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Diversity of culturable marine bacteria on the coastline of the central area of San Jorge Gulf, Argentina

Diversidad de las bacterias cultivables de la costa del centro del golfo San Jorge, Argentina

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Abstract.- This study aims to isolate and identify culturable marine bacteria from 4 locations in the San Jorge Gulf. A total of 1,532 strains were isolated using oligotrophic, heterotrophic and hydrocarbon-degrading bacteria media, and identified with reference to bacterial fatty acid methyl ester (FAME) profiles. Gram-negative bacteria were the most abundant, *Pseudoalteromonas* comprised 17% of the isolated bacteria, followed by *Pseudomonas*, with 11.14%. The Gram-positive bacteria genera identified were *Staphylococcus*, *Bacillus* and *Micrococcus*. In addition, those bacteria able to degrade hydrocarbons were: *Arthrobacter*, *Dietzia*, *Acinetobacter*, *Microbacterium*, *Micrococcus*, *Bacillus*, *Pseudomonas* and *Pseudoalteromonas*.

Key words: Marine bacterial diversity, fatty acids, sediments, San Jorge Gulf

INTRODUCTION

The composition of bacterial communities in estuaries and coastal regions of oceans is largely unknown, despite the substantial roles of many coastal, bacteria play in critical biogeochemical cycles and the potential utility for bioremediation and other biotechnological applications. The lack of knowledge of this important group of microorganisms can be attributed directly to their cultivability by standard microbiological techniques and to the reluctance on the part of marine microbiologists to study the small, potentially unrepresentative group of bacterioplankton that can be readily cultured from coastal seawater (Gonzales *et al.* 1997). 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 marine bacteria (Harayama *et al.* 2004). In fact, diverse petroleum degrading bacteria inhabit marine environments such as *Alcanivorax* (Head *et al.* 2006), *Cycloclasticus* (Kasai *et al.* 2002), *Marinobacter* (Gerdes *et al.* 2005), *Neptunomonas* (Hedlund *et al.* 1999) and 'non-professional' hydrocarbonoclastic bacteria such as *Vibrio*, *Pseudoalteromonas* and *Halomonas*.

The aim of this study was to determine the biodiversity of culturable bacteria in the San Jorge Gulf, where there is a long history of small spills generated by chronic

pollution (oil terminals, ships and inadequate sewage systems). In order to reach our objective, the bacteria were isolated using oligotrophic, heterotrophic and hydrocarbon degrading media and the bacteria were identified with reference to bacterial fatty acid methyl ester (FAME) profiles.

MATERIALS AND METHODS

Over the course of one year, in 2006, a total of 1,532 bacteria strains were isolated. These strains were obtained from samples taken in 4 different points of the San Jorge Gulf: Caleta Córdova (CC S45° 44.384 WO67° 22.633); Comodoro Rivadavia (CR S45° 52.954 WO67° 30.205); Rada Tilly (RT S45° 56.720 WO67° 33.154) and Caleta Olivia (CO S46° 27.583 WO67° 29.653). The 3 media used for culture were: BBR agar for oligotrophic bacteria (g L⁻¹: Triptone 0.5, yeast extract 0.5, K₂HPO₄ 1; (NH₄)₂SO₄ 2; agar-agar 15, sterile marine water 700 mL, sterile water 300 mL, pH 7.2); MBM-PG agar for hydrocarbon-degrading bacteria (g L⁻¹: NaCl 5 g, K₂PO₄H 0.5 g, NH₄PO₄H₂ 0.5 g, (NH₄)₂SO₄ 1 g, Mg SO₄ 0.2 g, KNO₃ 3 g, FeSO₄ 0.05 g, distilled water 1000 mL) which was 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 & Pucci 2003); and BRN agar for heterotrophic bacteria (g L⁻¹: Triptone 5, yeast extract 1, K₂HPO₄ 1; (NH₄)₂SO₄ 2;

agar-agar 15, sterile marine water 700 mL, sterile water 300 mL, pH 7.2). The isolates were identified based on whole-cell cellular fatty acids, derivatized to methyl esters, *i.e.*, FAMES and analyzed by gas chromatography (GC) using the MIDI system (MIDI, Newark, DE). The analysis was performed using the Sherlock Microbial Identification system TSBA 6.0 software and the library general system software version 6.0.

RESULT AND DISCUSSION

The present study employed Culture-dependent methods to assess the diversity of bacteria in marine sediment and seawater collected from the San Jorge Gulf. A total of 1,532 strains were isolated but only 745 were identified by MIDI. On the basis of MIDI, these 745 strains were classified as 65 genera and 150 species. There were 45 genera with less than 10 strains each. Gram-negative bacteria were abundant; *Pseudoalteromonas* represented 17% of total number of bacteria, followed by *Pseudomonas* with 11.14%. On the other hand, the Gram-

positive bacteria were *Bacillus* (6.98%); *Staphylococcus* (5.1%) and *Micrococcus* (4.7%). The other strains are shown in Table 1.

Gram-negative strains represented 75% from the total strains, predominating over the total number of bacteria identified (Table 2). The major percentage of Gram-positive bacteria was found during the winter, in the four different points sampled, being Rada Tilly the city with the highest percentage of bacteria (40.91% of Gram-positive bacteria and 59.09% Gram-negative bacteria). The Shannon diversity index were calculated, showed high values during autumn, in the city of Comodoro Rivadavia, when BRN culture medium was used (Table 2).

The number of strains found was quite equal on the BBR and BRN media; both without oil; but the number of strains isolated were lower in hydrocarbon culture media. The oil degrading bacteria were slow growing bacteria and colonies were visible after seven days; however, on the BBR and BRN media, the bacteria were visible after 24-48 h. *Pseudomonas* genus was more abundant on the

Table 1. Percentage composition of bacterial species from San Jorge Gulf, identified by isolates cultured in BBR, BRN and hydrocarbon degrading media / Composición porcentual de la identificación de las especies bacterianas del Golfo San Jorge, las cuales fueron aisladas de los medios de cultivo BBR, BRN y medio de cultivo con hidrocarburo

Identification	%	Identification	%
<i>Pseudoalteromonas</i> spp.	17.05	<i>Salmonella</i>	1.88
<i>Pseudomonas</i> spp.	11.14	<i>Microbacterium</i>	1.48
<i>Bacillus</i> spp.	6.98	<i>Zobellia uliginosa</i> , <i>Sphingopyxis</i> spp., <i>Escherichia</i> spp.	1.34
<i>Staphylococcus</i> spp.	5.10	<i>Brevibacillus</i> spp., <i>Arthrobacter</i> spp.	1.21
<i>Micrococcus</i> spp.	4.70	<i>Rhodovulum sulfidophilum</i> , <i>Neisseria</i> spp.	0.94
<i>Rhodobacter</i> spp.	4.16	<i>Paenibacillus</i> spp., <i>Flavimonas</i> spp.	0.81
<i>Acinetobacter</i> spp.	3.89	<i>Dietzia maris</i> , <i>Bradyrhizobium japonicum</i>	0.67
<i>Vibrio</i> spp.	3.89	A	0.54
<i>Psychrobacter immobilis</i>	3.76		
<i>Aeromonas</i> spp.	3.76	B	0.40
<i>Kocuria</i> spp.	2.95		
<i>Photobacterium</i> ,	2.68	C	0.27
<i>Oerhobactrum anthropi</i>			
<i>Shewanella putrefaciens algae</i> ,	2.01	D	0.13
<i>Brevundimonas</i> spp.			

A: *Rhodococcus* spp., *Gluconobacter asa/oxydans*, *Curtobacterium* spp., B: *Roseomonas* spp., *Pseudoxantomonas* spp., *Paracoccus denitrificans*, *Nocardia subflava*, C: *Xanthobacter* spp., *Virgibacillus pantothenicus*, *Stenotrophomonas maltophilia*, D: *Yersinia frederiksenii*, *Sporosarcina globispora*, *Rothia dentocariosa*, *Proteus vulgaris*, *Photobacterium luminescens luminescens*, *Pectobacterium carotovorum*, *Paucimonas lemoignei*, *Nocardia albus*, *Nesterenkonia halobia*, *Lysobacter enzymogenes enzymogenes*, *Lecheonella flava*, *Kurthia gibsonii*, *Flavobacterium ferrogineum*, *Enterococcus faecalis*, *Clavibacter michiganensis insidiosum*, *Cellulomonas* spp., *Burkholderia* spp.

BRN medium than on the hydrocarbon degrading bacteria medium, despite the fact that this genus is known by its metabolism in the use of hydrocarbon (Atlas 1981, Gerdes *et al.* 2005). In fact, *Ps. putida* and *Ps. stutzeri* were found in concordance with Röling *et al.* (2004). This genus has a rapid growth rate when conditions favour it, being widely known for its ability to use hydrocarbons as carbon source (Atlas 1981). The *Alcanivorax borkumensis* and *Pseudomonas stutzeri* strains dominated the initial stage of degradation of hydrocarbon. Another strain was the *Acinetobacter*, who is characterized by producing biosurfactants, which increased the bioavailability of water-insoluble products (Toren *et al.* 2001, Ron & Rosenberg 2002).

The major percentage of bacteria identified was 17% corresponding to *Pseudoalteromonas*, which were found in marine environments and have the ability to use hydrocarbons; this genus can be associated to *Alteromonas* and *Shewanella*, all marine heterotrophic Gammaproteobacterias (Ivanova *et al.* 2004). Other hydrocarbon degrading bacteria found were *Rhodococcus*, which have a greater capacity to survive in adverse conditions, are able to use different hydrocarbon and have catabolic versatility (Whyte *et al.* 1999, Ruberto *et al.* 2005). Besides, *Rhodococcus* is the most common genus of Nocardia recovered from marine sediment (Gontang *et al.* 2007). Hydrocarbon

degrading bacteria diversity on the San Jorge Gulf Coast has shown a genus that would be able to use hydrocarbons against possible oil spills on the coasts of the cities studied.

Nutrient concentrations in seawater and intertidal sediments were low and this affected the growth of enteric bacteria. However, we found and identified some *Escherichia*, *Salmonellas* and *Proteus*. In fact, the conditions of salinity, temperature and light can limit the survival of these species (Rozen & Shimshon 2001). However, some of them, *e.g.*, *E. coli*, can survive up to 13 days in water and marine sediment (Le Guyard *et al.* 1991). *E. coli* was present in the city of Comodoro Rivadavia, which is subjected to a wide spatial fluctuation of faecal contamination, caused by the pouring of untreated sewage water into the sea; enteropathogenic bacteria were not found in any other sampling point.

MIDI system identified *Shewanella algae* (2%), which is an opportunistic pathogen (Holt *et al.* 1997, 2005). Bacteria of the genus *Shewanella* have been studied in the past decade because of their important role in cometabolic bioremediation of halogenated compounds, petroleum products and because of their ability to reduce the magnesium and iron oxide. However, one of their main features is the ability to produce polyunsaturated fatty acids (Russell & Nichols 1999).

Table 2. Percentage of Gram (%) and Shannon diversity index (H') for season, media, and sites in San Jorge Gulf / Porcentajes de gram e índice de Shannon para las estaciones, medio de cultivo utilizado y ciudades estudiadas en el Golfo San Jorge

	% Gram positive bacteria	% Gram negative bacteria	Shannon diversity index (H')
Season			
Spring	17.16	82.09	3.165
Summer	21.63	79.18	3.707
Autumn	27.40	72.60	3.935
Winter	34.42	66.23	3.835
Media			
BBR	19.61	80.78	3.884
BRN	25.38	75.38	4.133
PGO	29.68	70.32	3.729
Sites			
Rada Tilly	40.91	59.09	3.73
Caleta Córdova	26.61	73.39	3.158
Caleta Olivia	22.33	79.13	3.255
Comodoro Rivadavia	15.06	84.94	3.886

Sampling site pollution determined the frequency of strains. In the correspondence analysis, Comodoro Rivadavia was associated with Enterobacterias due to the fact that these strains are poured into the sea by an inefficient sewage system. This type of microorganisms has the ability to withstand for long periods in sediments (Viera *et al.* 2001), their abundance demonstrates the degree of contamination of fecal origin that these beaches in the city contain. The latter is of great importance due to the risk of transmitting infectious diseases via fecal oral route. Comodoro Rivadavia sediment samples are related by strains that are able to use hydrocarbons; this is because in both sampling sites, there are potential sources of pollution by oil, a fuel discharge and untreated effluent discharges from the industrial area in the city of Comodoro Rivadavia. This shows the adaptive ability of microorganisms to exploit small-scale oil spills.

Comodoro Rivadavia and Rada Tilly are related to certain groups of bacteria e.g. *Pseudoalteromonas*, *Pseudomonas*, *Micrococcus* and *Photobacterium* (Fig. 1), possibly because the area between these two cities is greatly contaminated by the municipal garbage landfill, located in front to the beach area. Besides, this pollution is also increased by the effluents poured to the sea by an inefficient sewage system, which introduces a great number of bacteria into the ecological system, resulting in the highest diversity index, especially in Comodoro Rivadavia.

The present investigation contributes to the knowledge of culturable heterotrophic, oligotrophic and hydrocarbonoclastic bacteria in the sediment and seawater of San Jorge Gulf, where 65 genera and 150 species were found, most of which were hydrocarbon degrading bacteria, capable of using the hydrocarbon on small scale spills. There were many bacteria that MIDI could not identify.

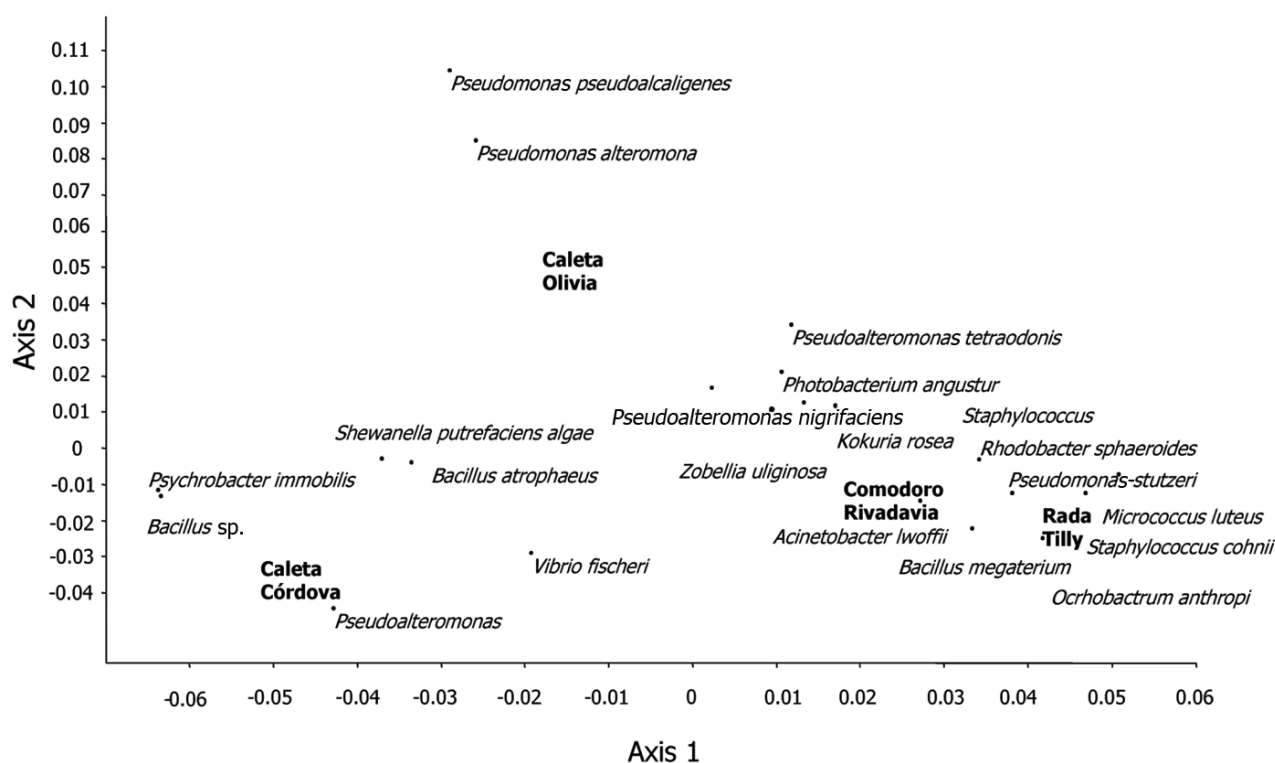


Figure 1. Correspondence analysis biplot between bacterial identities (according to fatty acids methyl ester (FAMEs) profiles) and four coastal cities of Argentina (in bold) / Análisis de correspondencia entre entidades bacterianas (identificadas por los ácidos grasos de sus membranas) y cuatro ciudades costeras de Argentina (en negrita)

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