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Article

Isolation, identification and antimicrobial activity of lactic acid bacteria from the Bahía Blanca Estuary

Aislamiento, identificación y actividad antimicrobiana de bacterias del ácido láctico del estuario de Bahía Blanca

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Resumen.- Este estudio analiza la biodiversidad de bacterias del ácido láctico presentes en el estuario de Bahía Blanca y su actividad antimicrobiana frente a patógenos relacionados con el cultivo de salmónidos. Se aislaron 21 cepas de bacterias del ácido láctico a partir de peces y sedimentos del estuario. Las especies de peces fueron seleccionadas entre aquellas que habitan la mayor parte de su ciclo en el estuario. De acuerdo al análisis de la secuencia 16S ADNr, los aislamientos correspondieron a los géneros *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus* y *Weissella*. La especie predominante en los aislamientos obtenidos de peces fue *Weissella viridescens*, la cual fue aislada de tres de las cuatro especies de peces estudiados. Ninguna de las especies aisladas de los peces fue encontrada en los sedimentos. El método de difusión en agar se empleó para detectar la actividad antimicrobiana de las cepas aisladas frente a patógenos de salmónidos: *Yersinia ruckeri*, *Aeromonas salmonicida* subsp. *salmonicida,* y dos cepas de *Lactococcus garvieae,* y frente a *Listeria monocytogenes*. Todas las cepas mostraron algún grado de inhibición frente a *L. monocytogenes, Y. ruckeri* and *Lc. garvieae*. Doce cepas mostraron actividad frente a *A. salmonicida*. Este estudio es el primer reporte sobre la diversidad de bacterias del ácido láctico en un ambiente marino costero y en peces de la Argentina. Las cepas de bacterias del ácido láctico obtenidas de peces y sedimentos con capacidad de inhibir *in vitro* los patógenos estudiados podrían tener aplicación en el control biológico en acuicultura y en la industria procesadora de pescado.

Palabras clave: Sedimentos, peces, patógenos

Abstract.- This study analyzed the biodiversity of lactic acid bacteria present in the Bahía Blanca Estuary and their antimicrobial activity against pathogens associated with the cultivation of salmonid. A total of 21 lactic acid bacteria (LAB) strains were isolated from superficial sediments and fish of the estuary. The fish species were selected from those that spend most of their life cycle in the estuary. According to 16S rDNA analysis, isolates were affiliated with the genera *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Enterococcus* and *Weissella*. The predominant LAB isolates from the fish species belonged to *Weissella viridescens*, which was isolated from three of the four species analyzed. None of the LAB species isolated from fish was found in sediments. The agar diffusion method was used for detection of antagonistic activity against *Listeria monocytogenes, Yersinia ruckeri*, *Aeromonas salmonicida* subsp. *salmonicida* and two strains of *Lactococcus garvieae*. All the isolates exhibited some degree of antagonistic activity against *L. monocytogenes, Y. ruckeri* and both strains of *Lc. garvieae*. Twelve strains were found to be inhibitory for *A. salmonicida*. This study is the first report on the diversity of lactic acid bacteria in a coastal marine environment and fish from Argentina. The sediments and fish analyzed showed microbial strains with the ability to suppress pathogen growth under *in vitro* conditions, suggesting their potential as biological control agents for aquaculture and fish processing.

Key words: Sediments, fish, pathogens

INTRODUCTION

Lactic acid bacteria (LAB) are characterized as Gram-positive, usually nonmotile and nonsporulating cells that produce lactic acid as a major or sole product of the fermentative metabolism. They are generally catalase-negative and usually lack cytochromes. LAB are nutritionally fastidious, requiring carbohydrates, amino

acids, peptides, nucleic acid derivates and vitamins for growth. Accordingly, they are commonly associated with nutritious environments like milk, dairy products, some plant surfaces, decaying material and the mucosal surfaces of animal gastrointestinal tract. Different species of LAB have adapted to grow under widely different environmental conditions, and they are widespread in nature.

In comparison to the wealth of information available on LAB in endothermic animals, fewer studies have been devoted to their occurrence in fish and their surrounding environment (Ringø *et al*. 1995, Ringø & Gatesoupe 1998, Ringø *et al*. 2000, Bucio *et al*. 2006). The sea, especially in coastal areas, is abundant in dead and living biota which is a rich source of nutrients necessary for the growth of heterotrophic microorganisms. Many ecological and taxonomic studies on marine bacteria have been carried out, but most of them focused on aerobic or facultative anaerobic Gram-negative bacteria. Isolation and taxonomic studies of LAB from marine environments have generally been confined to marine farmed fish (Franzmann 1991, Ringø & Gatesoupe 1998). In recent years, the isolation of marine LAB from living and decomposing marine organisms (Ishikawa *et al*. 2003, Itoi *et al*. 2008), and from deep sub-seafloor sediments have gained more attention (Toffin *et al*. 2005).

Moreover, LAB exerts strong antagonistic activity against many related and unrelated microorganisms, including food spoilage and pathogenic agents. Such antagonistic effects are mainly due to the decrease in the pH that they produce, to the competition for nutrients and to the production of inhibitory metabolites. Hence, LAB have acquired importance in the field of prophylaxis and their use was also proposed for aquatic species which may provide an alternative way to reduce the application of antibiotics in aquaculture (Gatesoupe 1994, 1999, Nikoskelainen *et al*. 2001, Irianto & Austin 2002, Panigrahi *et al*. 2004, Balcázar *et al*. 2006, Balcázar *et al*. 2008).

In Argentina, LAB have been intensively studied as probiotics in mammals and, more recently in freshwater amphibians (Pasteris *et al*. 2009). However, their diversity in coastal marine environments and in fish still remains unknown. Considering LAB biotechnological and probiotic potential, the investigation of this group of microorganisms in coastal marine environments becomes important and could provide new strains with application in aquaculture.

The aims of this study were to make a survey of the presence of LAB in fish and sediments from a coastal marine environment and to asses their antimicrobial activity against pathogens associated with aquaculture and fish processing industry.

Material and methods

Culture Media

MRS Broth pH 5.6 and 4.7: De Man, Rogosa and Sharpe broth (BK 070HA, Biokar Diagnostics, Beauvais, France) (De Man *et al*. 1960), the pH was modified in each case with 1N glacial acetic acid. Modified MRS Broth: MRS broth prepared from components, with sodium acetate and diammonium citrate omitted, pH adjusted to 8.0 with 1 N NaOH. This medium has been recommended for *Carnobacterium* sp. isolation (Schillinger & Holzapfel 1995, González *et al*. 2000). PCA: Plate Count Agar (105463, Merck, Darmstadt, Germany), pH 7.0. TSB: Trypticase Soy broth (105458, Merck, Darmstadt, Germany), pH 7.0. TSA: Trypticase Soy Agar, TSB amended with 1.2 % of purified agar-agar (1614, Merck, Darmstadt, Germany), pH 7.0.

Sampling and LAB isolation

Bahía Blanca is a mesotidal coastal plain estuary located between 38°45´ and 39°40´S, and 61°45´ and 62°20´W (south-west Atlantic Ocean, Buenos Aires Province, Argentina). The Bahía Blanca harbor system is one of the most important of the country. The estuary is a complex network of tidal channels, extensive tidal flats, low marsh areas and islands. It covers an area of 1900 km^2 at high tide which is reduced to 750 km^2 at low tide, and is classified as a salt marsh (Piccolo & Perillo 1990, Marcovecchio & Ferrer 2005). Surface sediment samples were collected with a bottom dredge from the first 10 cm, from three sites located in the Principal Channel: Galvan Harbor, the entrance to the Maldonado Channel and Cuatreros Harbor, in May and December 2008. Samples were put into sterile polyethylene bags (Nasco Whirl Pak™), immediately transported to the laboratory at 4°C and processed within 24 h of sampling. Ten grams of sediments were suspended in 90 mL sterile saline solution (NaCl 0.85% w/v) and homogenized in an orbital shaker at 450 rpm for 10 min. Aliquots of 10 mL of sediment suspensions were inoculated in 100 mL MRS broth pH 4.7, MRS broth pH 5.6 and in modified MRS broth. All broths were incubated at 25ºC for 48 h.

Coastal fish specimens were collected with standard fishing gear from the Principal Channel of Bahía Blanca

Estuary. These specimens included two *Scianidae* species: *Cynoscion guatucupa* (5 juvenile specimens) and *Micropogonias furnieri* (5 juvenile specimens), and two *Clupeidae* species: *Ramnogaster arcuata* (10 adult specimens) and *Brevoortia aurea* (5 juvenile specimens). Except for 5 adult specimens of *Ramnogaster arcuata* which were collected in December 2008, all fish were collected in May 2008. After the capture, specimens were dissected aseptically. The contents squeezed from the intestinal tracts were homogenized with 25 mL of sterile saline solution and homogenized with a vortex mixer for 2 min. Aliquots of 10 mL of fish-containing suspensions were inoculated in 100 mL MRS broth pH 4.7, MRS broth pH 5.6 and in modified MRS broth. The broths were incubated at 25ºC for 48 h.

For LAB isolation, broth samples were serially diluted in sterile saline solution and plated on MRS pH 5.6, MRS pH 4.7 and modified MRS broths, adding with 1.2 % (w/v) agar-agar (1614, Merck, Darmstadt, Germany). The plates were incubated at 6% of CO_2 and 25ºC for 72-96 h. The incubation temperature never exceeded 25ºC due to the sample origin and their potential use in aquaculture.

After incubation, bacterial colonies were selected from plates containing less than 300 colonies, based on differences of form, size, colour, elevation and border (Greco *et al*. 2005, Bucio *et al*. 2006, Itoi *et al*. 2008). The selected colonies were streaked out for pure culture on the respective MRS agar. Isolates were examined for Gram reaction, production of catalase and cytochrome oxidase. Selected strains were stored at -72ºC in MRS broth pH 5.6 supplemented with 20% v/v glycerol for further studies.

16S rDNA amplification and sequencing

DNA from selected isolates was extracted using the Wizard Genomic DNA Purification kit (Promega Corporation, Madison, WI). The 16S rRNA gene sequence (corresponding to positions 27-1492 in the *Escherichia coli* gene) was PCR-amplified as described by DeLong (1992), using a Multigene Gradient thermal cycler (Labnet International Inc., Woodbridge, NJ). Sequencing on both strands of PCR-amplified fragments was performed using the dideoxy chain termination method by the commercial services of Macrogen Inc. (Seoul, Korea).

The 16S rRNA gene sequences were compared to the GenBank nucleotide database with BLAST (Altschul *et al.* 1990) and identified using the EzTaxon program (Chun *et al*. 2007). Sequences were deposited at the GenBank database[1] under the accession numbers from FJ892726 to FJ892746**.**

Antimicrobial activity assay

Agar well diffusion method was used to detect antibacterial activity (Mishra & Prasad 2005, Balcázar *et al*. 2006, Van Hai *et al*. 2007). The indicator pathogen strains employed in this experiment were: *Yersinia ruckeri* ATCC 29473, *Aeromonas salmonicida* subsp. *salmonicida* ATCC 33658, *Listeria monocytogenes* ATCC 7644 and two *Lactococcus garvieae* clinical isolates 03/8460 and 03/8702 (*Lc. garvieae* strains were kindly provided by Dr. José Francisco Fernández-Garayzabal, School of Veterinary Medicine, Complutense University of Madrid, Spain). *L. monocytogenes*, *Y. ruckeri* and *Lc. garvieae* were cultured in TSB at 35ºC for 24 h. *A. salmonicida* was cultured in TSB at 25ºC for 30 h**.** The LAB isolates were grown in 10 mL of MRS pH 5.6 for 48 h at 25ºC. The supernatants were obtained by centrifugation at 6,000 x *g* for 10 min and filtered through a 0.45 µm pore-size filter (Millipore, USA). Each cell free supernatant was divided in three aliquots; 3.3 mL were neutralized (pH 6.8) with 5 N NaOH, 3.3 mL were neutralized and heated at 100ºC for 5 min, and the remaining portion was used without treatment (raw extract). Tubes with 20 mL of PCA melted and cooled to 48ºC, were inoculated with 200 µl of the TSB cultures of *Y. ruckeri*, *L. monocytogenes* and *Lc. garvieae*. The tube contents were mixed and spread into sterile Petri dishes. When the agar solidified, wells of 5 mm in diameter and 40 µl in capacity were made with a sterile glass tube. The wells were filled with 35 µl of the LAB culture cell free supernatants: a) raw extract, b) neutralized, c) neutralized and heated. Wells filled with neutralized MRS and MRS pH 5.6 were added as controls to determine possible inhibitory activity of the medium. The plates were placed at 4ºC overnight to allow the diffusion of the supernatants in the agar and then incubated at 35ºC for 48 h. Subsequently, the diameter of the clear zone around each well was recorded in millimeters with a Vernier caliper.

For *A. salmonicida* a disk diffusion assay in TSA was used because better conditions for growing were obtained (data not shown). An aliquot of 100 µL of a cell suspension in saline solution (~10^6 cell mL^{-1}) was spread onto TSA plates previously prepared. After air-drying the plates for 15 min, 5 mm sterile paper disks were placed on the agar surface. The disks were impregnated with 35 µl of treated and non-treated supernatants as well as neutralized MRS

[1]http://www.ncbi.nlm.nih.gov

and MRS pH 5.6 controls (Balcázar *et al*. 2008). The plates were placed at 4ºC overnight to allow the diffusion of the supernatants in the agar. The diameter of the clear zone around each disk was recorded in millimeters after incubating at 25ºC for 48 h. Four strains were tested per plate, and each plate was prepared in triplicate.

Results

Twenty one isolates presumptively corresponded to LAB based on phenotypic characteristics. All these isolations were Gram-positive cocci, coccobacilli or rod-shaped cells, nonsporeforming, oxidase and catalase negative. All the presumptive LAB strains were isolated in MRS pH 5.6 and 4.7. No isolate with LAB characteristics was obtained in the modified MRS, indicating that no strain corresponded to *Carnobacterium* sp. Table 1 shows the origin of each strain.

To further characterize LAB isolates, their 16S rRNA gene sequences (~ 1,400 bp) were analyzed (Table 2). The predominant LAB isolates from the fish species belonged to the genus *Weissella* (F1, F4, F7, F9, F10, and F11), with a 99.7 to 100% 16S rDNA sequence homology to *Weissella viridescens* (= *Lactobacillus viridescens sensu* Collins *et al*. 1993). The other fish isolates showed 16S rDNA homology values above 99% to *Enterococcus hirae*, *Leuconostoc mesenteroides*, *Leuconostoc citreum and Lactobacillus paracasei* (Table 2).

A different composition of the LAB community was obtained from sediment samples, which was represented by the genera *Lactobacillus*, *Enterococcus* and *Pediococcus* (Table 2). Thus, three isolates were closely related to *Lactobacillus pentosus*, two to *Pediococcus pentosaceus*, two to *Enterococcus mundtii* and single strains to *Lactobacillus graminis* and *Enterococcus durans* (Table 2).

When untreated supernatants were tested, all the LAB strains inhibited the growth of *L. monocytogenes* and *Y. ruckeri*, and most of them also inhibited both *Lc. garvieae* strains. Twelve LAB strains showed activity against *A. salmonicida*. The neutralized supernatants of 11 LAB strains maintained their inhibitory activity against *L. monocytogenes*, 12 against *Y. ruckeri*, one against *A. salmonicida*, eight against *Lc. garvieae* 03/8702 and two against *Lc. garvieae* 03/8460. The neutralized and heated supernatants of strains F2, S19, S20 and S21 conserved their activity against *L. monocytogenes*, and those from the strains S14, S15 and S19 against *Y. ruckeri* (Table 3).

Table 1. Lactic acid bacteria isolated from fish (F) and sediments (S) from the Bahía Blanca Estuary / Bacterias del ácido láctico aisladas de peces (F) y sedimentos (S) del estuario de Bahía Blanca

Isolates	Isolation source	Month (ºC)[c]
F1, F2, F3, F4, F6, F7	*Ramnogaster arcuata* [a]	December (22ºC)
F11, F12	*Ramnogaster arcuata* [a]	May (9ºC)
F8, F9	*Cynoscion guatucupa* [b]	May (9ºC)
F10	*Brevoortia aurea* [b]	May (9ºC)
F13	*Micropogonias furnieri* [b]	May (9ºC)
S14	Galván Harbor	December (22ºC)
S19, S22	Galván harbor	May (9ºC)
S15	Maldonado Channel	December (22ºC)
S20, S21	Maldonado Channel	May (9ºC)
S16, S17	Cuatreros Harbor	December (22ºC)
S18	Cuatreros Harbor	May (9ºC)

[a]Adult, [b]Juvenile, [c]water temperature

[a]Adultos, [b]Juveniles, [c]temperatura del agua

Discussion

It is important to emphasize that all fish collected corresponded to species that spawn, born and growth at least to the young phase in Bahía Blanca Estuary, except *Ramnogaster arcuata* which spends all its life cycle in the estuary.

Several species of *Leuconostoc* and *Enterococcus* have been frequently isolated from digestive tract and feces of fish (Ringø *et al.* 1995, Ringø & Gatesoupe 1998, Ringø *et al.* 2000), however, there is little information on the occurrence of *Weissella viridescens* in fresh marine fish (Leisner *et al.* 1994).

It is known that the microbiota of fish is affected by nutritional, physiological and environmental factors, and it is also expected that the microbial population of fish vary among species. Nevertheless, some authors have considered that season could be a decisive factor (Hagi *et al.* 2004). Bucio *et al*. (2006) observed seasonal changes of lactobacilli in the intestines of freshwater fish, with the highest counts in the summer and almost an absence of them in winter. Hagi *et al.* (2004) indicated that it is very likely that feeding habits did not have a significant influence on fish LAB composition, when the fish species were grown in the same conditions. Consistent with these findings, our results showed that all species collected in May had a similar LAB composition, represented by two species of *Leuconostoc* and *W. viridescens*. The specimens of *R. arcuata* colleted in December showed a different composition of LAB microbiota, except for the presence of *W. viridescens* (Tables 1 and 2).

Table 2. Taxonomic affiliation based on 16S rRNA gene sequence of LAB isolates from fish (F) and sediments (S) / Filiación taxonómica de los aislamientos de LAB a partir de peces (F) y sedimentos (S) basada en la secuencia del gen ARNr 16S

Isolate	GenBank accession number	Top species match (GenBank accession number)	Similarity (%)
F1	FJ892726	*Weissella viridescens* NRIC 1536^T (AB023236)	99.7
F2	FJ892732	*Lactobacillus paracasei* subsp. *tolerans* JCM 1171^T (D16550)	99.9
F3	FJ892739	*Enterococcus hirae* CECT 279^T (AJ420799)	99.5
F4	FJ892727	*Weissella viridescens* NRIC 1536^T (AB023236)	100.0
F6	FJ892740	*Enterococcus hirae* CECT 279^T (AJ420799)	99.9
F7	FJ892728	*Weissella viridescens* NRIC 1536^T (AB023236)	100.0
F8	FJ892744	*Leuconostoc mesenteroides* subsp. *mesenteroides* ATCC 8293^T (CP000414)	99.9
F9	FJ892729	*Weissella viridescens* NRIC 1536^T (AB023236)	100.0
F10	FJ892730	*Weissella viridescens* NRIC 1536^T (AB023236)	100.0
F11	FJ892731	*Weissella viridescens* NRIC 1536^T (AB023236)	100.0
F12	FJ892745	*Leuconostoc citreum* KM20 (DQ489736)	99.9
F13	FJ892746	*Leuconostoc mesenteroides* subsp. *mesenteroides* ATCC 8293^T (CP000414)	99.9
S14	FJ892733	*Lactobacillus pentosus* JCM 1558^T (D79211)	99.9
S15	FJ892734	*Lactobacillus pentosus* JCM 1558^T (D79211)	100.0
S16	FJ892735	*Lactobacillus graminis* DSM 20719^T (AM113778)	99.7
S17	FJ892737	*Pediococcus pentosaceus* DSM 20336^T (AJ305321)	99.7
S18	FJ892738	*Pediococcus pentosaceus* DSM 20336^T (AJ305321)	99.7
S19	FJ892736	*Lactobacillus pentosus* JCM 1558^T (D79211)	99.9
S20	FJ892741	*Enterococcus mundtii* CECT972^T (AJ420806)	99.9
S21	FJ892742	*Enterococcus mundtii* CECT972^T (AJ420806)	99.9
S22	FJ892743	*Enterococcus durans* CECT411^T (AJ420801)	99.7

Table 3. Antimicrobial activities of LAB isolated from fish and sediments of Bahía Blanca Estuary against pathogens / Actividad antimicrobiana de las bacterias acido lácticas aisladas de peces y sedimentos del estuario de Bahía Blanca

	L. monocytogenes			*Y. ruckeri*			*A. salmonicida*			*Lc. garvieae* (03/8702)			*Lc. garvieae* (03/8460)		
Treatment	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Strains	Inhibition zone														
F1	+	-	-	++	-	-	+	-	-	+	-	-	+	-	-
F2	+++	+	+	+++	+	-	+	-	-	++	+	-	+	-	-
F3	++	+	-	++	-	-	-	-	-	+	+	-	+	-	-
F4	++	-	-	+++	+	-	++	-	-	++	+	-	+++	+	-
F6	++	-	-	++	-	-	-	-	-	+	-	-	+	-	-
F7	+	-	-	++	+	-	+	-	-	-	-	-	+	-	-
F8	+	+	-	++	+	-	-	-	-	++	+	-	+	-	-
F9	+	-	-	++	+	-	+	-	-	+	-	-	+	-	-
F10	+	-	-	++	-	-	+	-	-	+	-	-	+	-	-
F11	+	-	-	+++	-	-	+	-	-	++	-	-	+	-	-
F12	++	-	-	+++	+++	-	-	-	-	-	-	-	+	-	-
F13	++	+	-	+++	+	-	-	-	-	+	-	-	+	-	-
S14	+++	+	-	+++	++	+	++	+	-	++	-	-	+	-	-
S15	+++	+	-	+++	++	+	+	-	-	++	-	-	+	-	-
S16	+++	-	-	+++	-	-	-	-	-	++	-	-	+	-	-
S17	+++	+	-	+++	+	-	++	-	-	+++	++	-	+	-	-
S18	+++	++	-	++	++	-	++	-	-	++	-	-	+	-	-
S19	+++	++	+	+++	++	+	++	-	-	++	-	-	+	-	-
S20	+++	+++	++	+++	-	-	-	-	-	++	+	-	++	-	-
S21	+++	+++	++	+++	-	-	-	-	-	+	+	-	++	-	-
S22	++	-	-	++	-	-	-	-	-	+++	++	-	++	+	-

A: Supernatants without treatment (raw), B: Neutralized supernatants, C: Neutralized Supernatants heated at 100ºC.
Diameter of Clear zone: + = 6-10 mm, ++ = 10.1-15 mm, +++ = more than 15 mm
A: Sobrenadante sin tratamiento (crudo), B: Sobrenadante neutralizado, C: Sobrenadante neutralizado y calentado a 100 ºC.
Diámetro del halo: + = 6-10 mm, ++ = 10,1-15 mm, +++ = más de 15 mm

However, none of the LAB species isolated from fish was found in sediments. As water flow passes through the digestive tract and gills of fish, it would be expected that the external environment influence fish microbiota. Most bacterial cells are transient in the gut, with continuous intrusion of microbes coming from water and food (Gatesoupe 1999). This is still more significant considering that in Bahía Blanca Estuary tidal energy and water turbulence strongly affect the water column, resulting in a high concentration of suspended sediment (Piccolo & Perillo 1990). Therefore, the observed differences in LAB species composition between fish and sediments could be indicating specific adaptations to each environment, and should be subject of future research.

The antimicrobial activity of the isolated LAB strains in the form of cell free spent broth was tested against *L. monocytogenes* and three common fish pathogens. *Listeria monocytogenes* was used as an indicator microorganism because fish and fishery products may be a vehicle for this pathogen and have been frequently isolated from these products (FAO 1999, Jensen *et al.* 2008, Parihar *et al*. 2008). The selection of fish pathogens reflected some of the main bacterial diseases encountered in salmonid culture. *Aeromonas salmonicida* is the causative agent of the destructive disease furunculosis (Jutfelt *et al*. 2006), *Y. ruckeri* is the causative agent of enteric redmouth disease (Hietala *et al*. 1995) and *Lc. garvieae* is an important Gram-positive coccus pathogen that causes serious economic losses in cultured marine and freshwater fish species (Brunt & Austin 2005, Vendrell *et al*. 2007). The antibacterial effect of LAB is generally due to the production of either one or a combination of the following factors: bacteriocins, siderophores, lysozymes, proteases and organic acids to alter the pH of the environment. The inhibition resulting from the application of the raw supernatant could be mainly attributed to the production of lactic acid, and consequently the reduction of the medium pH (Vázquez *et al*. 2005). This explains the decrease in the number of strains with inhibitory activity when neutralized supernatant was applied. The inhibitory effects of the neutralized supernatants could be caused by other compounds such as hydrogen peroxide, bacteriocins, siderophores, lysozymes, and proteases (Sugita *et al.* 2007). When the inhibitory activity persisted after heating at 100ºC for 5 min, the active component present in the spent broth could be a bacteriocin-like compound (Ammor *et al*. 2006), and further studies must be done.

To our knowledge, this study is the first report on the diversity of lactic acid bacteria in a coastal marine environment and in fish from Argentina. We report on the screening of antibacterial activity of 21 LAB strains isolated from fish and sediments with inhibitory effects against gram positive and negative pathogenic microorganisms related with aquaculture and fish processing. Our results also indicate that sediments and fish from Bahía Blanca Estuary would have a lactic microbiota which is proper to each environment.

Lactic acid bacteria originally isolated from coastal marine environment and fish are probably the best candidates because they should be well adapted to the environmental conditions of several fish culture and should therefore be more competitive than LAB from other sources. These results justify further studies to assess their potential as probiotic and biological control agents in aquaculture.

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