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BIOCHEMICAL AND ANTIBACTERIAL POTENTIAL OF AUTOCHTHONOUS *CARNOBACTERIUM* AND *LACTOBACILLUS* SPECIES ISOLATED FROM GASTROINTESTINAL TRACT OF COASTAL FISH

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ABSTRACT

A study was conducted to characterize 30 autochthonous lactic acid bacteria (LAB) strains isolated from the gastrointestinal (GI) tract of three fish species; Atlantic horse mackerel (*Trachurus trachurus*), European pilchard (*Sardina pilchardus*) and Atlantic bonito (*Sarda sarda*), on the basis of phenotypic characters. The strains were characterized according to their potential properties; antimicrobial activity, acidifying capacity, proteolytic and lipolytic activity and they belonged to; *Lactobacillus plantarum, L. sakei, L. coryniformis, L. fermentum, L. oris* and *Carnobacterium* spp. All strains revealed inhibitory activities against one or more of the following target strains: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), *Bacillus cereus* (LRSE 01), *Aeromonas hydrophila* (LRSE 04), *Listeria innocua* (LRSE 12), *Salmonella* sp. (LRSE 05) and *Vibrio* sp. (LRSE 23). Characterization of the antimicrobial substances showed that 4 out of 30 produce bacteriocin-like substances. The majority of the strains (26) revealed weak acidification, 15 strains were able to hydrolyze casein and twenty six out of 30 LAB strains revealed lipolytic activity .

Key words: costal fish, *Lactobacillus*, *Carnobacterium*, Potential properties.

INTRODUCTION

There are numerous investigations that have shown the presence of lactic acid bacteria (LAB) as a part of the indigenous intestinal microbiota of fish (Ringø 2004; Ringø *et al*. 2005, 2014a, 2014b; Lauzon and Ringø 2012; Merrifield *et al*. 2014). Among LAB colonizing the fish gut; Merrifield *et al*. (2014) reported eight genera, and two of them; *Carnobacterium* and *Lactobacillus* are commonly reported.

Several studies have demonstrated that lactobacilli and carnobacteria have the ability to inhibit *in vitro* growth of fish pathogens including; *Aeromonas salmonicida, Vibrio anguillarum* and *Vibrio salmonicida* (e.g. Ringø *et al*. 2005, 2010, 2014a; Robertson *et al*. 2000; Ringø 2008; Gopalakannan and Arul 2011). Accordingly, these bacteria species have been suggested to prevent colonization and proliferation of bacterial pathogens in the GI tract of fish and some of them have been used in *ex vivo* studies evaluating interactions between LAB and fish pathogens. It is also revealed that some species of *Lactobacillus* and *Carnobacterium* can be used as protective cultures to improve the product safety and quality such as vacuum-packed fresh fish and seafood (Brillet *et al*. 2004; Paari *et al*. 2011).

Atlantic horse mackerel (*Trachurus trachurus)*, European pilchard (*Sardina pilchardus*) and Atlantic bonito (*Sarda sarda*) are widely distributed in Mediterranean waters and exploited by different gears along the Algerian coasts (Grimes *et al*. 2004) and these fish species are important for the local community. As no study has been conducted to characterize the LAB community of costal fish in Algeria, the present study addressed to isolate autochthonous *Carnobacterium* and *Lactobacillus* species from the entire intestine of Atlantic horse mackerel, European pilchard and Atlantic bonito and to characterize their properties; acidification activity, enzymatic activities and their antimicrobial properties towards eight test bacteria.

MATERIALS AND METHODS

Isolation of LAB: Thirty-two two adult individuals from three fish species: mackerel, sardine and bonito were collected from the coast of Oran – Algeria. Each fish was dissected aseptically after capture. The intestines were cut free, weighed and 1 g intestinal content was homogenized with 9 ml of sterile saline solution and mixed for 2 min according to Ghiasi (2011).

Subsequently dilution series were performed up to 10^{-8}. Hundred µl of each dilution was inoculated into Man Rogosa Sharp (MRS; De Man *et al*. 1960) and tryptic soy bean (TSB) broth. After incubation at 30°C for 18 h, the enrichments were plated onto solid media;

MRS and D-MRS (Hammes *et al*. 1992). The MRS medium was modified by omitting acetate, substituting sucrose for glucose and adjusting the pH to pH 8.5 with 10N NaOH. Colonies were selected randomly and purified by re-streaking according to Leisner *et al*. (1997). Purified LAB isolates were investigated with regard to colony morphology, cell morphology, motility, Gram-staining, spore formation, oxidase and catalase tests as described by Harrigan and Mc Cance (1976).

Identification of isolates LAB: Gram-positive, oxidase – and catalase negative rods were further characterized according to the following criteria; gas production from glucose or gluconate, growth at different temperatures (0, 10, 15 and 45°C) and different pH (3.9, 4.8 and 9.6) as well as the ability to grow at different concentrations of NaCl (2, 4, 6.5 %) (Stiles and Holzapfel 1997; Carr *et al*. 2002). Furthermore, assimilation of gluconate, arginine dihydrolase, hydrolysis of esculin, the Voges-Proskauer test and growth on acetate agar were carried out. All strains were tested for their carbohydrates fermentation patterns using 21 key sugars (Carr *et al*. 2002). Tests for phenotypic characterization were conducted twice for each strain.

Potential properties of *Carnobacterium* and *Lactobacillus* strains

Antibacterial activity: All LAB strains were screened for their antibacterial activity by the agar spot test described by Schillinger and Lücke (1987). The indicator strains used were: *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), *Bacillus cereus* (LRSE 01), *Aeromonas hydrophila* (LRSE 04), *Listeria innocua* (LRSE 12), *Salmonella* sp. (LRSE 05) and *Vibrio* sp. (LRSE 23).

Screening for bacteriocin production: The most promising strains exhibiting antagonistic activities against the test bacteria by the agar spot test were further investigated for their potential to produce bacteriocin-like substances as described by Ammor *et al*. (2005). Sensitivity to proteolytic - and lipolytic enzymes of antibacterial compounds was investigated by the addition proteinase K, α-chymotrypsin and lipase at a final concentration of 1 mg/ml in phosphate buffer (pH 6.5). The supernatants were incubated with these enzymes at 37°C for 2 h and the antagonist activity was detected using the well diffusion agar method described by Corsetti *et al*. (2004).

Acidification activity and acidification rate: The isolates were grown in appropriate broth: MRS (*Lactobacillus*) and TSB (*Carnobacterium*) at 30°C for 24 h. The microbial culture was inoculated at a level of 1% in reconstituted sterile non-fat dry milk (10% w/v). The acidification activity was tested according to Nieto-Arribas *et al*. (2009) and was determined by measuring the Dornic acidity; that expresses the acidity developed in the medium by transformation of lactose into lactic acid. pH values were recorded after 2, 4, 6 and 24 h of incubation at 30°C using pH-meters (glass electrode, Hanna instruments, Padova, Italy) previously calibrated using two buffers (pH 4.0 and pH 7.0). The acidification rate was calculated as ΔpH; $\Delta pH = pH_{at\ time} - pH_{zero\ time}$ according to Ayad *et al*. (2004). The cultures were considered as fast, medium or slow acidifying when a ΔpH of 0.4U was achieved after 3, 3–5 and >5 h, respectively.

Proteolytic activity: Surface-dried plates of milk agar (PCA agar supplemented with 10% skimmed milk), were streaked with 24 h old cultures, after incubation at 30°C for 4 days, and examined for any clearing of casein around and underneath the growth for assessment of proteolytic activity (Thapa *et al*. 2006). Quantity of free amino acids released was determined according to the method of Church *et al*. (1983) but only of strains which possessed proteolytic activity. Results were expressed as glycine equivalents (mM) according to a standard curve, prepared using glycine in the range of 0.1 - 10 mM.

Lipolytic activity: Lipolytic activity was investigated using the method of Leuschner *et al.* (1997) with some modifications. Briefly, two wells were made on tributyrin agar for each isolate tested. One well was filled with overnight culture of the LAB and the 2nd well with phosphate buffer pH 7 as control. The plates were incubated at 30° C for 7 days and lipolytic activity was detected by the presence of a clear zone around the well. Strains revealed positive results were further tested by the titrimetric assay method (Kashmiri *et al*. 2006). Lipase unit (U) was defined as the amount of the enzyme that released one m mole fatty acid per min (Kumar *et al*. 2011).

RESULTS AND DISCUSSION

Identification of isolates: In the present study, totally 30 autochthonous LAB strains were isolated from the entire intestine of three costal fish species, of which 20 isolates belonged to genus *Lactobacillus* and 10 isolates were classified to genus *Carnobacterium.* All isolates were identified on the basis of carbohydrates fermentation patterns and growth on acetate agar as: *L. plantarum, L. sakei, L. coryniformis, L. fermentum, L. oris* and *Carnobacterium* sp. (Table 1). Presumptive lactobacilli species isolated in the present study revealed high similarity to *Lactobacillus* species previously isolated from intestinal contents of beluga (*Huso huso*) and Persian sturgeon (*Acipenser persicus*) (Ghanbari *et al*. 2009).

Table 1. Physiological and biochemical characteristics of *Lactobacillus* and *Carnobacterium* strains.

	Cb1	Cb2	Cb3	Cb4	Cb5	Cb6	Cb7	Cb8	Cb9	Cb10	L.1	L.2	L.3	L.4	L.5	L.6	L.7	L.8	L.9	L.10	L.11	L.12	L.13	L.14	L.15	L.16	L.17	L.18	L.19	L.20
Growth at different T(C°)																														
0	+	+	(+)	(+)	(+)	(+)	(+)	(+)	+	+	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
10	+	+	+	+	+	+	+	+	+	+	+	-	-	-		+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
15	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
40	(+)	-	-	(+)	(+)	-	-	-	-	-	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
45	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	(+)	-	(+)	-	-	-	-	-	-	-
Growth at different pH																														
3,9	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	(+)	(+)	-	-	+	-	+	+	(+)	+	+	+	+	+
4,8	+	+	-	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9,6	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-	-	-	-	(+)	-	-	-	-	-	-	-	(+)	-	-
NaCl Tolerance																														
2%	+	++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+
4%	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	++	+	+	+	+	+	+	+	+
6,5%	+	-	+	+	+	+	(+)	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gas production from																														
Glucose	-	-	-	-	-	-	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Gluconate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	+	-	-	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
ADH	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Acet. agar	-	(+)	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
V.P. test	+	-	+	+	-	+	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Hydr.Esculin	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+		+		+		+	+	+		+
Acid production from																														
Ribose	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+
Maltose	+	+	+	+	+	+	+	+	+	+	-	-	-	-	+	+	(+)	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	-	+	+	+	+	-	-	+	+	+	-	-	-	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-
Gluconate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	(+)	+	+	+	+
Cellobiose	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+
fructose	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	-	-	+	+	+	+	-	-	+	-	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+
mannose	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	-
salicine	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+	+	-	+	-	+	+	+	+	+	-	+
L.arabinose	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	-	-	+	-	+	-	+	+	-	-	+
D-xylose	-	+	+	+	+	-	-	+	-	+	-	+	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
Rhammose	-	-	-	(+)	(+)	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	+	-	-	+	+	-	-
Inositol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	(+)	-	-	-	-	+	+	-	+	-	-	-	-	-
Sorbitol	(+)	-	-	-	-	(+)	-	(+)	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	+	(+)	+	+	+	+	-
amygdaline	+	-	+	+	+	+	(+)	+	+	-	+	-	-	-	+	+	+	-	-	+	-	+	+	+	+	+	-	+	-	+
melibiose	+	+	+	+	-	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+
trehalose	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+
starch	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	+	-	-	-	+	-	-	-	-
saccharose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+
raffinose	+	-	(+)	(+)	(+)	-	-	+	+	-	-	+	+	+	-	-	-	+	+	-	+	+	+	+	-	+	+	-	-	-

L. plantarum (L. 06, L. 07, L. 09, L.10, L.12, L. 14, L.16, L.17, L. 18), *Lb. fermentum* (L. 02, L.03, L.04), *L. sakei* (L. 01, L.05, L.20), *L. coryniformis* (L. 11, L.19) and *Carnobacterium* sp. (Cb1,Cb2 to Cb10). VP - (Voges Proskauer), ADH (arginine hydrolysis), Acet. Agar (growth in acetate agar).

+ Positive reaction; (+) low reaction; – negative reaction, ND – not determined

Table 2. Inhibitory spectra of LAB isolate exhibiting antimicrobial activity.

	S. aureus **(ATCC 25923)**	***E. coli*** **(ATCC25922)**	***P. aeruginosa*** **(ATCC27853)**	***B. cereus*** **(LRSE 01)**	***A. hydrophila*** **(LRSE 04)**	***L. innocua*** **(LRSE 12)**	***Salmonella*** **sp (LRSE 05)**	***Vibrio*** **sp. (LRSE 23)**
L. plantarum								
L.06	+++	-	-	++	++	+++	++	++
L.07	++	++	-	++	-	++	++	-
L.08	++	++	++	++	++	+++	++	-
L.09	++	+++	++	++	+++	++	++	++
L.10	++	+++	++	++	++	+++	-	++
L.12	++	++	-	++	++	-	-	-
L.14	++	-	-	+++	+	-	-	-
L.15	++	++	++	++	++	++	++	++
L.16	++	-	-	+++	++	+++	++	-
L.17	+++	++	++	++	++	+++	++	++
L.18	++	+++	+++	+++	+++	+++	++	-
L.sakei								
L.01	+++	+++	++	++	+++	++	+++	+++
L.05	++	+++	+++	+++	-	++	++	+
L.20	+++	++	+++	++	-	++	-	+
L.fermentum								
L.02	+++	++	+++	++	++	++	+++	+++
L.03	+++	++	++	++	++	+++	-	+++
L.04	+++	++	++	++	+	+++	++	++
L.coryniformis								
L.11	+++	++	+++	+++	-	+++	++	++
L.19	+++	+++	++	++	++	++	-	++
L.oris								
L.13	+++	+++	++	++	++	++	-	++
Carnobacterium **sp.**								
Cb01	+++	++	+++	+++	-	++	++	++
Cb02	+++	+	++	+	-	++	-	-
Cb 03	++	-	++	++	+	++	-	-
Cb 04	++	-	++	+	++	+++	++	-
Cb05	++	++	++	-	++	+++	++	++
Cb 06	++	++	-	-	++	++	-	-
Cb 07	++	++	-	++	-	++	-	-
Cb08	+++	-	-	++	++	++	+++	+
Cb 09	+++	++	++	++	+	++	+	-
Cb 10	++	-	++	++	++	++	+++	+++

– = no antimicrobial activity; + = inhibition zone < 10 mm ; ++ = inhibition zone > 11 mm; +++ = inhibition zone > 20 mm

Antibacterial activity: All LAB strains isolated in the present study revealed antagonistic activity against one or more of the target strains tested (Table 2). The diameters of the inhibition halos were within the range of 12±0.4 – 31±0.09 mm. The most promising isolates revealing high inhibitions towards the test bacteria, five out of eight, were identified as *L. plantarum* (L.18), *L. sakei* (L.01) and *L. coryniformis* (L.11).Generally, the antibacterial activities of *Carnobacterium* were less than that of lactobacilli, and *Carnobacterium* (Cb01) revealed best activity against the test bacteria, three out of eight. The inhibitory effect, which was observed by the formation of clear and distinct zones around the wells, may be due to the production of several antimicrobial compounds; organic acids, H_2O_2 or bacteriocins (Corsetti *et al*. 2004). The present study revealed inhibition of the foodborne pathogens; *L. innocua*, *Salmonella* sp. and *Vibrio* sp. bacteria commonly reported in ready-to-eat foods and lightly preserved seafood products (Huss *et al*. 2000; Matamoros *et al*. 2009).

Screening for bacteriocin production: The activity of the inhibitory agent of some promising isolates was tested under conditions which eliminate possible effect of organic acids by adjusting the pH of the cells-free supernatant to 6.5 and of H_2O_2 by catalase treatment. Four out of thirty strains; *L. coryniformis* (L.11), *L. fermentum* (L.03), *Carnobacterium spp.* (Cb04, Cb10) revealed inhibition zones for neutralized culture supernatants and catalase treated supernatants. This antibacterial activity was lost after proteinase K and α-chymotrypsin treatment but not affected by lipase. The sensitivity to proteolytic enzymes is a proof of its proteinaceous nature reflecting the production of bacteriocins (Klaenhammer 1993).

Acidification activity: Evaluation of acidification during fermentation is a potential characteristic of LAB that is of paramount importance to investigate (Corsetti and Settanni 2007).

Results for acidifying activity in milk at 30°C are displayed in Figure 1 and 2. Acidification kinetics revealed that the decrease in pH, after 24 h of incubation of *Lactobacillus* was lower (pH 5.27) compared to that detected of *Carnobacterium* spp.; lowest value detected = 5.41. *L. plantarum* (L.06), (L.14), (L.17) and *L. coryniformis* (L.11) showed fast acidifying activity whose values of ΔpH ranged from 0.40±0.01 to 0.48±0.02 after 2h (Table 3). Four tested strains of *L.. fermentum* L.03, *L. oris* L.13, *L..plantarum* L.07 and L.16 showed medium acidification rate (ΔpH ranged from 0.41±0.01 to 0.49±0.01 at 4h). Three strains *L. sakei* (L.01), (L.05) and *L. plantarum* (L.15) showed a slow acidification with ΔpH values ranging from 0.51±0.01 to 0.57±0.02.

Table 3. ΔpH values of 30 strains of lactic acid bacteria isolated from marine fish. Data are based on means of 3 experiments.

Strains	**ΔpH after 2h**	**ΔpH after 4h**	**ΔpH after 6h**	**ΔpH after 24h**
L. plantarum				
L.06	0.48±0.02	0.62±0.02	0.71±0.01	1.21±0.02
L.07	0.23±0.04	0.42±0.02	0.43±0.03	0.98±0.03
L.08	0.04±0.01	0.11±0.02	0.09±0.01	1.32±0.03
L.09	0.03±0.01	0.06±0.54	0.03±0.01	1.32±0.01
L.10	0.04±0.02	0.37±0.54	0.07±0.00	1.32±0.03
L.12	0.03±0.01	0.03±0.01	0.07±0.01	1.36±0.02
L.14	0.47±0.01	0.59±0.01	0.71±0.01	1.31±0.01
L.15	0.23±0.03	0.34±0.01	0.57±0.02	1.17±0.02
L.16	0.29±0.01	0.49±0.01	0.54±0.01	0.88±0.02
L.17	0.40±0.01	0.48±0.01	0.52±0.02	1.03±0.03
L.18	0.2±0.30	0.24±0.05	0.36±0.02	0.86±0.01
L. sakei				
L.01	0.37±0.02	0.39±0.00	0.52±0.01	1.04±0.10
L.05	0.03±0.02	0.32±0.05	0.51±0.01	1.46±0.25
L.20	0.30±0.01	0.38±0.01	0.39±0.01	0.99±0.01
L. fermentum				
L.02	0.03±0.01	0.04±0.02	0.12±0.02	1.27±0.02
L.03	0.24±0.03	0.41±0.01	0.46±0.01	1.32±0.01
L.04	0.06±0.00	0.26±0.20	0.18±0.02	1.14±0.02
L.coryniformis				
L.11	0.41±0.00	0.42±0.02	0.66±0.20	1.13±0.03
L.19	0.03±0.01	0.04±0.03	0.12±0.53	0.38±0.01
L. oris				
L.13	0.11±0.03	0.45±0.01	0.50±0.02	1.09±0.09
***Carnobacterium* sp.**				
Cb01	0.02±0.02	0.07±0.01	0.11±0.02	1.11±0.01
Cb02	0.06±0.02	0.16±0.02	0.19±0.01	1.12±0.02
Cb 03	0.62±0.01	1.33±0.03	1.61±0.02	1.22±0.02
Cb 04	0.27±0.00	0.43±0.02	0.50±0.01	0.38±0.53
Cb05	0.39±0.53	0.21±0.02	0.26±0.01	1.08±0.01
Cb 06	0.03±0.01	0.13±0.03	0.21±0.02	1.35±0.02
Cb 07	0.19±0.02	0.37±0.01	0.52±0.02	0.74±0.01
Cb08	0.37±0.02	0.41±0.01	0.58±0.01	1.21±0.01
Cb 09	0.33±0.02	0.45±0.01	0.49±0.01	1.09±0.01
Cb 10	0.36±0.01	0.41±0.02	0.44±0.01	1.23±0.01

The amount of lactic acid produced as function of time is slightly variable from strain to strain. At the end of incubation; 24h, it was noted that *L. coryniformis* (L.19) had the highest acid production (81.33±0.88

Dornic acidity D°) while, *L. plantarum* (L.08) revealed the lowest (42±1.15 Dornic acidity D°) (Figure 1). Only one strain of *Carnobacterium* (Cb03) showed fast acidifying activity (ΔpH: 0.62±0.01 at 2h), four strains (Cb04, Cb08, Cb09 and Cb10) showed medium acidification with ΔpH values ranging from 0.41±0.01 to 0.45±0.01 at 4h (Table 3) and one strain (Cb07) showed slow acidification (ΔpH: 0.52±0.02 at 6h). These results revealed that *Carnobacterium* strains were able to grow in milk and produce lactic acid from lactose confirming the presence of ß-galactosidase. Thirteen strains (L.02, L. 04, L.08, L.09, L.10, L.12, L.18, L.19, L.20, Cb01, Cb02, Cb05 and Cb06) revealed very slow acidifying activity with ΔpH values ranging from 0.03±0.01 to 0.39±0.04 after 6h of incubation (Table 3).

The generally low acidification activity of the LAB strains isolated in the present study is in agreement with Mauguin (1991) and Sahnouni *et al*. (2012) reporting low acidifying activity of LAB isolated from fishery products and intestine of marine fish, respectively. Some of the LAB isolated in the present study may be commercially used in the future for the production of lactic acid, and to decrease the pH of the medium which may contribute to the development of texture, color and taste, prevents the growth of pathogenic microorganisms and thus improves safety and stability of the final product. Additionally, the poor acidifiers strains particularly those possessing antibacterial activity, may be used to preserve slightly acidified products as seafood (Matamoros *et al*. 2009).

Proteolytic activity: Proteolytic activities have been reported in numerous bacteria isolated from fish gut (Ray *et al*. 2012) as well as for carnobacteria (Askarian *et al*. 2013). Fifteen strains revealed proteolytic activity (Table 4), and the activities of the selected *Lactobacillus* and *Carnobacterium* strains, determined by the OPA test, were in the range 0.25±0.023 and 1.95±0.015 mM Gly/L (Figure 3). *Carnobacterium* (Cb08) revealed highest activity (1.95±0.015 mM Gly/L) followed by *L. sakei* L.20 (1.93±0.024 mM Gly/L), *Carnobacterium* Cb07 (1.89±0.062 mM Gly/L) *and L. plantarum* L.06 (1.82±0.064 mM Gly/L), while *L. plantarum* (L.14) showed the lowest (0.25±0.023Mm Gly/L). The results revealed in the present study are in agreement with that reported by Bonomo *et al*. (2008) reporting middle-to-low proteolytic activity of most LAB isolated from meat products.

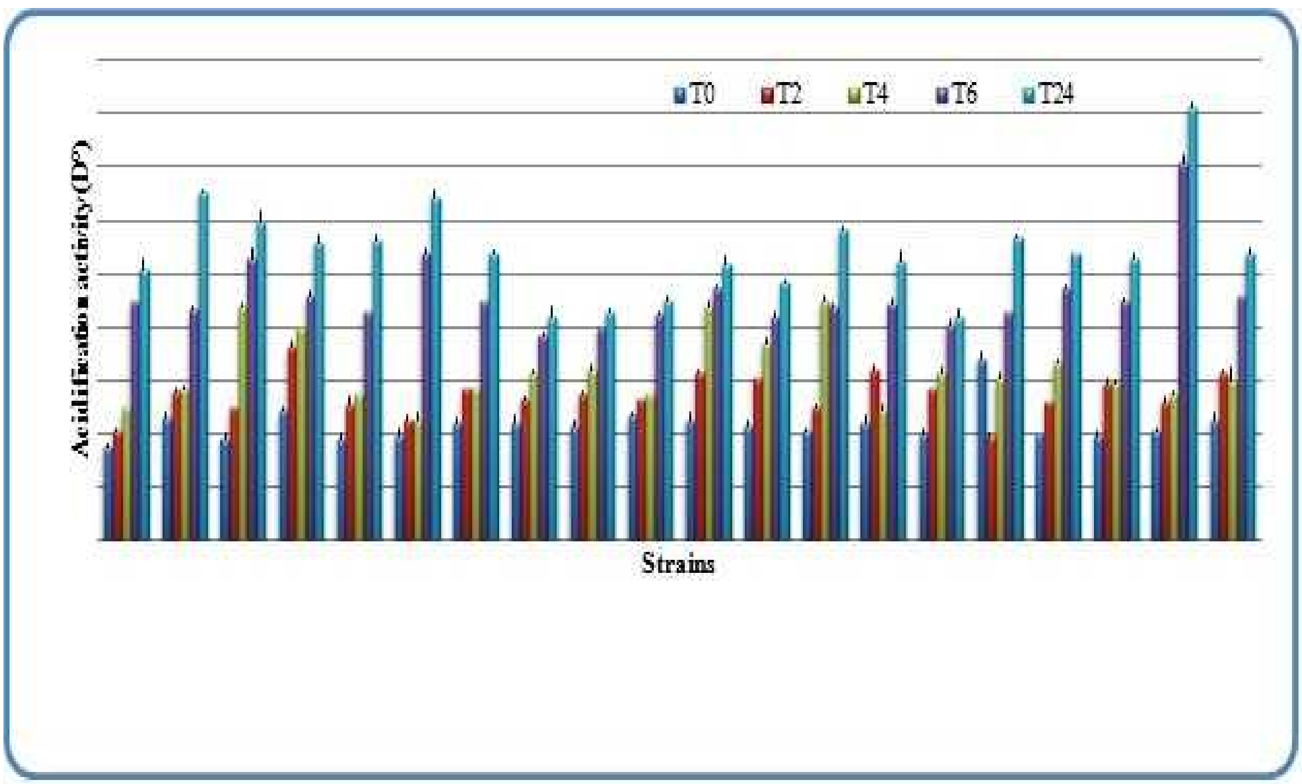

Figure 1. Acid production ability of the *Lactobacillus* strains after 2 h, 4h, 6h and 24 h. Values are mean ± standard deviation.

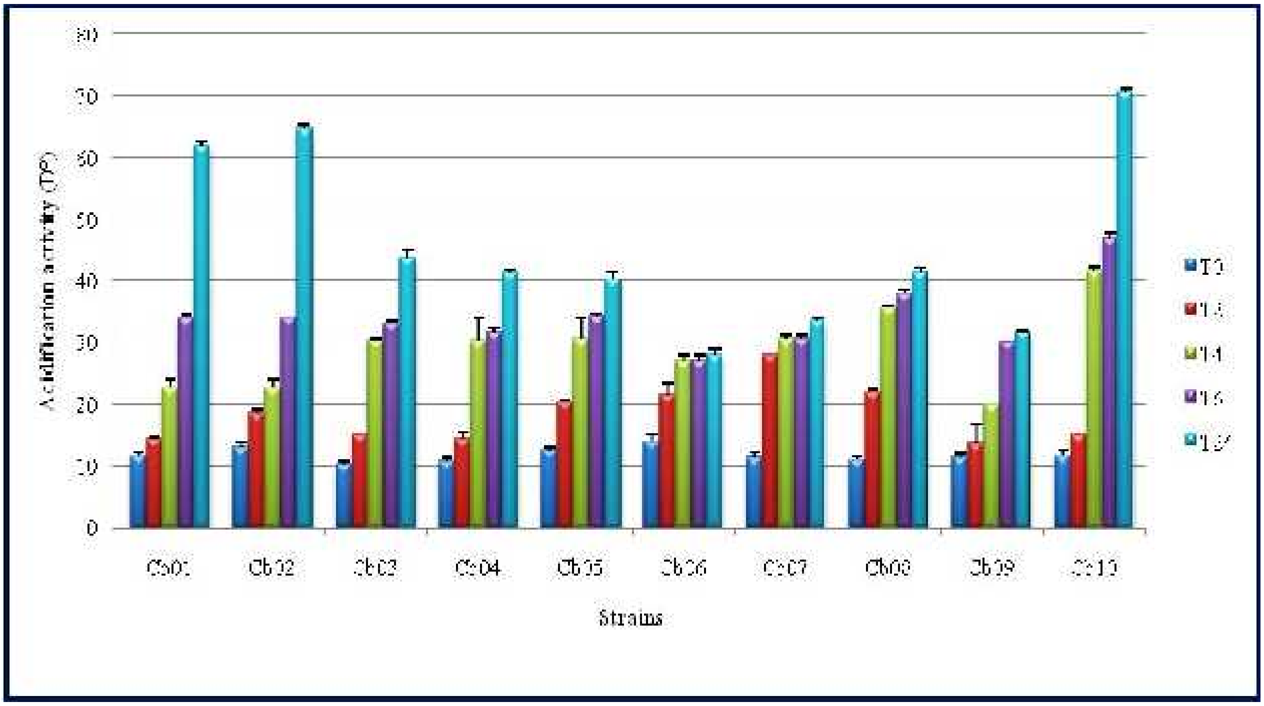

Figure 2. Acid production ability of the *Carnobacterium* strains after 2 h, 4h, 6h and 24 h. Values are mean ± standard deviation.

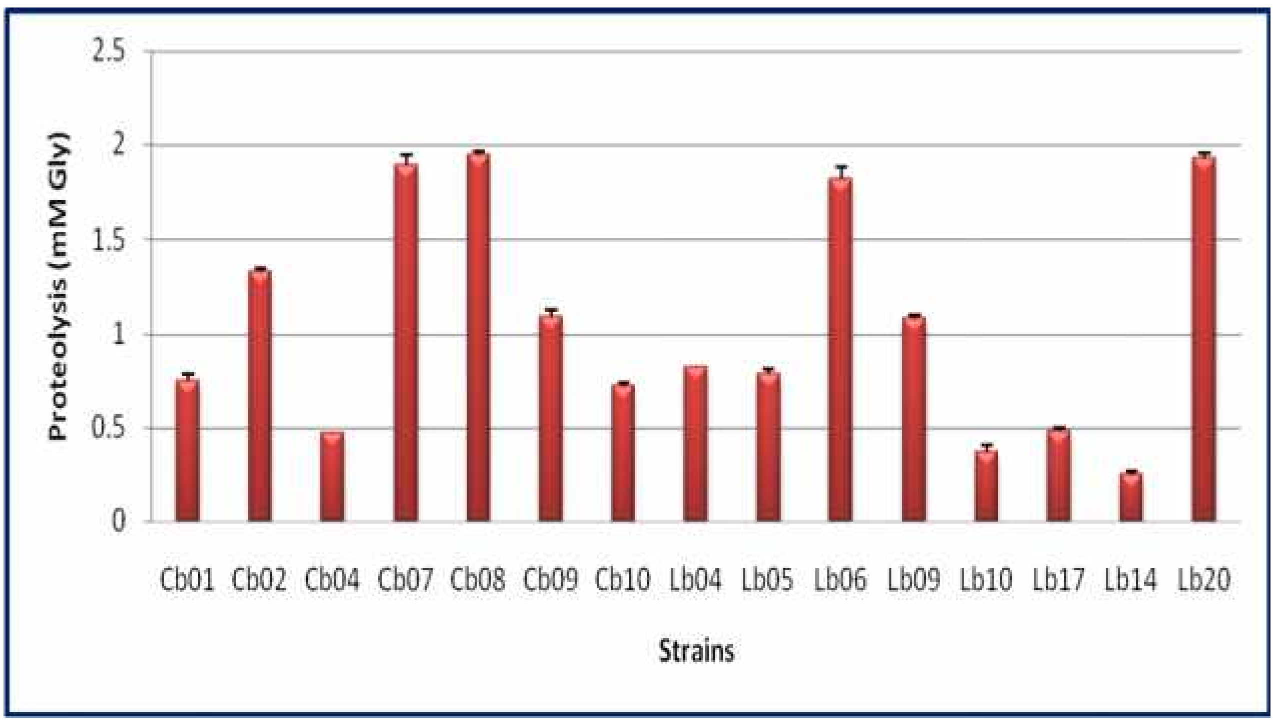

Figure 3. Proteolytic activity *of Lactobacillus* and *Carnobacterium* strains isolated from marine fish. Values are mean ± standard deviation

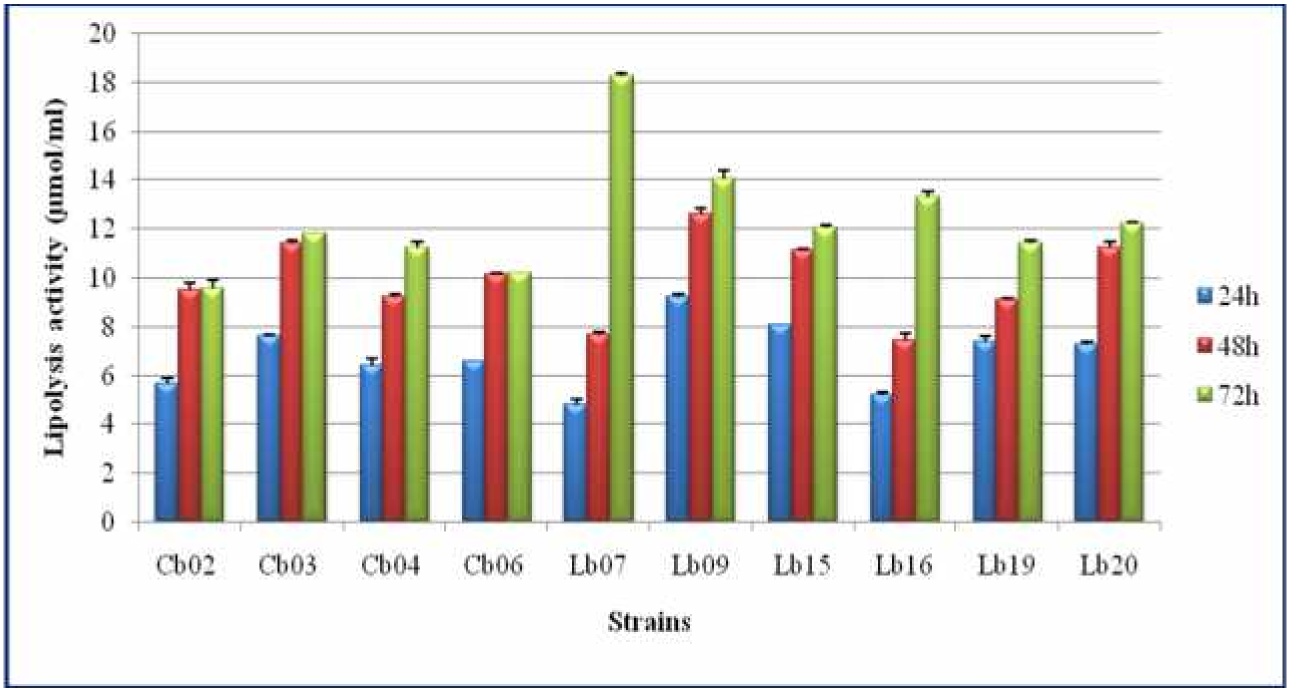

Figure 4. Lipolytic activity of *Lactobacillus* and *Carnobacterium* strains isolated from marine fish. Values are mean ± standard deviation.

Lipase activity: Lipase activity is seldom reported of LAB isolated from fish intestine (Jini *et al*. (2011), however, in the present study twenty-six LAB strains revealed lipolytic activity (Table 4). Quantitative analysis of ten LAB isolates revealed that *Lactobacillus plantarum* (L.09) had the highest lipolytic activity (9.21±0.11 µmol/ml) followed by *Lactobacillus plantarum* (L.15) (8.05±0.035µmol/ml) and *Carnobacterium* Cb03 (7.6±0.1µmol/ml) after 24h incubation (Figure 4). However, after 72h incubation, *Lactobacillus plantarum* (L.07) revealed highest lipolytic activity (18.25 µmol/ml ± 0.13). A general finding was that *Carnobacteria* revealed lower lipolytic activity than *Lactobacillus* strains. Due to their immense genetic and biochemical diversity, marine microorganisms are of interest as a promising new source of enzymes with unique properties, including salt tolerance, hyperthermostability, barophilicity and cold adaptation (Sabu 2003; Ranjitha *et al*. 2009).

Conclusion: The present study revealed that autochthonous *Lactobacillus* and *Carnobacterium* strains isolated from three costal fish species possessed important properties. All strains revealed inhibitory activities against challenging food borne pathogens of which four strains produced bacteriocin-like substances. A low acidification activity was generally observed in most strains. *Carnobacterium* (Cb08) revealed highest proteolytic activity compared to the others strain. However, *Lactobacillus plantarum* (L.09) had the highest lipolytic activity. These results justify the importance of indigenous intestinal microbiota of fish which may be further explored.

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