Selection and Evaluation of Anti-Listerial Activity of *Leuconostoc Mesenteroides* Wild Strains Isolated from Algerian Raw Dromedary Milk

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**Abstract**

Camel milk is a key food in arid and semi-arid regions for their nutritional needs qualitative and quantitative. Beneficial microflora of camel milk represented by lactic acid bacteria is a potential source of biological material used in food technology. A total of five samples of individual dromedary raw milks were collected from three camels nomad herd in south Algeria and were analysed for bacterial load. A total of sixty different colonies characterized as Lactic acid bacteria, they were classified by phenotypic and biochemical analysis as *Leuconostoc mesenteroides* subsp *mesenteroides* and *Leuconostoc mesenteroides* subsp *dextranicum*. Then, they were tested for antimicrobial compounds production. Out of these, twenty two strains were determined to be able to inhibit the growth of the indicator pathogens strains *Listeria innocua* ATCC 33090 by the agar spot test. Two strains were selected by the large and clear zones of inhibition when tested by the well diffusion assay. The two strains Y44 and Y46 show a high antimicrobial activity against *Listeria innocua* ATCC 33090 by inhibitory substances with proteinaceous nature confirmed after treatment with proteases and Urea, it was proved by kinetics growth in mixed culture. This fact suggests that bacteriocin-like produced by the both *Leuconostoc* strains are specific against *Listeria* sp and may find application as biopreservatives in food products. Therefore, the bio-protective cultures technology would be an ideal solution for enhanced the microbiological safety of food products.

**Keywords:** Dromedary milk; Lactic acid bacteria; *Leuconostoc mesenteroides*; Bacteriocin-like substances; Antimicrobial activity; *Listeria innocua*; Biopreservatives

**Introduction**

New nutrients sources should be more exploited to varied food and take advantage of new functional ingredients and natural food components. Algeria is among the African and Arab countries, where farming conditions of cows are severe in order to get out of this situation by developing a culture system for local animals like camels.

Camel milk is a key food in arid and semi-arid regions or their nutritional needs and covers both qualitative and quantitative. The ancient nomadic tribes think that camel milk has several therapeutic applications. This empirical observation has been scientifically proved; it has a strong antimicrobial activity compared to that of other mammals and its ability to inhibit Gram-positive and Gram-negative pathogens of concern to food safety. Beneficial microflora camel milk represented by lactic acid bacteria is a potential source of biological material used in food technology [1].

A major concern for the food industry is the psychrotrophic pathogenic micro-organisms, ubiquitous in nature, *Listeria innocua*. Its ability to survive in various environmental conditions, such as refrigeration at pH as low as 3.6 in Food, salt concentrations up to 10%, contributes significantly to its risk status. *Leuconostoc*'s capacity to produce anti-listerial bacteriocins has gained a lot of interest in improving safety of various foods.

The use of protective strains could resolve these problems because of the constant and localized delivery of antibacterial compound, which will add to other advantages, such as space colonization by strain, leading to no longer effective protection of the food product. The bioprotective effect of lactic acid bacteria and bacteriocins was widely tested in dairy products [2,3].

For these Reasons we suggest in this work the isolation and characterization of autochthonous *Leuconostoc* strains producing antimicrobial substances active against *Listeria* sp. The aim was therefore to use the cells and or metabolites to control pathogenic microorganisms and to improve the safety of food product, pathogen inactivation and stability in order to extend its shelf life, and secondly, a better exploitation of food sources to vary the food and take advantage of new functional ingredients.

**Literature Review**

**Verification of antagonistic activity**

Inhibitions between bacteria can have various causes (presence of phage lysogens, nutrient deficiency, production of inhibitor, ...) [4,5]. We looked for inhibitions reaction that we have highlighted. Twenty one *Leuconostoc* isolates were submitted to an initial screening to verify the presence of antagonist activity using two methods: spot agar test and well diffusion assay.

**Spot agar test**

The detection of the inhibitory ability was tested against pathogens indicator bacteria *Listeria innocua* (ATCC 33090) provided by the Spanish Laboratory (LICHCA). The antimicrobial activity of the isolates was assessed on solid medium according to Barefoot and Klaenhammer [6] method. MRS medium has inoculated into spot by the 21 strains. After incubation, the plates were overlayed with 8 ml of BHI semi-solid

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ag (Fluka analytical, Sigma Aldrich, Steinheim, India) (0.8 g/100 ml of bacteriologic agar (Merck, Darmstadt, Germany) inoculated with 100 µl of our indicator strains including microbial load which was adjusted in advance to an A 600 nm located in the interval 0.08-0.1 corresponding to a cell concentration of 1 to 2×10^6 CFU was spotted on the surface then incubated for 24 to 48 hours at 37°C. Strains with a clear zone around were considered as positives Leuconostoc.

**Well diffusion assay**

This method allowed us to contact the supernatant of Leuconostoc isolates producing antimicrobial substances with the indicator strains. The strains were grown in MRS liquid and were incubated for 6 to 8 hours [7], after incubation, the medium was centrifuged (6000 rpm for 30 min) and the supernatant was stored at 4°C. The supernatants thus obtained were subjected to a heat treatment of 10 min at 80°C in a water bath in order to eliminate vegetative forms, followed by neutralization of the pH to 6.5 by the addition of 5 mol/l NaOH, then, supernatants were filtered through 0.22 µm filters. A Petri dish containing BHI 9 mm solid was inoculated by our indicator strains and left for 30 min at room temperature; wells of 5 mm diameter were formed by a punch and covered by 10 µl of BHI solid. Wells receive 40 µl of CSF (Cerebrospinal Fluid), our strains and plates were incubated at 37°C for 24 hours. Then, the plates were examined for the formation of inhibition zones round wells, strains with inhibition zone diameter higher than 0.5 mm were considered as positive.

**Materials and Methods**

**Sampling**

Four samples of camel milk were analyzed and stored at 4°C and transported in iceboxes until analysis, the samples were collected from southwest region of Algeria called Béchar (Abadla region). The samples animals differ in ages (4 and 9 years old) and color (grey and brown). Sampling was done in aseptic conditions by washing the teats using warm water containing 2% of bleach and collecting the milk in sterile bottles after hand washing with diluted alcohol. Samples were maintained at 4°C and were transported in iceboxes to the laboratory for analysis. Milk samples were vortexed and serially diluted in sterile saline solution (0.85% w/v NaCl) before being spread onto MRS agar plates supplemented with 30 µg/ml of vancomycin and on M7E agar plates and then incubated at 30°C for 24-72h lactic acid bacteria, usually found in decomposing plants and milk products, produce lactic acid as the major metabolic end-product of carbohydrate fermentation.

**Isolation, purification and characterization**

Sixty colonies were randomly transferred with the sterile toothpicks to MRS agar (Conda, Pronadisa, Spain) and M7E agar (Conda, Pronadisa, Spain) plates incubated at 30°C for 24 h. on the basis of phenotypic and morphological criteria, Gram staining, morphology and catalase production were determined. Colonies with catalase negative and Gram-positive were presumed to be lactic acid bacteria. After purification, colonies were examined for CO2 production from glucose, growth on different temperatures 4°C, 15°C, 30°C, 37°C and 45°C, growth on different pH 4.8, 6.8 and 9.6, growth on different NaCl concentration 3% and 6.5%, strains thermo resistance at 63°C. In addition, the isolates were subjected to biochemical tests such as: Dextran production from sucrose on M7E medium, Arginine hydrolysis using M16BCP (Oxoid Ltd., London, UK) medium, citric acid degradation on Kempler and Mc Kay solid medium which differentiate between Leuconostoc strains genus and the hetero fermented Lactobacillus genus. Carbohydrates fermentation was performed on MRSBCP-ME medium using the following sugars in order to differentiate the subspecies of Leuconostoc strains: Arabinose, maltose, rhamnose, esculine, mannitol, sorbitol, dulcitol, galactose, lactose, fructose, glucose, sucrose, xylose, cellulbiose.

**Verification of antimicrobials type and proteineous nature**

To determine antimicrobials types in this study, five samples were prepared; sample 1 with MRS broth, the 2nd sample with a buffered MRS broth in order to exhibited acids effect, the 3rd sample with buffered MRS broth treated with catalase exhibiting hydrogen peroxide action [8].

The production of antimicrobial substances nature was confirmed using proteolytic enzymes in 2 different protocols: spot-on-the-lawn [9] and well-diffusion assay, which uses the neutralized supernatant of LAB cultures [10]. In both protocols, L. innocua ATCC33090 was used as indicator pathogens, along with the following proteolytic enzymes (all from Sigma, at 20 mg/ml): α-chymotrypsin (C4129) and trypsin TPCK (T1426). For proteolytic enzymes, sterile distilled water was used as a negative control.

Using the spot-on-the-lawn protocol of Tagg and McGoven; each LAB culture identified as antagonistic was recovered in MRS broth (30°C for 24 h) and an aliquot of 2 ml was spotted onto plates containing 10 ml of MRS agar and incubated at 30°C for 24 h. The agar plates was covered with an overlayer of semi-solid BHI (8 ml) inoculated with 10° CFU/ml of the indicator strain and were then incubated at 37°C for 24 h.

Otherwise, using the well-diffusion-assay protocol of Lacroix et al., described that LAB strains identified as antagonists were recovered in 50 ml of MRS broth (30°C for 24 h) and centrifuged at 6800 g for 20 min at 4°C. Supernatants obtained from this process were neutralized with NaOH at 1 mol equi/l (to eliminate the inhibitory effect due to acid production) and sterilized by filtration (Millipore, Bedford, MA, USA). Plates containing 20 ml of semi-solid BHI (0.8 g/100 ml of bacteriologic agar) previously inoculated with 10° CFU/ml of the indicator were prepared. Adjacent wells with a diameter of 5 mm were cut on these plates, and 40 µl of the supernatant and 20 µl of each enzyme, respectively, were deposited. After absorption (4°C for 2 h), the plates were incubated at 37°C for 24 h. For this step, 9 cm diameter plates were used and two LAB cultures inoculated in equidistance cut wells were tested per plate. For each culture, 4 wells of 5 mm were cut (2 proteolytic enzymes and the negative control). In the center of each plate 2 wells of 5 mm were cut for the bacteriocin producer supernatant inoculation (1 for the negative control).

The thermostability of the antimicrobial substances was determined by heating neutralized CFS (Chronic fatigue syndrome) prepared from the six selected isolates at 60°C, 70°C, 80°C, 90°C for 30 min and 100°C, 121°C for 15 minutes.

The effect of pH on the CFS antimicrobial activity was verified by incubation for 2 h with pH values ranging from 2 to 10.

The sensitivity of the antimicrobials compounds to detergents was tested by incubating neutralized CFS in the presence of 1% of tween-80 and Urea for 2 h at 37°C.

**Kinetics growth**

The growth kinetics of pathogenic indicator "Listeria innocua ATCC 33090" in the presence of test isolates Y44 most efficient, such determination seemed necessary for quantitative information on the metabolite’s impact produced by isolates selected on the dynamics
of the pathogen in question. Isolates the most efficient producer of antimicrobial substances: Y44, Y46 and indicator strain *Listeria innocua* were subcultured routinely in 10 ml of skimmed milk with 0.3% yeast extract, and after 18 h, 3 ml was inoculated into 100 ml of skimmed milk for monitoring pure cultures, whereas, 1.5 ml of the overnight culture of the indicator strain was mixed with 1.5 ml of the test strain for those of mixed cultures. The whole was incubated at 30°C for 24 h, the pH reading, acidity and enumeration were performed every 3 hours.

**Results**

**Phenotypical Identification of isolates**

The physiologic characteristics of the isolates are shown in Table 1. A total of sixty were phenotypically identified as belonging to the *Leuconostoc* genus based on the following criteria: ovoid shape, Gram positivity, catalase negativity, vancomycin-resistance, production of gas from glucose, lack of arginine hydrolysis, and fermentation profiling [11-14].

Production of dextran on MSE medium is an important character to differentiate between the *Leuconostoc* species. 21 isolates are capable to hydrolyze sucrose and produce dextran [15]. The use of carbohydrates (Table 2) show that Two out of 21 isolates could produce acid from arabinose, glucose, sucrose, fructose, maltose and xylose and then were characterized as *Leuconostoc mesenteroides* subsp mesenteroides. Otherwise, the other isolates were characterized as *Leuconostoc mesenteroides* subsp dextranicum.

The strains present a variable character about the citrate utilization. 18 strains from 21 were able to form blue colonies on KMK medium, reflecting their ability to use the precursor of aromatic compounds which is taken as an important character in the selection of technological interest of LAB species. This variability according to Bellengier et al. and [16] may be due to the loss of plasmids encoding the genes responsible for the degradation of citrate.

**Antagonistic activity**

A total of six strains showed more inhibition zones than the other strains against indicator microorganisms. Inhibitory spectra of these strains are presented in Table 3. These strains isolated from dromedary milk revealed a strong inhibitory activity towards pathogenic microorganisms like *Listeria* sp. The antimicrobial activities of these strains have been evaluated by measuring the diameter of the inhibition zones around the wells. Clearly, they were limiting the growth of the pathogen (*Listeria innocua* ATCC 33090) with diameters of inhibition slightly variables having values between 15.5 ± 0.30 to 11 ± 1mm.

**Characterization of the antimicrobial compounds**

The result in Table 4 showed that six selected *Leuconostoc* strains, exhibiting antagonistic activities against *L. innocua* (ATCC 33090), were characterized for their antimicrobial compounds like organic acids, hydrogen peroxide, carbon dioxide, phages, diacetyl and acetoin then low molecular mass antimicrobials known as bacteriocins. All strains exhibited inhibition zones for sample 1. They also displayed similar inhibition zones for samples 2 and 3. The inhibition zones for sample 3 and sample 3 were smaller than that displayed by sample 1 (Figure 1 and Table 4).

**Antimicrobials sensitivity to proteases, detergents, heat and pH**

The effects of various proteases on the inhibitory agent were investigated (Table 3). The antimicrobial compounds from all strains were completely inactivated by treatment with α-chymotrypsin and trypsin affected the activity of cell-free supernatants derived from strains Y44 and Y46. The antimicrobial activities expressed by the CFS (of these strains were sensitive to proteases, indicating that the active compounds have a proteinaceous nature, a general characteristic of bacteriocin. Such proteinaceous compounds that inhibit closely related bacteria can be included in the category of bacteriocins [17].

Heating for 30 min at 60, 70, 80, 90 and 100°C did not affect the antimicrobial activity of CFS since the residual activity was detected by the agar well diffusion assay (Table 4) but inactivated at 121°C for 15 min. The results suggest that all strains produced thermostable compounds.

Stability of antimicrobial activity at different pH values was evaluated after incubation of the CFS at 37°C for 2h at pH 2, 4, 6, 8, 10 and its readjustment at pH 6.5. All samples retained full antimicrobial activity against *L. innocua* (ATCC 33090) in the pH range (2 to 10). The antimicrobial compounds can be used in foods because they are stable at pH and temperatures chosen on the basis of their usual levels in foods and processing operations.

Detergents like tween-80 doubled the inhibitory activity of the CFS obtained from *Ln. mesenteroides* subsp mesenteroides Y44 and *Ln. mesenteroides* subsp dextranicum Y46 strains [18,19]. However, Urea reduced the activity of the CFS for both strains. Controls were done to verify the antimicrobial potential of each enzyme and other agents assayed during this work.

**Kinetik monitoring of pH evolution, acidity and kinetics growth**

The determination of lactic acid showed various rates (0.645 ± 0.06 to 1.455 ± 0.021 g/l) which indicate quite variety of acidification characters may have an inhibitory effect on the typical alteration flora by low pH.

Acidification kinetics of *Leuconostoc* isolates allowed us to conclude that although the performance of the antagonistic effect was positively influenced by incubation time. The results showed in the Figure 2 revealed that the amount of produced acid is modified according to the life stage of the bacteria cells that can be explained by a defect in the transport system fermentable compounds to the cell cytoplasm [20].

The result illustrated in Figure 3 was derived from the kinetics monitoring of pH of *Leuconostoc mesenteroides* strain Y44 in pure and in mixed culture indicate that the indicator pathogens strain *Listeria innocua* is less acidifying in pure culture in milk medium, observed by a drop in pH from 6.96 ± 0.17 to 5.08 ± 0.15 after 24 h. Moreover, the potential of mixed culture had more acid pH after 24h (3.97 ± 0.19).

Following the study of the growth kinetics of pathogenic indicator strains in pure and mixed culture with *Leuconostoc* Strains Y44 (Figure 4) showed that the cultures tested had relatively slow growth, promoted their own growth to control pathogens by inhibiting pathogenic growth [21]. The reductions in the listerial loads were approximately 4.56 log units for *L. innocua* (ATCC 33090) by the strains Y44; a smaller reduction is compared with the addition of test strains. This change brings us closer to the effectiveness of bacteriocins produced by *Leuconostoc* against *Listeria* species especially *Listeria innocua* [22].

**Discussions**

The antagonistic effect of LAB dairy strains on pathogenic microorganisms could be used for expanding the range of healthful...
foods. LAB, originally isolated from raw camel’s milk or artisanal dairy products, are probably the best candidate for improving the microbiological safety of these foods, because they are well adapted to the conditions of the substrate.

The present study was focused on the control of *L. innocua* as a target pathogen to be inhibited in food. *L. innocua* has been recognised as a foodborne pathogen with the ability to survive in raw foods with various environmental conditions, such as refrigeration, low pH, high salt concentrations and modified atmospheres. Several bacteriocins have been tested for their inhibitory activity against *L. innocua* (Table 3) and their potential to be used as biocontrol agents in food products. The results showed that the neutralized supernatants of some *Leuconostoc* strains isolated from camel’s milk possess inhibitory activity against *L. innocua* as shown in Table 3.

### Table 1: Physiological tests and fermentation profiling of bioactive *Leuconostoc* strains isolated from camel milk.

| Strains | Catalase | Growth at different T° | Growth with NaCl | Growth at different pH |
|---------|----------|------------------------|------------------|------------------------|
|         |          | T°4       | T°15     | T°30     | T°37     | T°45     | NaCl 3% | NaCl 6.5% | pH4.8 | pH6.8 | pH9.6 |
| Y01     | –        | –         | +        | +        | +        | +        | +       | +         | –     | –     | –     |
| Y02     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y03     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y04     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y05     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y06     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y07     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y08     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y09     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y35     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y37     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y38     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y39     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y40     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y41     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y42     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y43     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y44     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y45     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y46     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y47     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y48     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y49     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |
| Y50     | –        | –        | +         | +        | +        | +        | +       | +         | –     | –     | –     |

### Table 2: Carbohydrates fermentation profiling of *Leuconostoc* strains isolated from camel’s milk.

| Glu | Suc | Fru | Ara | Gal | Lac | Rha | Raf | Mal | Xyl | Cel | Manl | Sorl | Dul | Esc |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Y01 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | –   |
| Y02 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y03 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y04 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y05 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y06 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y07 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y08 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y09 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y35 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y37 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y38 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y39 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y40 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y41 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y42 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y43 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y44 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y45 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y46 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y47 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y48 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y49 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |
| Y50 | +   | +   | –   | +   | +   | –   | +   | ±   | ±   | –   | –   | –   | –   | ±   |

### Table 3: Inhibitory activity of neutralized supernatant of *Leuconostoc* strains isolated from camel’s milk.

| Test isolates | Clear zone diameter (mm) |
|---------------|--------------------------|
| *L. innocua* (ATCC 33090) | |
| Y39           | 12.9 ± 0.14              |
| Y40           | 12.85 ± 0.21             |
| Y41           | 13.0 ± 0.14              |
| Y44           | 14.4 ± 0.14              |
| Y46           | 15.5 ± 0.23              |
| Y47           | 10.9 ± 0.14              |
Table 4: Inhibitory activity of neutralized supernatant of Leuconostoc strains Y44, Y46 under heat, pH, proteases and detergents effect.

| Temperature | Strain Y44 | Strain Y46 |
|-------------|------------|------------|
| 60°C / 30 min | 14.4 ± 0.14 | 15.5 ± 0.23 |
| 70°C / 20 min | 14.4 ± 0.14 | 15.5 ± 0.23 |
| 80°C / 20 min | 14.4 ± 0.14 | 15.5 ± 0.23 |
| 100°C / 15 min | 10.8 ± 0.21 | 11.2 ± 0.18 |
| 121°C / 15 min | - | - |
| pH | - | - |
| α-chymotrypsine | - | - |
| Trypsine | - | - |
| Detergents | Urea 10.9 ± 0.17 | 11.9 ± 0.21 |
| | Tween 80 19.25 ± 0.23 | 20.37 ± 0.16 |

Figure 1: The inhibition spectra of Leuconostoc mesenteroides strains against indicator pathogens microorganisms Listeria innocua; Y39, Y40, Y41, Y44 and Y47: Leuconostoc mesenteroides isolated from camel’s milk.

A1: Inhibition of Listeria innocua (ATCC 33090) by Leuconostoc mesenteroides using a direct method.
A2: Inhibition of Listeria innocua (ATCC 33090) by Leuconostoc mesenteroides using a direct method with buffered medium.
A3: Inhibition of Listeria innocua (ATCC 33090) by Leuconostoc mesenteroides using a direct method with buffered medium treated by catalase.
B1: Antibacterial activity of Leuconostoc mesenteroides vs Listeria innocua (ATCC 33090) using an indirect method.
B2: Antibacterial activity of Leuconostoc mesenteroides Y44, Y46 vs Listeria innocua (ATCC 33090) using a buffered medium treated with Tween-80.
B3: Antibacterial activity of Leuconostoc mesenteroides Y44, Y46 vs Listeria innocua (ATCC 33090) using a buffered medium treated with α-chymotrypsin.

Figure 2: The acidity kinetics of Leuconostoc mesenteroides Y44 strain and indicator pathogens Listeria innocua (ATCC 33090) in pure and mixed cultures. Acidity (μmole) produced by LAB with a wide spectrum of activity against bacteria have been reported that are active against Listeria [23,24].

Twenty two Leuconostoc bioprotective strains obtained from raw camel’s milk were distinguished from other LAB strains by physiological and biochemical tests. Strains of L. mesenteroides from camel’s milk clustered together indicating high relationship among strains of this origin. From those, bacteriocinogenic strains Y44 and Y46 clustered separately because it presented a high antagonistic response against indicator pathogen strain L. innocua (ATCC 33090). A characterization of the antimicrobial activity of the supernatants from strains Y44 and Y46 showed similar action spectrum to that reported for Class IIa bacteriocins, especially with respect to the high anti-listerial activity and high resistance to pH and temperature. Our finding on bacteriocinogenic strains from camel’s milk is in agreement with other works reporting that most of the bacteriocins produced by Leuconostoc strains belong to the Class II bacteriocins, small heat-stable non-modified peptides, and active against Listeria.

The origin of the strains reported here broadens the spectra of habitats of Class IIa bacteriocins producing lactic acid bacteria. Previously to our work its presence was reported only in dairy and meat products [25,26].

Bacteriocins activity from these strains showed not only an effect against Listeria, but also against other pathogens likes Staphylococcus aureus which are found in food. This fact is very interesting, as the bioprotective strains would inhibit pathogenic competitors which may compromise the protection ability of the strain in the site action or cause spoilage reactions.

The evolution of pH in pure and mixed cultures can be observed in Figures 2 and 3. A significant pH decrease was observed in the mixed cultures after 72 h for the indicator pathogens strain L. innocua in pure cultures were less acidifying in milk medium as compared to the mixed cultures. Accordingly, significant pH decreases were determined.

The fickleness of kinetics of acidification can be revealed by several factors, the most important is the ability of isolates to possess proteolytic activity that is encoded by an extra-chromosomal material [27-31].

The kinetics of synthesis of antimicrobials compounds of Leuconostoc mesenteroides Y44 was in agreement with that reported...
the bioprotective effect depends on the relative concentrations of both skimmed milk medium. We have observed that the efficacy of innocua strains as bioprotective agents against Leuconostoc bacteriocinogenic strains are able to control, we have studied the needed to confirm the relationship among the presence of sugar and sugar contents to produce bacteriocins, this second exponential phase of incubation). As we know that bacteriocinogenic strains needed high produced during the late exponential phase of growth (after 9 hours has been observed at the late exponential phase of growth. Our results for preservation in the food industry. Furthermore, a genomic and proteomic characterization of the isolated bacteriocins.

**Conclusion**

In conclusion, the use of Leuconostoc strains as bio protective agents in food provided positive and encouraging results especially with anti-listerial activity, reinforced by the safe nature of this bacterial group. As shown, two strains were selected as bio protective cultures and it can be considered as safe (GRAS statute), although longer assays should be done to confirm that the application of these bio protective strains do not alter shelf life of the product. Moreover, two strains produced Class Ila bacteriocins with high anti-listerial activity. However, further research is needed to confirm the applicability of the potential bio protective strains under different conditions used for preservation in the food industry.

To determine the range of pathogen concentrations that the bacteriocinogenic strains are able to control, we have studied the efficacy of Leuconostoc strains as bioprotective agents against L. innocua skimmed milk medium. We have observed that the efficacy of the bioprotective effect depends on the relative concentrations of both the pathogen and the antagonist cells. In addition, it is of relevance capital to arrive at a truth which affirms the specificity of the bacteriocin called mesenterocin to the species Listeria sp. The technology that can be derived from the present study seems to be prospective [33-35].

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