

# Isolation and Identification of Homolactic Bacteria from *Solanum melongena* L. with Antibacterial Activity that Improve Vegetable Fermentation

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**Abstract**— The aim of this work was the isolation and identification of lactic acid bacteria with homolactic metabolism from eggplant; the lactic acid production and antibacterial activity were adopted as selected criteria to be included in the elaboration of fermented carrots as starter cultures. Between 50 isolated colonies, 14 were identified as lactic acid bacteria with homolactic metabolism, but only lactic acid bacteria identified phenotypic and genotypic as *Lactobacillus plantarum* were effective to produce cellular death and inhibit biofilm formation of five pathogenic bacteria. *L. plantarum* SB1 and SB2 were included in carrot fermentation on the basis of the best lactic acid production, antibacterial activity as well as the lowest C4 compounds and H<sub>2</sub>S formation. The scalded process was not enough effective to reduce Gram negative bacteria, but the addition of the selected bacteria isolated from eggplant to fermentation was effective to reduce all Gram negative population at 7 day. The big finding of this work was the isolation and identification of *L. plantarum* SB1 and SB2 from eggplant that could adapted in an different ecological niche and their addition to fermented carrots increase stability and microbiological safety of final product, preventing infectious diseases, with optimal sensorial attributed.

**Keywords**— Lactic acid bacteria, carrot fermentation, culture started, antibacterial activity, biofilm inhibition.

## I. INTRODUCTION

Fresh vegetables have a very short shelf-life since subjected to rapid microbial spoilage, among the various technological options; lactic acid fermentation may be considered as a simple and valuable biotechnology for maintaining and/or improving the safety, nutritional, sensory and shelf-life properties of vegetables [1,2]. Lactic acid bacteria (LAB) are mainly responsible for the fermentation of vegetable, spontaneous fermentation thus leads to variations of the sensory properties of the products [3]. It was shown that the use of a starter cultures helps to standardize the fermentation by controlling the microbial flora [4]. Although, there are very few cultures designed for vegetable fermentations.

Besides, numerous studies have been carried out on cabbage, olive and cucumber fermentation [5], but little is known about the lactic fermentation of carrots. There are no commercial starter cultures available for the inoculation of carrots and thus there is a need to evaluate starters for this purpose.

Two main options may be pursued for the controlled lactic acid fermentation of vegetables and fruits: the use of autochthonous or allochthonous starters [6,7]. Autochthonous starters means isolated from and re-used on the same raw matrix. Allochthonous starters means isolated from certain raw matrices but used to ferment various products. More than for other food matrices, this differentiation between starters is of fundamental importance for plant species, which represent completely different niches [8].

Eggplant (*Solanum melongena* L.) is an economically important common vegetable grown and consumed throughout the world. At the present, there are no reports in the scientific literature regards to lactic acid population in Argentinean eggplant, their metabolic activity, the antibacterial activity against pathogenic bacteria or the possible use of them as starter cultures of carrot fermentation to improve the final product.

The aim of this work was the isolation and identification of lactic acid bacteria with homolactic metabolism from eggplant produced in Argentina, with high lactic acid production and ability to inhibit the growth and biofilm formation of five pathogenic bacteria, *Escherichia coli* ATCC 35218, 25922 and 700, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212, to be included as starter cultures in the elaboration of fermented carrot.

## II. MATERIALS AND METHODS

### A. Isolation of LAB from eggplant

Eggplant fruits (*Solanum melongena* L.), purple type, provided by INTA (2012 harvest, Lules, Tucumán, Argentina) farmer, were used for this study.

All tree-harvested eggplants manually were placed in sterile bags and immediately transported to the laboratory under refrigeration, being analyzed on arrival. For isolation of LAB, skins of each eggplant were washed three times with sterile distilled water. Each water washing was collected under sterile conditions. From water washing samples, 0.1 ml aliquots (diluted if necessary) were placed on MRS agar (Oxoid Ltd., London, England), pH=6.0, supplemented with 1.0 % of cycloheximide (MRS-C) (Sigma Chemical Co., St. Louis, MO) to suppress yeasts growth. MRS-C plates were incubated anaerobically for 72 h at 30 °C. The isolates LAB were routinely propagated in MRS broth and incubated at 30 °C.

#### *B. Phenotypic characterization of LAB isolates at species level*

Selected isolates considered as LAB on the basis of their results for Gram staining, cell morphology, motility, spore formation and catalase reaction were tested for fermentative catabolism of glucose, gas and D- or L-lactic acid isomers production from glucose in Gibson medium [9] and by using an enzymatic method, Boehringer Kit (Mannheim, Germany), respectively. The glucose fermentation in MRS broth containing glucose 1 % (v/v), bromocresol purple (0.04 g/L) and inverted Durham tube was carried out. Ability of growth, under microaerophilic conditions in BBL GasPak jars in which the content of oxygen was reduced by use of a lighted candle, was determined on MRS agar plates incubated at 15, 30, 37 and 45 °C. The ability of growth at different NaCl concentrations (2, 4, 6 and 8 %, w/v) and at different pH (4.0, 5.0, 5.5 and 6.5) was also evaluated. The formation of ammonia from arginine was tested in medium that contained in g/L: L-arginine,3; triptone,4; yeast extract,4; glucose,1; peptone,6; tween 1; pH=6.5). Diacetyl, acetoin and 2,3-butanediol concentration was analyzed as a combined value according to the colorimetric method of Hill et al. [10] modified by Branen and Keenan [11].

#### *C. Antibacterial activity of LAB supernatants*

##### *1) Strain used and preparation of the inocula*

The bacteria used as test organism were *Escherichia coli* ATCC 35218 (American Type culture collection), *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 700, *Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 29212. Bacteria were grown aerobically at 30°C in brain heart infusion (BHI) broth (Britania, Argentina) medium, pH=7.0.

The bacteria were grown at 37 °C in nutrient broth and agar medium. pH=6.8. Before experimental use, cultures from solid medium were sub-cultured in liquid media, incubated for 24 h and used as the source of inocula for each experiment.

##### *2) Influence of LAB supernatants on the growth and viability of pathogenic bacteria.*

The liquid growth medium used in this experiment was BHI broth. Supernatants of each LAB were added individually to the medium (10 %). The media were inoculated 7 % with overnight culture of pathogenic bacteria. Bacterial growth was followed by incubation for 24 h at 37 °C in a tunable microplate reader (Versamax, Molecular Devices). The plates used were microtitre plate flat form. Bacterial growth measurement was determined indirectly by measuring absorbance at 540 nm by the microplate reader and directly by enumerating the number of viable cells by plating serial dilutions in the Muller-Hilton agar medium. The inhibitory effects of different supernatants on the bacteria were measured by comparing the control growth curves, without supernatant added.

##### *3) Influence of LAB supernatants on the biofilm formation*

All pathogenic bacteria were inoculated in BHI medium and incubated for 24 h, the overnight culture were used for the biofilm assay. The biofilm assay was performed as previously described by O'Toole and Kolter [12] with the following modifications. Briefly, 200 µL of the overnight culture and 50 µL of LAB supernatants were added to the wells of sterile flat bottom 96-well polystyrene microtiter plates and incubated for 24 h at 20 °C for biofilm formation. Then, the non-adherent cells were taking off and the wells washed twice with distilled water in order to remove all non adherent cells, and 200 µL of 0.01 % (w/v) Crystal Violet (CV), were added to the wells for 30 min in darkness. The stained biofilms were rinsed with distilled water and extracted with 200 µL of 96 % ethanol. The amount of biofilm was quantified by measuring the OD 595 nm of dissolved CV using the microplate reader. A control of biofilm formation of each bacterium was made and uninoculated medium controls were included.

##### *D. Lactic acid and H<sub>2</sub>S production by homofermentative bacteria with antibacterial activity*

The initial and final concentration of glucose in MRS media inoculated with selected homofermentative bacteria was determined by colorimetric assay and the production of lactic acid bacteria was determined by using an enzymatic method, Boehringer Kit (Mannheim, Germany).

The ability to produce was determined by a qualitative method described by Giudici and Kunkee [13]. Selected bacteria was inoculated in media supplemented with different concentrations of cystein (0.0, 0.5 and 1.0 g/L), at the top was a filter paper soaked with a lead acetate solution (5 %).

#### *E. Genotypic identification of homolactic LAB*

Pure cultures were grown and the DNA was extracted as described Reguant and Bordons [14]. *Lactobacillus plantarum*, *Lactobacillus pentosus* and *Lactobacillus paraplantarum* strains were further distinguished by means of partial amplification product comparison of the *recA* gene, according to the method of Torriani et al. [15]. Preparation of bacteria for this PCR reaction involved growing of a bacterial colony on MRS agar plates at 30 °C until colonies could be clearly distinguished. The 20 µL PCR reaction mix consisted of 0.25 µM of each *paraF*, *pentF*, and *pREV* primers and 0.12 µM of *plan F* primer, 0.2 mM of each dNTP (Amersham Pharmacia Biotech, Inc., Piscataway, NJ, USA), 1.5 mM of MgCl<sub>2</sub>, 5 µL of 10X reaction buffer, 0.5 U of Taq DNA polymerase (Amersham Pharmacia Biotech, Inc.) and a single colony. The profile of amplification was: one cycle of 94 °C for 3 min, 30 cycles of 94 °C for 30 s, 56 °C for 10 s and 72 °C for 30 s. A final extension of 72 °C for 10 min was also included. The amplification products were visualized by ethidium bromide (5 µg/mL) staining after gel electrophoresis. A Biometra TRIO-Thermoblock machine was used for all PCR reactions.

#### *F. Experimental design for carrot inoculation and fermentation*

##### *1) Preparation of selected LAB inocula for fermentation*

Isolated strains with high lactic acid production and antibacterial activity were selected to be used as starter culture of carrot fermentation. Selected bacteria were cultivated in MRS media, pH=5.5 during 20 h at 30 °C. Cultures in logarithmic phase of growth were centrifuged at 10 000 g, washed twice in saline (0.85 % w/v NaCl), and finally resuspended in 200 mL sterile saline solution. The inoculums were adjusted to obtain an initial inoculation of 10<sup>5</sup> CFU/mL in fermentation assay.

##### *2) Carrot preparation for fermentation*

The assay was conducted with whole peeled carrot, washed in sterile distilled water, and cut into 1 mm thick pieces of equal size. The samples were separated into two groups.

The first was not subjected to any treatment, and the second under a scalding process, which consisted of heating at 80 °C for 10 min, then scalded samples were placed in sterile bags and sealing immersed in sterile distilled water at 20 °C for cooling. 30 grams of untreated and scalded carrot were weighed and placed them in sterile containers containing 70 mL of sterile saline in a concentration used commercially (2.5 % NaCl). One series served as control and was not scalded or inoculated with LAB, allowing a spontaneous fermentation (SF). The second series served as scalded control, it was scalded but not inoculated with LAB, allowing a spontaneous fermentation in scalded carrot (SSF). The third series, scalded carrot were inoculated with selected cultures of *Lact. plantarum* SB1 or *Lact. plantarum* SB2 (FSB1 and FSB2).

##### *3) Analytical and Microbiological determinations during fermentation*

Samples were taken at 0, 7, 14 and 21 days for determine viable count in MRS and Mc Conkey media, pH, lactic acid and phenolic compounds concentrations. The fermentation was initiated at 20 °C for 21 days. The LAB viability was carried out by enumerating the number of viable cells by plating serial dilutions in MRS agar medium (Oxoid Ltd., London, England) (pH 5.5) incubated anaerobically for 72 h at 30 °C. The enterobacteria viability was determine by plating dilutions in Mc Conkey agar (Britania, Argentina) incubated for 24 h at 37 °C. The pH value of each sample was measured with a microprocessor pHmeter (HANNA Instruments, Milan, Italy). D- or L-lactic acid isomers production during fermentation was determine using an enzymatic method, Boehringer Kit (Mannheim, Germany).

##### *G. Sensory analysis*

The sensory analysis of fermented vegetables was determined by using the descriptive model of acceptability, odour, appearance and fragrance were the sensory attributes considered by using a scale 0–5. A non-trained panel consisting of 10 judges was used. Results were classified as preferable (total points: 13.9–20), barely acceptable (total points: 11–12.9), needs modification (total points: 8–10.9) and not acceptable (total points: 0–7.9).

##### *H. Statistical analysis*

All determinations are the means of three independent experiments carried out in duplicate. One-way analysis of variance was applied to experimental data.

Variable means showing differences with statistical significance were compared using Tukey's test. Comparisons between viability data were made using Student's t test.

### III. RESULTS

#### A. Isolation and phenotypic characterization

A total of 50 colonies from eggplants that exhibited distinct morphological differences in color, shape and size were randomly picked up from MRS-C agar plates and incubated at 30 °C for 48 h. Cultures that exhibited gram positive and catalase negative reactions were considered as LAB and selected for phenotypic characterization. They were also characterized for cell morphology using a phase contrast microscope (Olympus CX41, Japan). Bacterial cultures were maintained at -20 °C in MRS medium and glycerol (20 %, v/v). Working cultures were prepared from the frozen cultures by two consecutive transfers in MRS broth at 30 °C, pH=6.0.

From 50 colonies randomly picked up from MRS-C agar plates, a total of 34 strains were assigned as presumed LAB group because they were Gram positive, catalase negative, non-spore forming, non-motile bacteria, the ability of growth at different NaCl concentration and pH.

The morphological and biochemical characteristics of selected strains were determined as shown in Table 1. Isolates were divided in 4 groups according to their morphological and physiological features: Group I: Consisted in seven rod homofermentative bacteria. Group II: Consisted in twelve rod heterofermentative bacteria. Group III: seven coccus homofermentative. Group IV: Consisted of eight coccus heterofermentative bacteria.

**Table 1.**  
Group of lactic acid bacteria isolates from eggplant on the basis of their physiological and biochemical characteristics.

| Group                           | I<br>(N=7) | II<br>(N=12) | III<br>(N=7) | IV<br>(N=8) |
|---------------------------------|------------|--------------|--------------|-------------|
| Morphology                      | Rod        | Rod          | Coccus       | Coccus      |
| Gram                            | +          | +            | +            | +           |
| Catalase                        | -          | -            | -            | -           |
| Metabolism                      | Homof.     | Heterof.     | Homof.       | Heterof.    |
| Glucose fermentation            | +          | +            | +            | +           |
| Gas from glucose                | -          | +            | -            | +           |
| Growth at Salt (NaCl):          |            |              |              |             |
| 2%                              | +          | +            | +            | +           |
| 4%                              | +          | +            | +            | +           |
| 6%                              | +          | +            | +            | +           |
| 8%                              | +          | +            | +            | +           |
| Growth at pH:                   |            |              |              |             |
| 4,0                             | +          | +            | +            | +           |
| 5,0                             | +          | +            | +            | +           |
| 5,5                             | +          | +            | +            | +           |
| 6,5                             | +          | +            | +            | +           |
| Ammonio formation from arginine | -          | -            | -            | -           |

(+): positive, (-): negative. All strains were non-motile and non-spore forming. The data were determined in three independent experiments.

### B. Antibacterial effect of LAB supernatants

The antibacterial activity was carried with homofermentative lactic acid bacteria isolates from eggplant (Group I and III). Lactic acid bacteria of group III did not show antibacterial activity against *E. coli* ATCC 25922, *E. coli* 700, *E. coli* 35218, *Enterococcus faecalis* 29212 and *Staphylococcus aureus* 25923, whereas LAB of group I showed antibacterial activity.

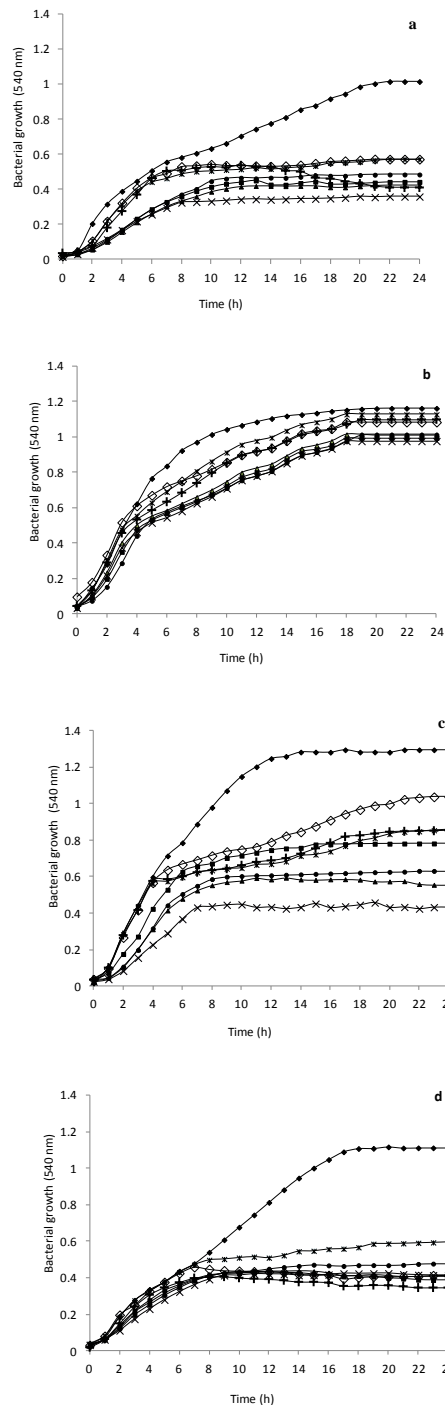
Figure 1 (a) shows that all LAB supernatants decreased the final cell density of *E. coli* ATCC 25922 between 44 % and 65 %, the maximal growth rate in 59 %, 26 %, 56 %, 54 %, 59 %, 21 % and 58 % and viable counts by 2.23, 2.71, 2.5, 3.72, 2.58, 2.7 and 4.49 log cycle by JB1, JB2, SB1, SB2, SB5, SB6 and SB12 supernatant, respectively, compared to control (Fig. 2).

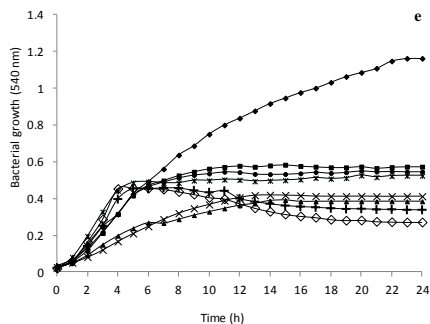
The growth inhibition of *E. coli* 700 was the lowest (Figure 1b), the final cell density and the growth rate was slight decreased by the addition of all LAB supernatant. The addition of JB1, JB2, SB1, SB2, SB5, SB6 and SB12 supernatants at medium decreased the number of viable cells by 1.37, 0.93, 2.51, 0.96, 0.92, 1.47 and 0.37 log cycle with respect to control, respectively (Fig. 2).

Figure 1 (c) shows that final cell of *E. coli* 35218 decreased 34 %, 20 %, 40 %, 57 %, 67 % and 51 %, the growth rate diminished 5 %, 6 %, 32 %, 45 %, 61 % and 41 % with the addition of JB1, JB2, SB1, SB2, SB5 and SB12, respectively. SB6 supernatant produced a 34 % of inhibition of final biomass with an increase of 5 % in the growth rate. The addition of JB1, JB2, SB1, SB2, SB5, SB6 and SB12 supernatants reduced the viable cells of *E. coli* 35218 in control medium, by 0.85, 0.39, 1.5, 2.2, 2.32, 1.32 and 1.8 log cycles, respectively (Fig. 2).

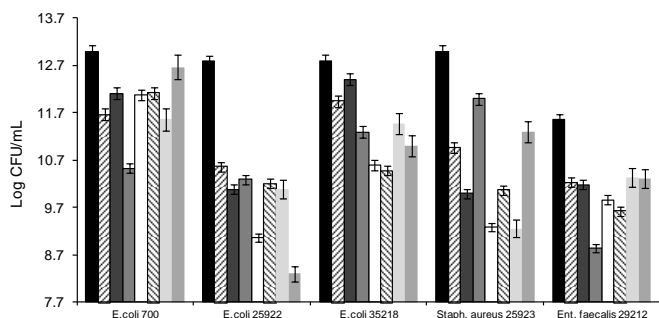
*Enterococcus faecalis* 29212 growth was high inhibited by all LAB supernatants (Figure 1d), the final cell density was reduced around 46% and 69% and the growth rate between 20 % and 44%. JB1, JB2, SB1, SB2, SB5, SB6 and SB12 supernatants produce the diminution in viable cells by 1.33, 1.38, 2.71, 1.7, 1.93, 1.22 and 1.25, respectively (Fig. 2).

Figure 1 (e) shows that the addition of LAB supernatants produce a strong inhibition on the final biomass (around 51 % and 71 %) of *Staphylococcus aureus* 25923. The number of viable cells was reduced 2.03, 2.71, 2.5, 3.75, 2.58, 2.7 and 4.49 with JB1, JB2, SB1, SB2, SB5, SB6 and SB12 supernatants, compared to control, respectively (Fig. 2).





**Figure 1:** Curve Growth of *Escherichia coli* ATCC 25922 (a), *Escherichia coli* ATCC 700 (b), *Escherichia coli* ATCC 35218 (c), *Enterococcus faecalis* ATCC 29212 (d) and *Staphylococcus aureus* ATCC 25923 (e) in Mueller-Hilton broth media supplemented with supernatants of LAB isolated from eggplants: JB1 (+), JB2 (◇), SB1 (■), SB2 (▲), SB5 (x), SB6 (Ж) and SB12 (●). The control media was made for each bacterium without supernatant added (◆).



**Figure 2:** Final log CFU/mL achieved at 24 h incubation of five bacteria in the media supplemented with supernatants of LAB isolated from eggplants. (■) Control, (▨) JB1, (□) JB2, (▤) SB1, (▥) SB2, (▧) SB5, (▩) SB6, (▪) SB12.

All LAB supernatants produce the inhibition of biofilm formation by the five bacteria selected (Table 2). The highest inhibition was observed against *E. coli* 35218, whereas the lowest inhibition was found against *Enterococcus faecalis* and *E. coli* 700. JB1 supernatant was the most effective to inhibit the biofilm formation of *St. aureus* and *E. coli* 35218, but produce a low biofilm inhibition against *E. faecalis*. JB2 supernatant was the most effective to reduce the biofilm the formation of *E. coli* 25922 and 700, while SB12 supernatant was the best inhibitory against *E. faecalis*.

**Table 2.**  
**Inhibition of biofilm formation of pathogenic bacteria by supernatants of lactic acid bacteria isolated from eggplant.**

|              | <i>Escherichia coli</i> 25922 | <i>Escherichia coli</i> 700 | <i>Escherichia coli</i> 35218 | <i>Enterococcus faecalis</i> 29212 | <i>Staphylococcus aureus</i> 25923 |
|--------------|-------------------------------|-----------------------------|-------------------------------|------------------------------------|------------------------------------|
| <b>JB 1</b>  | +++                           | ++                          | ++++                          | +                                  | +++                                |
| <b>JB 2</b>  | ++++                          | +++                         | ++++                          | ++                                 | +++                                |
| <b>SB 1</b>  | +++                           | ++                          | +++                           | +                                  | +++                                |
| <b>SB 2</b>  | +++                           | +++                         | +++                           | ++                                 | ++                                 |
| <b>SB 5</b>  | +++                           | +++                         | ++++                          | ++                                 | +++                                |
| <b>SB 6</b>  | +++                           | ++                          | ++++                          | +                                  | +++                                |
| <b>SB 12</b> | +++                           | +++                         | ++++                          | +++                                | ++                                 |

(-) no inhibition, (+) low biofilm inhibition (between 1-25 %), (++) moderate biofilm inhibition (between 25-50 %), (+++) high biofilm inhibition (between 50-80 %) or (++++) strong biofilm inhibition (between 80-100 %).

### C. Genotypic identification of LAB isolates

We selected the group I for genotypic identification and for further studies. The multiplex PCR assay of the seven selected bacteria (group I), using species specific primers for *Lactobacillus plantarum*, *Lactobacillus paraplantarum* and *Lactobacillus pentosus* gave a single product of 318 bp, the expected size of the PCR fragment for *Lactobacillus plantarum*, only when one colony from *Lactobacillus plantarum* isolates and the reference strain were used as target for specific PCR reaction. This fact confirmed the morphological, biochemical and physiological characterization of *Lactobacillus plantarum* isolates.

### D. Lactic acid and H<sub>2</sub>S by selected homofermentative LAB with antibacterial activity

In the selected group, SB1, SB2, SB5 and SB6 strains produce D and L lactate isomers, but SB12, JB1 and JB2 produce only D lactate isomer (Table 3).

The production of H<sub>2</sub>S by LAB, in media supplemented with different concentration of cystein was observed in table 4. Only a low production of H<sub>2</sub>S was observed with the inoculation of SB5 and JB2 strain, in media with 1.0 mg/L of cystein. The concentration of compounds of C4 by all LAB of group I was lower than 1.5 mg/L, SB1 and SB2 strains produce the lowest concentration (0.70 mg/L), whereas SB12 and JB2 produce the highest concentration (1.4 mg/L).

The selection was made on the basis of the future application of these lactic acid bacteria with homofermentative metabolism as started culture of fermentation of vegetables.

**Table 3.**  
**Glucose consumption and lactic acid production by isolated LAB of Group I, in MRS media.**

| Strains     | Glu residual (mmol/L) |                                 | L.A. (D) <sup>a</sup> (mmol/L)  | L.A. (L) <sup>a</sup> (mmol/L)  | Y      | Q    |
|-------------|-----------------------|---------------------------------|---------------------------------|---------------------------------|--------|------|
|             | T <sub>0</sub>        | T <sub>final</sub> <sup>a</sup> | T <sub>final</sub> <sup>a</sup> | T <sub>final</sub> <sup>a</sup> | LA/glu | LA/h |
| <b>SB1</b>  | 55.6±2.78             | 5.10±0.25                       | 61.00±3.05                      | 47.5±2.37                       | 2.10   | 4.52 |
| <b>SB2</b>  | 55.6 ±2.78            | 4.00±0.19                       | 50.40±2.52                      | 46.91±2.35                      | 1.89   | 4.05 |
| <b>SB5</b>  | 55.6±2.78             | 4.20±0.21                       | 52.22±2.61                      | 58.00±2.9                       | 2.14   | 4.59 |
| <b>SB6</b>  | 55.6±2.78             | 4.30±0.21                       | 50.40±2.5                       | 25.90±1.3                       | 1.49   | 3.18 |
| <b>SB12</b> | 55.6±2.78             | 4.71±0.23                       | 40.33±2.01                      | 30.40±1.9                       | 1.39   | 2.95 |
| <b>JB1</b>  | 55.6±2.78             | 10.04±0.5                       | 47.67±2.38                      | 19.70±1.7                       | 1.48   | 2.81 |
| <b>JB2</b>  | 55.6±2.78             | 7.82±0.39                       | 48.78±2.43                      | 25.90±1.3                       | 1.18   | 2.36 |

Glu: Glucose  
L.A.: Lactic acid  
Y= Yield – mmol of L.A. produced by mmol of glucose consumed.  
Q= Productivity of L.A. per hour.  
<sup>a</sup>D or L lactic acid isomers were not detected at initial time.  
<sup>a</sup>Values determine at the end of exponential growth phase (24 h).

**Table 4.**  
**Production of H<sub>2</sub>S by LAB of Group I**  
**H<sub>2</sub>S production: High (+++), moderate (++), low (+) o negative (-).**

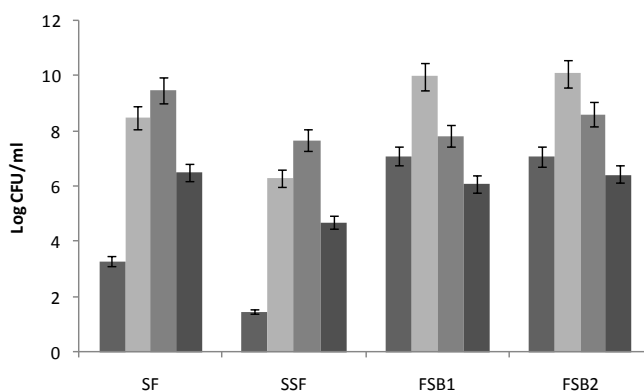
| Production of H <sub>2</sub> S in media supplemented with cystein |     |     |     |     |     |     |      |
|---|-----|-----|-----|-----|-----|-----|------|
| g of cystein / L  | JB1 | JB2 | SB1 | SB2 | SB5 | SB6 | SB12 |
| 0   | -   | -   | -   | -   | -   | -   | -    |
| 0.5   | -   | -   | -   | -   | -   | -   | -    |
| 1.0   | -   | +   | -   | -   | +   | -   | -    |

### E. Carrot fermentation

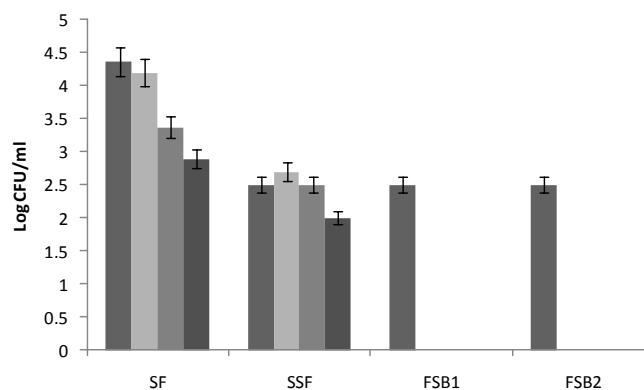
SB1 and SB2 strains were selected to be used as starter cultures in carrot fermentation, for the highest lactic acid production, the lowest production of compounds of C4 and for not produce H<sub>2</sub>S in media supplemented with cystein, and at last for the high reduction of the viable cells of pathogenic bacteria.

In SF the number of microorganisms that growth in MRS medium increases approximately 6 log cycles during 7 days, then a reduction of 3 log cycles was observed at 21 days (Figure 3). In this condition, the growth in the selective media Mc Conkey, an initial population of 4.36 log cycle was observed, which decreased 2 log cycle at 21 days (Figure 4). The blanching process reduce 1.83 and 2.0 log cycle the bacterial population determined by enumeration in MRS and Mc Conkey media during SSF, respectively. The viability of bacteria in MRS media increased at 14 days and then the population decreased by 3.0 log cycles at 21 days.

In FSB1 and FSB2, the initial population of gram positive rod and catalase negative bacteria, increased 5.13 and 5.80 log cycles with respect to SSF, respectively. The population increased approximately 3.0 log cycles at 7 days, and then a reduction of 4.27 and 4.06 log cycles was observed at 21 d in FSB1 and FSB2, respectively (Figure 3). Both in the FSB1 and FSB2, the population of enterobacteria, plating in the selective Mc Conkey media was not detected at 7, 14 and 21 days (Figure 4).



**Figure 3: Viability of lactic acid bacteria (Log CFU/ml) plating in MRS media incubated in microaerophilic conditions, during fermentation in different conditions: SF, SSF, FSB1 and FSB2, at 0 d (■), 7 d (□), 14 d (□) and 21 d (■).**



**Figure 4: Viability of aerobic bacteria (Log CFU/ml) plating in Mc Conkey media during fermentation in different conditions: SF, SSF, FSB1 and FSB2, at 0 d (■), 7 d (□), 14 d (□) and 21 d (■).**

Table 3 showed the final lactic acid concentration and pH variation during carrot fermentation in different conditions. During all fermentation process, the pH decreased, in general the initial pH decreased from 6.83 to final values between 3.3 and 3.9.

The production of D and L lactic acid reached a 27.44 and 11.37 mmol/L in SF and SSF, respectively. There was observed that in SSF, L- lactic acid isomer was detected in a lower concentration than in SF. In FSB1 and FSB2, the lactic acid production increase respect to SSF, with high values of L-lactic acid. The FSB1 showed the highest values of lactic acid concentration.

**Table 5.**  
**Lactic acid production and Ph variation during carrot fermentation in different conditions.**

|             | pH        |           | Final LA (mmol/L) |              |
|-------------|-----------|-----------|-------------------|--------------|
|             | To        | T 21d     | D-LA              | L-LA         |
| <b>SF</b>   | 6.83±0.27 | 3.33±0.15 | 17.22 ± 2.06      | 10.22± 0.92  |
| <b>SSF</b>  | 6.83±0.21 | 3.98±0.18 | 11.33 ± 1.36      | 0.04 ± 0.001 |
| <b>FSB1</b> | 6.82±0.25 | 3.38±0.15 | 14.33 ± 1.59      | 14.22 ± 0.59 |
| <b>FSB2</b> | 6.83±0.27 | 3.40±0.13 | 9.33 ± 0.33       | 4.03 ± 0.13  |

LA: Lactic acid

At the end of storage, fermented carrot was subjected to sensory analysis (Table 6). All the sensory attributes were scored the highest for carrot fermented with the addition of *Lactobacillus plantarum* SB1.

**Table 6.**  
**Sensory assay during carrot fermentation in different conditions.**

|      | Acceptability | Appearance | Odour | Fragrance |
|------|---------------|------------|-------|-----------|
| SF   | BA            | BA         | BA    | BA        |
| SSF  | BA            | BA         | BA    | BA        |
| FSB1 | P             | P          | P     | P         |
| FSB2 | P             | BA         | BA    | P         |

P: preferable; BA: Barely acceptable; NM: Needs modification and NA: not acceptable.

#### IV. DISCUSSION

The eggplants used in this study, characteristic of this region of the world, were traditionally cultivated and consumed in Argentina from centuries.

In the present study, seven homofermentative LAB were isolated, identified and evaluated for their lactic acid production and the ability to inhibit the growth and biofilm formation of five pathogenic bacteria.

In this investigation, seven *Lactobacillus plantarum* (SB1, SB2, SB5, SB6, SB12, JB1 and JB2) were isolated and identified by biochemistry characterization and their identification was confirmed with PCR assays. All *L. plantarum* isolated consumed glucose and produce both isomers of lactic acid, the highest productivity and yield of lactic acid was found with *L. plantarum* SB1, SB2 and SB5. The production of compounds of C4, such as diacetyl, acetoin and 2,3 butilenglicol by the seven isolated bacteria was low and the lowest values were found with *L. plantarum* SB1 and SB2, this fact is beneficial by sensorial characteristics of final product. *L. plantarum* SB1, SB2, SB6, SB12 and JB1 did not produce H<sub>2</sub>S in media added with two concentrations of cystein, thus, these strains could be suitable for use for vegetable fermentation because the production of H<sub>2</sub>S is undesirable.

In this work, the seven LAB supernatants were effective to decrease the number of viable cells and biofilm formation of the five pathogenic bacteria, such as *E. coli*, *S. aureus* and *Enterococcus faecalis* that have been involved in outbreaks of food-borne disease and adhere and form biofilms on numerous surfaces. With respect to the number of viable cells, the highest reduction of *E. coli* 700 and *Enterococcus faecalis* were observed with *L. plantarum* SB1 supernatants, whereas *L. plantarum* SB12, SB5, and SB6, were the most effective against *E. coli* 25922, *E. coli* 35218 and *Staphylococcus aureus*, respectively. All LAB supernatant produce inhibition on the biofilm formation, the highest inhibition was observed against *E. coli* 35218 and 25922. The lowest inhibition was observed against *Enterococcus faecalis*, with the exception of SB12 supernatant that produce a high inhibition. Our results showed that the seven LAB isolated from eggplants are capable to inhibited the growth and biofilm formation of pathogenic bacteria, in different degree.

So, LAB with homolactic metabolism and antibacterial capacity could be a good strategy to be probe as starter cultures in the fermentation of carrot, which could be provide an additional benefit to control the growth of undesirable microorganisms. Our results are in agreement with those reported by Ammor et al. [16], who observed that certain LAB may repress the growth of some undesirable microorganisms in biofilms and thus may be used as barrier flora against the settlement of these undesirable microorganisms on the processing surfaces equipment in meat small-scale facilities.



Guerrieri et al. [17] reported that *L. plantarum* possesses antilisterial activity in biofilms.

*L. plantarum* SB1 and SB2 were selected to be included in carrot fermentation, on the basis of the best lactic acid production, the lowest formation of C4 compounds and H<sub>2</sub>S, and the best antibacterial activity. Scalded treatment, demonstrated that the LAB that produce D isomer of lactic acid was more resistant than LAB that produce L-lactic acid. The lactic acid production was directly related with pH decreased. The scalded process was not enough effective to reduce all Gram negative bacteria, but carrot fermentation with the addition of two selected bacteria isolated from eggplant was effective to reduce all Gram negative bacteria at 7 days. Sajur et al. [18] demonstrated that lactic acid bacteria species from tomatoes surface produce organic acids that contributed to the diminution in the natural microflora cells number. Steinkraus [19] demonstrated that lactic acid bacteria inhibited the aerobic mesophilic organisms growth that might destroy crispness in the cabbages or cucumbers and that this inhibition could be related with metabolic products, such as lactic and acetic acids, produced by the inoculated microorganism during storage. On the other hand, Vescoso et al. [20] reported that inhibitory effect of LAB on microflora associated with ready to use vegetables was due to bacteriocin production.

#### V. CONCLUSIONS

The big finding of this work was the isolation and identification of *Lactobacillus plantarum* SB1 and SB2 from eggplant that could adapt in different ecological niche, such as carrot fermentation. The addition of selected LAB increase stability and microbiological safety of fermented carrot, preventing infectious diseases, with optimal sensorial attributed.

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