were maintained at 250ºC and 300°C respectively. The Sample (2 µL) was injected in split mode and the column temperature was raised from 170 to 270°C at a rate of 5°C.min^{-1}.

Bacterial identification by FAMEs determination

Fatty acids were determined as methyl esters from whole-cell hydrolysates according to the procedure of the SHERLOCK microbial identification system (MIDI Inc., Newark, Del.). Samples of about 40 mg of cell (dry mass) were subjected to derivatization in Teflon screw-cap test tubes. For the preparation of esters, cells were saponified at 100°C for 30 min with 1 mL of reagent I (45g of sodium hydroxide, 150 mL of methanol, 150 mL of water), methylated at 80°C for 10 min with reagent II (325 mL of 6 M hydrochloric acid, 275 mL of methanol), extracted with 1.25 mL of reagent III (200 mL of n-hexane, 200 mL of methyl tert-butyl ether), and base washed with 3 mL of reagent IV (10.8 g of sodium hydroxide, 40 g of sodium chloride, 900 mL of water). Methylation following saponification was preferred for total fatty acid analysis because transesterification procedures are suspected to cause underestimations of cyclopropane acids and are less effective at releasing cellular hydroxyl fatty acids. The separation and determination of the fatty acid methyl esters were performed with a 6890 GC gas chromatography system (Agilent, Palo Alto, Calif.) equipped with a flame ionization detector (GC-FID). An automatic sample inlet was used (split injection, split ratio of 100:1, 250°C), and the separation was carried out in an Ultra2 nonpolar capillary column (length, 25 m; inside diameter, 0.20 mm; film thickness, 0.33µm; Agilent), with hydrogen as the carrier gas (9 lb of constant pressure/in2). The temperature program was as follows: 170 to 260°C at 5°C min-1, 260 to 310°C at 40°C min^{-1}, and 310°C for 1.5 min. Data acquisition and data analysis were controlled by ChemStation software (version 10.01; Agilent) and the Sherlock software package (version 6.0; MIDI). Fatty acid assignments were checked by comparisons of the retention times with those of known standards measured on columns with different polarities and were analyzed further after common derivatization procedures, including hydrogenation and silylation. The fatty acid composition was calculated from fractions of individual peaks of the total peak area. Replicate determinations indicated reproducible fatty acid profiles with relative errors of 2 to 5%, calculated as standard errors of the means.

Statistical analysis

Results of hydrocarbons mineralization and bacterial count were analyzed using ANOVA with BIOM program (Applied Biostatistics Inc.,11711 USA). The fatty acid data were analyzed by redundancy analysis (RDA) to analyze the fatty acids profiles and environmental factors using PAST.

RESULTS

Bacterial count

The bacterial count on RT showed a change in total counts and hydrocarbon degrading bacteria with a significant difference between count values in water and sediment samples, the latter had the highest values (Table 1). Both samples presented their highest values in summer season ($P< 0.05$). While in the rest of the seasons; quantity of bacteria count did not show a significant difference ($P > 0.05$).

Hydrocarbons mineralization

The capacity to mineralize was observed in all the samples taken and for all tested hydrocarbons (Fig. 2). In seawater samples, the highest values was presented in summer, it was

Table 1. Bacteria number on samples. SW: seawater, S: intertidal sediment, BBR: oligotrophic bacteria media, MM-PGO: hydrocarbon degrading bacteria media

Site	Spring		Summer		Autumn		Winter	
	BBR	MM-PGO	BBR	MM-PGO	BBR	MM-PGO	BBR	MM-PGO
SW1	$2.0x10^2$	$3.5x10^2$	$1.4x10^4$	$2.6x10^4$	$1.3x10^3$	$1.5x10^2$	$4.2x10^2$	$2.3x10^2$
SW2	$1.1x10^3$	$3.0x10^2$	$1.0x10^4$	$1.0x10^5$	$1.4x10^3$	$2.6x10^2$	$7.6x10^2$	$4.9x10^2$
SW3	$4.1x10^2$	$2.0x10^2$	$7.0x10^4$	$1.7x10^4$	$1.1x10^3$	$1.3x10^3$	$1.6x10^2$	$2.8x10^2$
S1	$1.2x10^4$	$5.9x10^3$	$4.3x10^4$	$2.2x10^4$	$8.1x10^3$	$4.6x10^2$	$2.6x10^2$	$1.2x10^3$
S2	$1.1x10^4$	$2.0x10^3$	$1.3x10^4$	$1.8x10^4$	$5.6x10^3$	$5.0x10^3$	$1.1x10^3$	$3.4x10^3$
S3	$9.6x10^3$	$3.1x10^3$	$9.1x10^5$	$2.1x10^6$	$1.1x10^3$	$2.2x10^3$	$2.2x10^3$	$3.5x10^3$

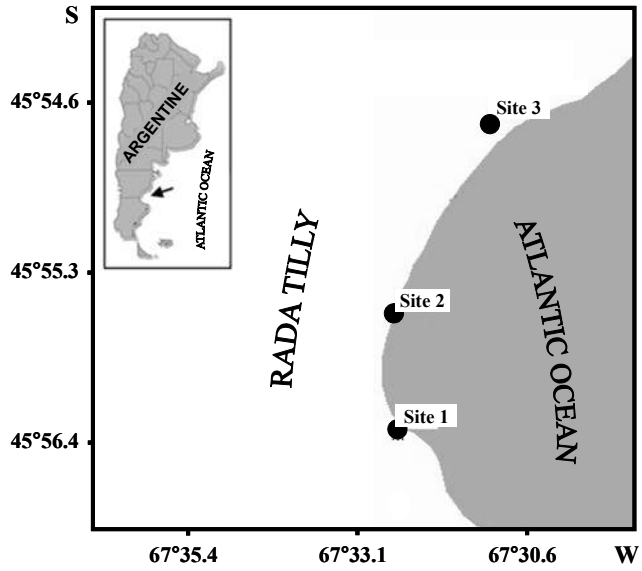

Fig. 1. Map of the coast showing sampling locations

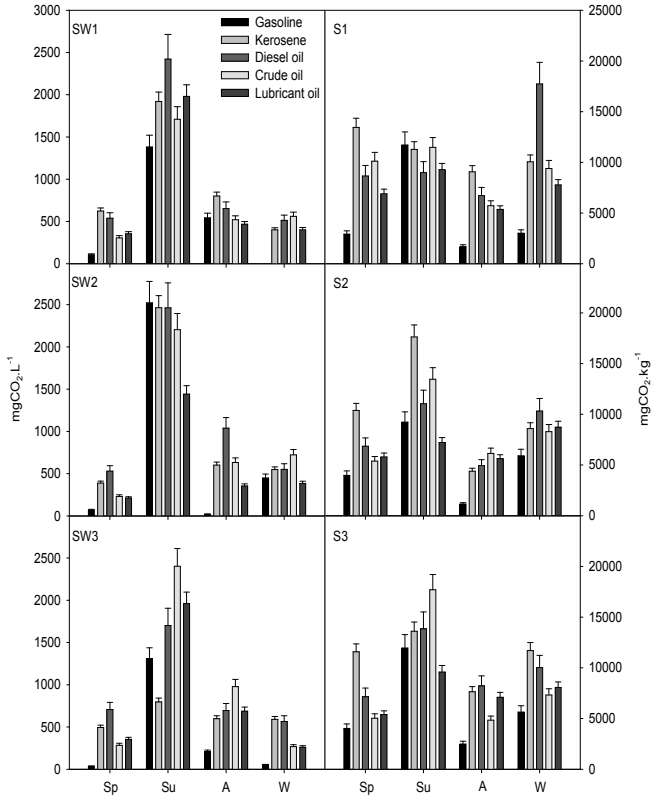

Figure 2. Accumulated CO_2 taken in day 50 with oil and different distillates oil in seawater samples and intertidal sediment samples. SW: seawater, S: intertidal sediments, Sp: spring, Su: summer, A: autumn, W: winter.

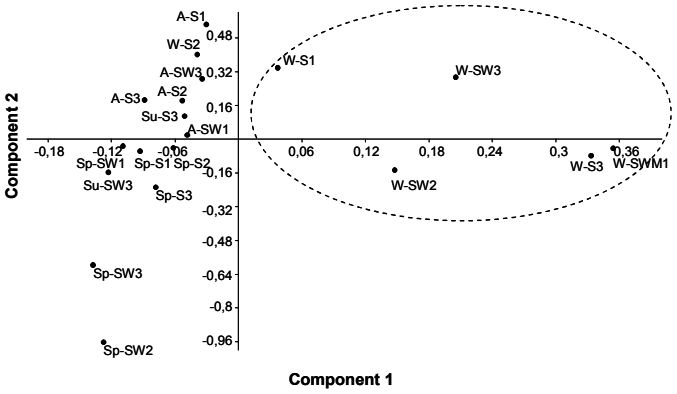

Figure 3. Principal component analysis of bacterial communities fatty acids grown on BBR medium (oligotrophic bacteria) and season. SW: seawater, S: intertidal sediments, Sp: spring, Su: summer, A: autumn, W: winter.

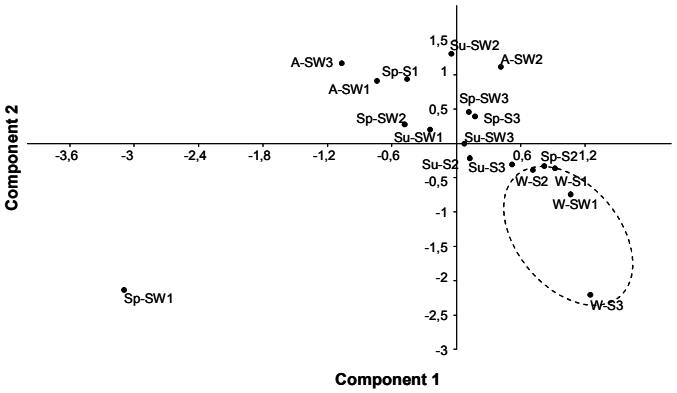

Figure 4. Principal component analysis of bacterial communities fatty acids grown on mineral medium with hexadecane as carbon and energy source and season. SW: seawater, S: intertidal sediments, Sp: spring, Su: summer, A: autumn, W: winter.

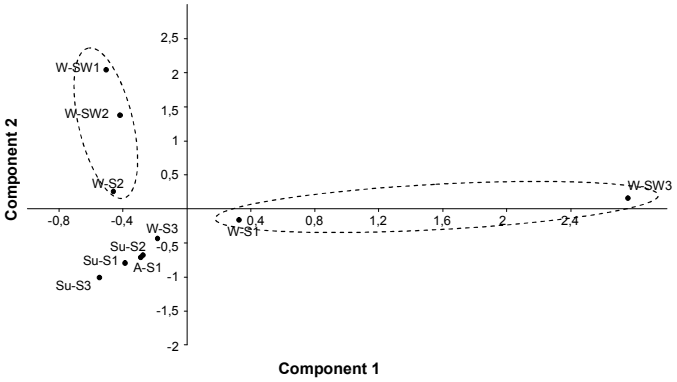

Figure 5. Principal component analysis of bacterial communities fatty acids grown on mineral medium with phenanthrene as carbon and energy source. SW: seawater, S: intertidal sediment, Sp: spring, Su: summer, A: autumn, W: winter.

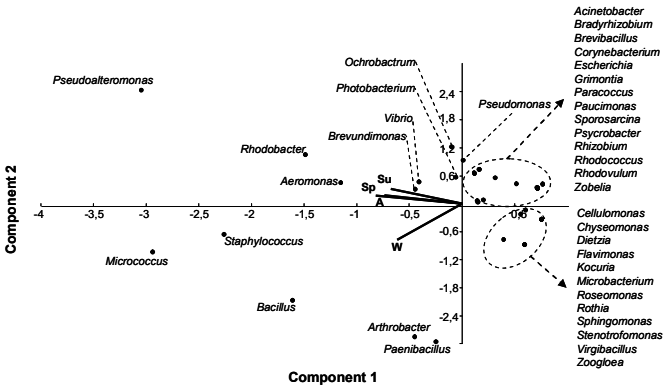

Figure 6. Principal component analysis identified microorganisms and seasons. Sp: spring, Su: summer, A: autumn, W: winter.

higher than 1000 $mgCO_2.L^{-1}$ from tested hydrocarbons which can be used as a sole source of carbon and energy, with only an exception in kerosene from site 3. The mineralization results were obtained from oil followed by diesel oil, kerosene and lubricant oil; whereas gasoil was the less efficient with one exception on site 2, seawater sample in summer season, in fact, this value was 522 $mgCO_2.L^{-1}$. In intertidal sediment samples, the values of the result were 10 times higher than the values of seawater samples ($P < 0.05$). In site S1 there was not a significant different between the seasons ($P > 0.05$). In the sites S2 and S3, the best potential of mineralization was obtained in summer season. As to what was observed with the seawater samples, the sediments samples studied, when gasoline was incorporated as a carbon source, the values of mineralization presented a decrease of CO_2 produced at the time of study.

Bacterial communities

The bacterial communities, which grow on BBR medium, presented different fatty acids compositions during the four seasons of the year (Fig. 3). The seawater samples and the intertidal sediments samples taken on site 1 and 2 in summer season and SW2 sample taken in autumn did not develop. Despite this, the samples taken in winter season had a different fatty acids composition on microbial communities, and this was especially different in comparison to the rest of the season. Bacterial communities' development in presence of hexadacane as contaminant presented problems to grow, the samples Sp-S1, A-S1, A-S2, A-S3, W-SW1 and W-SW3 did not have biomass. From the study of the principal component (Fig. 4), the bacterial communities showed, during the period of the study, a homogeneous community, which tended to differ only during winter. When phenanthrene was used as contaminant, its presence inhibited the development of all samples taken in spring; seawater samples in summer and all seawater samples, S2 and S3 in autumn. The winter samples were different from the rest of the samples (Fig. 5).

Strain identification

From the count media, 213 strains were isolated and identified by FAMEs. The most frequently found genera was *Pseudoalteromonas* and *Micrococcus* (9.85 %) followed by *Staphylococcus* (8.4 %) and then by *Bacillus* and *Rhodobacter* (6.5%), *Aeromonas* (6.10%), *Arthrobacter* (3.7%), *Brevundimonas*, *Photobacterium* and *Paenibacillus* (3.2%); *Ochobactum* and *Vibrio* (2.8%), the rest of genera present in the Figure 6 were less than 2.34% each. The principal component analysis between quantity of strain and season (Fig. 6) showed that the members of bacterial communities were similar during spring, summer and autumn associated with *Pseudoalteromonas*, *Rhodobacter*, *Aeromonas*, *Ochobactum*, *Photobacterium*, *Brevundimonas* and *Vibrio*. On the other hand, in winter, the members were *Micrococcus*, *Staphylococcus*, *Bacillus*, *Arthrobacter* and *Paenibacillus*.

DISCUSSION

Previous study done on the coast of RT beach showed there was not hydrocarbon contamination (Commendatore *et al.,* 2000). However, it is important to know the response capacity of bacterial communities to an accidental spill (Iantanos *et al.,* 2008). In summer samples, the capacity to use oil and distilled oil was the best and this was accompanied by oligothrophic and hydrocarbon degrading bacteria in seawater and sediment samples. The large number of bacteria may, of course be the responsible of the biodegradation (Atlas and Bartha, 1972). One of the response factors in the increment of the counting number can be the environmental temperature (Head *et al.,* 2006), which in summer is around 20ºC.

The temperatures of the rest of the seasons were 15ºC, 10ºC and 6ºC in spring, autumn and winter respectively. Mineralization values in seawater samples were lower than in sediments samples with no significant difference in hydrocarbon degrading- bacteria counts. This may be motivated by the hydrophobicity between water and hydrocarbons, which makes this type of contaminant bioavailable for microorganisms but limiting their use as carbon and energy source (Atlas and Bartha, 1972). On the other hand, the good use of hydrocarbons in intertidal sediments is that they are best support for hydrocarbons and micro-organisms (Stoeck *et al.,* 2002), favoring the contact between the two by their absorption on the particles to promote its use by the present hydrocarbons degrading bacteria (Head and Swannell, 1999). The biodegradation profile observed for petroleum distillates in general terms showed that the major degradation was with lubricating oil and diesel, followed, in descending order, by kerosene, oil and ending with gasoline. This may be influenced by the type of compounds that comprise each. Lubricating oils and diesel, are composed mainly of paraffins and aromatic compounds (Speight, 1991) that are bioavailable and utilizable substrates as carbon and energy source by microorganisms (Atlas, 1995). Kerosene and gasoline are made up of lower molecular weight compounds, including hexane, benzene, toluene, xylene and ethylbenzene (Wang *et al.,* 2006), which are toxic to the bacterial membrane by inhibiting the growth of microorganisms (Sikkema *et al.,* 1995). These compounds, those of kerosene and gasoline, would be responsible for exerting a toxic effect on bacterial community, showing less efficient growth kinetics in the presence of these oil distillates. The bacterial community composition in spring, summer and autumn was homogeneous, showing that it is modified in winter. This was because during the warmer times of the year the predominant organisms were gram negative belonging to the genera *Pseudoalteromonas*, *Rhodobacter*, *Ochrobactrum*, *Photobacterium*, *Brevundimonas*, *Aeromonas* and *Vibrio*. On

the other hand, during winter, the predominant organisms were gram positive members of the genera *Micrococcus*, *Staphylococcus*, *Bacillus*, *Arthrobacter* and *Paenibacillus*. These bacterial genera often coincide with those found in waters and marine sediments in the study region (Gallardo *et al.,* 2004), observing that in the cities of Comodoro Rivadavia and Caleta Olivia, near Rada Tilly, were also found to be prevalent in samples water and marine sediment (Pucci *et al.,* 2009a, b).

The literature cites the *Aeromonas* (Ilori *et al.,* 2005), *Arthrobacter* (Seo *et al.,* 2006), *Brevundimonas* (Jiajun *et al.,* 2010), *Micrococcus* (Pantaroto de Vasconcello *et al.,* 2009), *Ochrobactum* (Al-Mailem *et al.,* 2010) and *Paenibacillus* (Obuekeve *et al.,* 2009) as hydrocarbon degrading bacteria. From the results obtained in this work, we can show that on the coast of Rada Tilly there is a bacterial community capable of biodegrading oil and distillates, observing that this activity was optimal during the warmer seasons of the year. On the other hand, it was determined that the bacterial community was homogeneous throughout the seasons of spring, summer and autumn, composed mainly of gram negative, whereas in winter it was constituted by gram positive organisms. This shows that in the event of contamination of these coasts from micro hydrocarbon spills, environment recovery is possible by means of the natural process of biodegradation by microorganisms.

REFERENCES

Al-Mailem, D.M., Sorkhoh, N.A., Marafie, M., Al-Awadhi, H., Eliyas, M. and Radwan, S.S. 2010. Oil phytoremediation potential of hypersaline coasts of the Arabian Gulf using rhizosphere technology. Bioresour. Technol., 101: 5786-5792.

Atlas, R. 1995. Bioremediation of petroleum pollutans. Int. Biodeterior. Biodegrad., 35: 317-327.

Atlas, R.M. and Bartha, R. 1972. Biodegradation of petroleum in seawater at low temperatures. Can. J. Microbiol. Biotechnol., 18: 1851-1855.

Commendatore, M., Esteves, J.L. and Colombos, J.C. 2000. Hydrocarbons in coastal sediments of Patagonia Argentina: levels and probable source. Mar. Pollut. Bull., 40: 989-998.

Gallardo, A.A., Risso, S., Fajardo, M.A. and Estevao-Belchior, S. 2004. Caracterización de poblaciones microbianas presentes en la macroalga comestible *Monostroma undulatum*, Wittrock. Arch. Latin. Nut., 54: 337-345.

Gerdes, B., Brinkmeyer, R., Dieckmann, G. and Helmke, E. 2005. Influence of crude oil on changes of bacterial communities in Arctic sea-ice. FEMS Microbiol. Ecol., 53: 129-139.

Harayama, S., Kasai, Y. and Hara, A. 2004. Microbial communities in oil-contaminated seawater. Curr. Opin. Biotechnol., 15: 205-214.

Head, I.M., Jones, D.M. and Röling, W.F. 2006. Marine microorganisms make a meal of oil. Nature Rev., 4: 173-182.

Head, I.M. and Swannell, R.P. 1999. Bioremediation of petroleum hydrocarbon contaminants in marine habitats. Curr. Opin. Biotechnol., 10: 234-239.

Hedlund, B.P., Geiselbrecht, A.D., Bair, T.J. and Staley, J.T. 1999. Polycyclic aromatic hydrocarbon degradation by a new marine bacterium, *Neptunomonas naphthovorans* gen. nov., sp. nov. Appl. Environ. Microbiol., 65: 251-259.

Iantanos, N., Pucci, G., Acuña, A. and Pucci, O. 2008. Derrame de hidrocarburos en la playa de Caleta Córdova, ciudad de Comodoro Rivadavia, Argentina: su evolución. Ing. Sanit. Amb., 100: 113-117.

Ilori, M.O., Amobi, C.J. and Odocha, A.C. 2005. Factors affecting biosurfactant production by oil degrading *Aeromonas* spp. isolated from a tropical environment. Chemosphere., 61: 985-992.

Jiajun, X., Linjuna, G., Shipenga, W. and Yitong, L. 2010. Comparative impact of cadmium on two phenanthrene-degrading bacteria isolated from cadmium and phenanthrene co-contaminated soil in China. J. Hazard. Mat., 174: 818-823.

Obuekwe, C.O., Al-Jadi, Z.K. and Al-Saleh, E.S. 2009. Hydrocarbon degradation in relation to cell-surface hydrophobicity among bacterial hydrocarbon degraders from petroleum-contaminated Kuwait desert environment. Int. Biodeterior. Biodegrad., 63: 273-279.

Pantaroto de Vasconcellos, S., Crespim, E., Feitosa da Cruz, G., Barbosa Senatore, D., Marques Simioni, K., Vaz dos Santos Neto, E., Marsaioli, A. and Oliveira, V. 2009. Isolation, biodegradation ability and molecular detection of hydrocarbon degrading bacteria in petroleum samples from a Brazilian offshore basin. Org. Geochem., 40: 574-588.

Pucci, G., Acuña, A., Llanes, M.L., Tiedemann, M.C. and Pucci, O.H. 2009a. Diversidad de bacterias cultivables de la costa de Caleta Olivia, Patagonia, Argentina. Acta Biol. Colom., 14: 121-134.

Pucci, G.N., Acuña, A.J., Llanes, M.L., Tiedemann, M.C. and Pucci, O.H. 2009b. Identificación de bacterias marinas cultivables de la ciudad costera Comodoro Rivadavia, Argentina. Rev. Biol. Mar. Oceanogr., 44: 49-58.

Seo, J.S., Keum, Y.S., Hu, Y., Lee, S.E. and Li, O.X. 2006. Phenanthrene degradation in Arthrobacter sp. P1-1: Initial 1,2-, 3,4- and 9,10-dioxygenation, and meta- and ortho-cleavages of naphthalene-1,2-diol after its formation from naphthalene-1,2-dicarboxylic acid and hydroxyl naphthoic acids. Chemosphere., 65: 2388-2394.

Sikkema, J., De Bont, J. and Poolman, B. 1995. Mechanisms of membrane toxicity of hydrocarbons. Microbiol. Rev., 59:201-222.

Speight, J. 1991. The chemistry and technology of petroleum. Marcel Dekker Press, USA.

Stoeck, T., Kröncke, I., Duineveld, G. and Palojärvi, A. 2002. Phospholipid fatty acid profiles at depositional and nondepositional sites in the North Sea. Mar. Ecol. Prog. Ser., 241: 57-70.

Wang, Z., Fingas, M., Yang, C. and Christensen, J. 2006. Environmental forensics. ElSevier Science, Press, USA.

****