Evaluation of technological properties of *Leuconostoc mesonteroides* (V1) strain isolated from Algerian goat’s milk

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**ABSTRACT**

This study was to evaluate probiotic characteristics by in vitro tests of *Leuconostoc mesonteroides* strain (V1) isolated from Algerian goat’s milk collected from El Bayadh which is located in the west of Algeria. This strain V1 were identified by phenotypic and biochemical methods, were tested for their antimicrobial activity against *Staphylococcus aureus*, *Listeria innocua*, *Listeria ivanovii* and was evaluated for certain properties relevant to probiotic including: acid pH, bile salts, pepsin resistance, hemolytic activity and antibiotics sensitivity. The results revealed the strain V1 showed antagonistic activity against *Staphylococcus aureus*, *Listeria innocua* and *Listeria ivanovii*, due to a production of proteinous nature substances. The strain were resistant to pH 3 and 4, bile salts at 0.5%, 1% and 2% and pepsin at pH 3. Was γ-hemolytic and was susceptible to four antibiotics: Chloramphenicol, pristinamycin, Clindamycin and Licomycin. *Leuconostoc mesonteroides* strain V1 showed desirable properties as potential probiotic strains, which can be considered the strain V1 as suitable probiotic candidate.

**KEYWORDS:** *In vitro*, goat’s milk, probiotic, *Leuconostoc*

**INTRODUCTION**

Goat’s milk has a compositional most identical to that of cow’s milk. It provides the same type of protein (80% casein, 19% soluble proteins and 1% enzyme), vitamins and minerals. In contrast, the composition of their fat is different, a particularity that makes the milk of goat more digestible [1].

It contains four times more oligosaccharides than cow’s milk (about 23 mg / 100g against 6mg / 100g). These sugars act as prebiotics and promote the development and the growth of the probiotic flora which eliminate the pathogenic bacterial flora.

Goat milk is alive as any milk, even if it comes from a milking done in normal conditions of cleanliness and hygiene it contains many germs which its rapid development is ensured by the temperature at the exit of the udder (35 ° C) as well as its richness in water and carbohydrates [2].

Currently dairy industries are indeed very concerned by the use of lactic acid bacteria as process agents they have essential roles to acidify the milk and the curdled, to participate in the formation of taste (proteolysis, flavor production) texture and the opening of dairy products (cheese, butter, yogurt, fermented milk) and reducing alterations micro flora and pathogenic flora.
Among the lactic acid bacteria used in the dairy industries, we may mention the genus *Leuconostoc* isolated from goat milk for several reasons such as the ability to metabolize more carboxylic acids fumarate, gluconate, malate, pyruvate and oxoglutarate [3] and their production of dextran, a product used as an additive food as well as increasing the viscosity and as a stabilizer through strengthening the rigidity of the casein network "fermented milks, creams, dessert made of milk and flavored milk” [4-8]. As the citrate metabolism and the sugars by *Leuconostoc* involved in aromatization of fermented dairies. In addition; the production of acid acetic, diacetyl and CO₂ inhibits the growth of pathogenic bacteria in food [9].

Lactic acid bacteria have also been used in probiotic food, as they produce bacteriocins that inhibit harmful bacteria [10]. However, the probiotics have not been shown to inhibit harmful or spoilage fungi, necessitating the use of PCR and ELISA methods for identification of fungal contaminants [11]. Probiotics have recently been investigated in regards to health effects besides preventing harmful bacterial growth in the gut. Probiotics have been shown to stimulate the immune system through activating immune cells [12]. These bacteria have also been shown to lower total cholesterol levels and LDL cholesterol levels in diabetes patients [13] as well as lower bile acid levels [14].

The antimicrobial effect due to bacteriocins produced by *Leuconostoc* sp. was observed for the first time in the 90’s [15]. Later, different bacteriocins were producing from *Leuconostoc mesenteroides* subsp. *mesenteroides* FR52 class II such as Mesenterocin52A and Mesenterocin52B [16] also MesénertéicineY105 and MésénértéicineB105 from *Leuconostoc mesenteroides* subsp. *mesenteroides* Y105 Class IIa [17], the determination of the peptide sequences of these bacteriocins shows that they exhibit antimicrobial activity against pathogenic bacteria including in particular *Listeria* sp.[18].

**MATERIAL AND METHODS**

1. Bacterial strains and culture conditions:

*Leuconostoc mesenteroides* (V1) strain were isolated from raw goat milk (milk is collected aseptically goat race Arbia, her wool is colored "black and gray" and 11 months age), from the west of Algeria (El kheiter is a town in the wilaya of El Bayadh, which is located 370 km southeast of Oran, 520 km southwest of Algiers and 500 km northeast of Bechar).

The indicator strains of *Staphylococcus aureus* ATCC 43300 were collected from Oran hospital and the following strains: *Listeria ivanovii* ATCC 19119 and *Listeria innocua* ATCC 33090 were collected from CECT Spain. They were stored as frozen stocks at -20 ° C. Before use, the indicator strains were transplanted into the brain heart infusion broth (BHI) at 37 ° C.

2. Isolation of *Leuconostoc* strain:

Isolation of *Leuconostoc* was carried out on MRS solid medium at pH 6.8, supplemented with vancomycin (30µg / ml) [19], were incubated at 30 ° C for 48h. Among the isolated colonies, only those Gram-positive and Catalase-negative were retained [20].

3. Identification of strain:

Identification of *Leuconostoc* strain was carried out according to [21]; Based on the following criteria: CO2 production, arginine hydrolysis on M16BCP medium, growth at different temperatures (4°C, 15°C, 37°C, and 45°C), growth at different pH (4.8 and 6.5), and growth at different NaCl concentrations (3% and 6.5%). Dextran production on MSE medium [22], citric acid degradation on Kempler and McKay solid medium, esculin hydrolysis and production of acetoin on Clark and Lubs medium [23,24]. Carbohydrate fermentation was tested on MRS supplemented with bromocresol purple as a pH indicator using the following sugars to differentiate the subspecies of *Leuconostoc*: Arabinose, Glucose, galactose, lactose, fructose, sucrose, sorbitol, mannitol, maltose Rhamnose, Raffinose, xylose [25, 26].

2.4. Technological characteristics of strain:

2.4.1. Antimicrobial activity:

The search for possible production inhibiting substance against the strain *Leuconostoc mesenteroides* indicator bacteria (*Staphylococcus aureus* ATCC 43300, *Listeria innocua* ATCC 33090, *Listeria ivanovii* ATCC 19119) was carried out by the direct method according to[27]. An 18h culture strain was spotted onto the surface of MRS agar and incubated at 37 °C for 24 hours. After incubation, the indicator bacteria was stricted in superposition of 8ml Mueller-Hinton semisolid medium then incubated for 24h to 37°C. The inhibition was considered positive when the width of the inhibition halos was clear ≥0.5 cm.

2.4.2. Detection of protein nature antimicrobial substance:

The detection was performed by an indirect method. From 18h culture of *Leuconostoc mesenteroides* strain, 1ml was centrifuged at 8000 rpm for 10 minutes. Then 100 µl of the supernatant was incubated on wells formed
on solid MRS medium that were seeded by indicator strains, and incubated at 37 °C for 24h. The colonies surrounded by a clear zone with a diameter greater than 2 mm are considered positive. Several factors were eliminated to confirm the proteinous nature of the inhibitory substance, such as lactic acid using a buffered medium and was incubated at 30 °C for different organic (1 %)olytic enzyme pepsin to a final concentration of 1 mg/ml.

To identify the proteinaceous nature of the inhibitory substance, heat treatments of the supernatant at different temperatures (60°C, 80°C, and 100°C), different pH (1 or 9) and effect of the solvant organic (1 % Tween 80, 1 % Urée) were carried out.

2. Acidity and Growth Kinetics in Pure and Mixed Cultures:

To study the growth kinetics of L. mesenteroides (V1)in pure and mixed cultures with the alterations and / or pathogens strains (Staphylococcus aureus ATCC 43300, Listeria innocua ATCC 33090 and Listeria ivanovii ATCC 19119). Strain V1 was selected and inoculated by streaking on solid MRS medium and incubated at 30°C for 18 h. After incubation, a colony was inoculated in to MRS liquid and was incubated at 30°C for 18 hours. Then, 100 μL of the 18 h culture was inoculated into 10 ml of skimmed milk containing 0.3% yeast extract and was incubated at 30°C for 18h [28].

The bacterial population measurement with the indicator strains in pure and mixed cultures was performed by counting in the selective medium for each strain (MRSV for Leuconostoc at 30 °C; BHI for both strains of Listeria at 37 °C and Baird Parker for Staphylococcus aureus at 37° C.

Strain V1 was most efficient the strain in the production of antimicrobial substances, and the four indicator strains (Staphylococcus aureus ATCC 43300, Listeria innocua ATCC 33090 and Listeria ivanovii ATCC 19119), were routinely sub cultured in 10 ml of skim milk with 0.3% yeast extract.

The four strains were inoculated separately into 100 ml of skim milk for monitoring pure cultures, and the mixed culture was prepared by mixing a culture of the indicator strains with the strain V1. The cultures were divided into tubes, and incubated at 30°C for 24 h. Every three hours, the samples were aseptic call removed from the tubes to determine the pH, titratable acidity, and the growth rate [29].

2.4.4. Probiotic effect of Leuconostoc strain:

2.4.4.1. The pH tolerance:

Bacterial cells grown in MRS broth at 30 °C overnight were harvested by centrifugation and were washed twice with phosphate buffer saline (PBS) of pH 7 and resuspended in PBS of pH 2, 3 and 4. The viable counts were enumerated on MRS agar after incubation at 30 °C for 0 and 3h.

2.4.4.2. Test of resistance and Hydrolysis bile salt:

Bacterial cultures were grown at 30 °C overnight and the bacterial cells were harvested by centrifugation, washed twice with PBS of pH 8 before being resuspended in PBS (pH8) solution containing 0.3% bile salts. The viable counts were enumerated on MRS agar after incubation at 30 °C for 0 and 4h.

The hydrolysis bile salt test is based on the determination of bile hydrolase enzyme that catalyzes the hydrolysis of bile salt. On Petri dishes containing modified MRS prepared with 0.5% bile salt, 0.1 ml the culture of Leuconostoc strain was inoculated on the surface and incubated at 30 °C for 48h.

2.4.4.3. Pepsin resistance:

Bacterial cells grown in MRS broth at 37 °C overnight were recovered by centrifugation, were washed with sterile PBS (pH 8) and resuspended in PBS of pH 2 and 3 supplemented with 3 mg / ml of pepsin respectively. Strain resistance was determined by counting the MRS agar initial viable cells after 3 hours of incubation at 37°C.

2.4.4.4. Hemolytic activity:

Hemolytic character was investigated by streaking the culture of Leuconostoc strain on Columbia agar containing 5% human blood. After incubation for 24 hours, leuconostoc may be α hemolytic (green color around the colonies); β-hemolytic (clarification around the colonies) or γ hemolytic (the medium is not changed)

2.4.4.5. Antibiotic sensitivity of Leuconostoc strain:

L. mesenteroides strains were cultured in MRS broth at 30°C for 18h and were then adjusted to a 0.5 McFarland scale and smeared homogeneously on MRS culture plate. Antibiotic discs were placed on the plates and incubated for 24 h at 37°C. The antibiotics included Ampicillin 10 μg, Streptomycin 10 μg, Erythromycin 15μg, Kanamycin 30μg, Chloramphenicol 30μg, Pristinamycin15μg, Tobramycin 10 μg, Clindamycin 2μg, Spiramycin 100μg, Amikacin 30μg, Licomycin 2μg, Cefotoxin 30μg, Naldixic acid 30μg, Gentamicin 10 μg.
The inhibitory circles emerging after 24 h of incubation were measured. Activity was assessed as sensitive (≤21 mm), intermediate (16–20 mm), and resistant (≥15 mm), as previously described by [30].

RESULTS AND DISCUSSION

1.1. Isolation and identification:
Our isolate were Gram-positive, catalase negative, able to produce gas from glucose (heterofermentative), unable to degrade arginine (Table 1). This isolate were considered as the genus of Leuconostoc [31-34]. The strain V1 were able to grow at 15 and 37 °C but not at 4 and 45 °C, which confirms that she were mesophilic bacteria, no able to resist at 63.5 °C for 30 min, but to resist at 55 °C for 15 min, our results seem to those found by [35, 36]. Were able to stand a concentration of 3% of NaCl; but not grow at 6.5% NaCl and were able to grow at pH 6.5 and not at pH 4.8, this results agree with [37]. This strain V1 were able to hydrolyze sucrose to produce dextran, was able to hydrolyze esculin catalyzed by a β-glucosidase through the presence of esculine which degrades it to glucose and esculinite [24, 32]. The strain V1 have a positive character to the use of citrate, appear form blue colonies on medium KMK, reflecting the presence of citratase enzyme and the ability to use this precursor aromatic compound which is taken as an important criterion in the selection of species for technological interest, this was already demonstrated by [24, 38,39]. The strain V1 were unable to produce acetoin from glucose, which corresponds to the characters of the two subspecies Leuconostoc mesenteroides subsp mesenteroides and Leuconostoc mesenteroides subsp dextranicum [32, 34]. According to the fermentation profile (Table 2), isolate strain V1 belong to the species Leuconostoc mesenteroides subsp. mesenteroides which arabinose sugar is the key of distinction between the subspecies of Leuconostoc mesenteroides, according to the work done by [32, 40].

3.2. Technological characteristics of strain:
3.2.1. Antimicrobial activity of Leuconostoc mesenteroides:
Our strain exhibits inhibitory activity against all tested indicator strains: Staphylococcus aureus ATCC 43300, Listeria innocua ATCC 33090 and Listeria ivanovii ATCC 19119. (Figure 1) show the different zones of inhibition. This antimicrobial activity is described by the synthesis of molecules such as organic acids, hydrogen peroxide, carbon dioxide, diacetyl and bacteriocins [41].

In order to ensure that the inhibiting substance is proteinaceous product, and in order to eliminate the lactic acid and hydrogen peroxides effect; Culture were treated with the proteolytic enzyme (pepsin) which led to the disappearance of the inhibition zones, This result indicate that the inhibition is due to a protein nature substance (Date not show)

The production of bacteriocins by Leuconostoc mesenteroides strain already was confirmed by [42-45].

3.2.2. Kinetic Monitoring of pH Evolution and Acidity:
The strain V1 that followed indicate a producing of organic acid (lactic and acetic acid), which pacing 65°D (Figure 3 and 4) and decrease of pH from 7.00 to 4.88 after 24h of incubation (Figure 2).

The indicator strains so decrease of pH was observed, in pure culture pH decrease from 7.00 to 5.79 for Listeria innocua, from 7.00 to 5.88 for Listeria ivanovii and from 7.00 to 6.2 for Staphylococcus aureus. However in mixt culture observed decrease from 7.00 to 5.41 for Listeria innocua, from 7.00 to 5.12 for Listeria ivanovii and from 7.00 to 5.23 for Staphylococcus aureus (Figure 2).

3.2.3. The Growth Kinetics of Strains in pure cultures and mixed cultures with Leuconostoc Strain:
The growth of indicator strains in the presence of strain V1 was observed (Figure 5).
The growth of Listeria innocua ATCC33090 after 24 hours of culture which the decrease was of the order of 34.61%, Listeria ivanovii ATCC 19119 of which the decrease was of the order of 24% and Staphylococcus aureus ATCC 43300 of which the decrease was of the order of 20%. This decreases may be explain by presence combined with the production of acidity and/or production of antimicrobial substances similar results was measured by [46, 47].

3.2.4. Probiotic effect of Leuconostoc strain:
3.2.4.1. The pH resistance:
Ph tolerance of our results indicates that Leuconostoc mesenteroides strain viable at pH 3 and 4 but no viability has been observed at pH 2 (Table3). This result are in agreement with by [45]. The L. mesenteroides strain Z8 isolated from Algerian camel milk was not viable at pH 2, but marked an increases of 7.49% and 2.43% at pH3 and 4, respectively and according to other authors for other probiotic strains, [48] The strains of Pediococcus acidilactici (P2), Lactobacillus curvatus (RM10), and P. pentosaceus (FF) showed tolerance to acid (pH 3.0). [49] The strain of Entérocooccus faecium isolated from non fermented animal foods, although no
viable cells were detected at pH 2, the reduction ranges after 3h of exposure to pH3. The survival of *Leuconostoc mesenteroides* strain V1 at pH 3 and 4 indicated that it could transit through the stomach.

3.2.4.2. Hydrolysis resistance and bile salt:

The results of tolerance to bile salt are shown in (Table 4). It appears that our strain (V1) presented variable sensitivity to various bile salt concentrations with slight reduction in viability after 4 h. No activities of bile salt hydrolysis were observed. This result reported by other authors for the selection of probiotic [49]. Exposure to different concentrations of oxgall (0.5%, 1% and 2%) affected the viability of strain *E. faecium* LHICA 28.4 isolated from non fermented animal foods and none of the *E. faecium* strains under study hydrolysed oxgall. ; [45] *L. mesenteroides* strain (Z8) was able to hydrolyse bile salt and were able to grow in the presence of 0.5%, 1%, and 2% oxgall.

3.2.4.3. Resistance to pepsin:

The results are illustrated in (Table 5). No effect and no viability were observed after 3 hours at added 3 mg/ml of the pepsin at pH 2. However a small decrease in growth of the strain at Ph 3 with the added 3 mg/ml of pepsin. Our results indicate a resistance to pepsin at pH 3. This result was reported by [45], the strain *L. mesenteroides* was able to survive at pH 2 when 3mg/ml of pepsin was added. However, a remarkable resistance was observed at pH 3 when 3mg/ml of pepsin was added, also [49], reported that no viability had been observed for any of the strains after exposure to pH 2, the addition of pepsin at this pH value had no detectable effect. In contrast, all four *E. faecium* strains survived well when exposed to pepsin at pH 3.

### Table 1:

<table>
<thead>
<tr>
<th>Isolates</th>
<th>V1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase</td>
<td>-</td>
</tr>
<tr>
<td>Gram</td>
<td>+</td>
</tr>
<tr>
<td>ADH</td>
<td>-</td>
</tr>
<tr>
<td>CO₂ Production</td>
<td>+</td>
</tr>
<tr>
<td>4°C</td>
<td>-</td>
</tr>
<tr>
<td>15°C</td>
<td>+</td>
</tr>
<tr>
<td>37°C</td>
<td>+</td>
</tr>
<tr>
<td>45°C</td>
<td>+</td>
</tr>
<tr>
<td>63.5/30 min</td>
<td>-</td>
</tr>
<tr>
<td>55/15 min</td>
<td>+</td>
</tr>
<tr>
<td>pH 4.8</td>
<td>-</td>
</tr>
<tr>
<td>pH 6.5</td>
<td>+</td>
</tr>
<tr>
<td>3% NaCl</td>
<td>+</td>
</tr>
<tr>
<td>6.5% NaCl</td>
<td>-</td>
</tr>
<tr>
<td>Dextran</td>
<td>+</td>
</tr>
<tr>
<td>Hydrolysis of esculin</td>
<td>+</td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>+</td>
</tr>
<tr>
<td>HydrolysateProduction</td>
<td>-</td>
</tr>
</tbody>
</table>

*: positive reaction, -: negative reaction.

### Table 2:

<table>
<thead>
<tr>
<th>Carbohydrates</th>
<th>V1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabinose</td>
<td>+</td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
</tr>
<tr>
<td>Galactose</td>
<td>+</td>
</tr>
<tr>
<td>Lactose</td>
<td>+</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
</tr>
<tr>
<td>Saccharose</td>
<td>+</td>
</tr>
<tr>
<td>Sorbitol</td>
<td>-</td>
</tr>
<tr>
<td>Mannitol</td>
<td>-</td>
</tr>
<tr>
<td>Maltose</td>
<td>+</td>
</tr>
<tr>
<td>Rhamnose</td>
<td>-</td>
</tr>
<tr>
<td>Raffinose</td>
<td>-</td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
</tr>
</tbody>
</table>

V1: *Leuconostoc mesenteroides subsp mesenteroides*

### Table 3:

<table>
<thead>
<tr>
<th>pH2</th>
<th>pH3</th>
<th>pH4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
These results are expressed as log CFU.

Table 4: Effect of different concentrations of bile salts on the *Leuconostoc* strain.

<table>
<thead>
<tr>
<th></th>
<th>0.5 %</th>
<th>1 %</th>
<th>2 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0h</td>
<td>4h</td>
<td>0h</td>
<td>4h</td>
</tr>
<tr>
<td>0h</td>
<td>4h</td>
<td>0h</td>
<td>4h</td>
</tr>
<tr>
<td>0h</td>
<td>4h</td>
<td>0h</td>
<td>4h</td>
</tr>
<tr>
<td>0h</td>
<td>4h</td>
<td>0h</td>
<td>4h</td>
</tr>
</tbody>
</table>

These results are expressed as log CFU.

Table 5: Effect of pepsin in the presence of different pH on the viability of the strain *Leuconostoc*.

<table>
<thead>
<tr>
<th>Pepsin (pH2)</th>
<th>0h</th>
<th>3h</th>
<th>Pepsin (pH3)</th>
<th>0h</th>
<th>3h</th>
</tr>
</thead>
<tbody>
<tr>
<td>0h</td>
<td>4h</td>
<td>0h</td>
<td>4h</td>
<td>0h</td>
<td>4h</td>
</tr>
<tr>
<td>0h</td>
<td>4h</td>
<td>0h</td>
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<td>0h</td>
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<td>0h</td>
<td>4h</td>
<td>0h</td>
<td>4h</td>
<td>0h</td>
<td>4h</td>
</tr>
</tbody>
</table>

These results are expressed as log CFU.

Table 6: Antibiotic sensitivity test.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Symbol</th>
<th>µg/ disc</th>
<th>Clear zone diameter (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ampicillin</td>
<td>AM</td>
<td>10</td>
<td>08</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>S</td>
<td>10</td>
<td>07</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>E</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>K</td>
<td>30</td>
<td>06</td>
</tr>
<tr>
<td>Chloramphénicol</td>
<td>C</td>
<td>30</td>
<td>22</td>
</tr>
<tr>
<td>Pristinamycin</td>
<td>PT</td>
<td>15</td>
<td>21</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>TOB</td>
<td>10</td>
<td>09</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>DA</td>
<td>2</td>
<td>23</td>
</tr>
<tr>
<td>Spiramycin</td>
<td>SP</td>
<td>100</td>
<td>17</td>
</tr>
<tr>
<td>Amikacin</td>
<td>AK</td>
<td>30</td>
<td>12</td>
</tr>
<tr>
<td>Licomycin</td>
<td>L</td>
<td>2</td>
<td>25</td>
</tr>
<tr>
<td>Cefotaxim</td>
<td>CTX</td>
<td>30</td>
<td>00</td>
</tr>
<tr>
<td>Nalidixique acid</td>
<td>NA</td>
<td>30</td>
<td>00</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>CN</td>
<td>10</td>
<td>08</td>
</tr>
</tbody>
</table>

Fig. 1: The inhibition spectra of *Leuconostoc mesenteroides* strains against indicator microorganisms.
(a1) Inhibition of *Staphylococcus aureus* ATCC 43300 by *Leuconostoc mesenteroides* using a direct method.
(a2) Inhibition of *Listeria innocua* ATCC 33090 by *Leuconostoc mesenteroides* using a direct method.
(a3) Inhibition of *Listeria ivanovii* ATCC 19119 by *Leuconostoc mesenteroides* using a direct method.
(b1) Antibacterial activity of *Leuconostoc mesenteroides* versus *S. aureus* ATCC 43300 using a buffered medium.
(b2) Antibacterial activity of *Leuconostoc mesenteroides* versus *L. innocua* ATCC 33090 using a buffered medium.
(b3) Antibacterial activity of *Leuconostoc* versus *Listeria ivanovii* ATCC 19119 using a buffered medium.

3.2.4.4. Hemolytic activity:

The *Leuconostoc mesenteroides* strain was incapable to hydrolysis human blood on the Columbia medium. So the strain is non-hemolytic bacteria (γ-hemolytic). This character has been confirmed by [45, 50, 51].

3.2.4.5. Antibiotic resistance:

The *Leuconostoc mesenteroides* strain resisted 9 antibiotics: Ampicillin, Kanamycin, Erythromycin, Streptomycin, Tobramycin, Amikacin, Cefatoxin, Gentamicin and Nalidixic acid. However, the profile of sensitivity was observed for :Chloramphenicol, Pristinamycin, Clindamycin, Licomycin and Spiramycine as an intermediary. Our results are consistent with those obtained in other studies author for *Leuconostoc mésenteroides* strain [45, 52].
The results of this study showed that the strain *Leuconostoc mésonteroides* V1 can be a good potential probiotic candidate. Therefore, it seems that strain V1 has a potential probiotic needed in foods system for développement of foods production.

(A)  

(B)  

(C)  

**Fig. 2:** Kinetics of evolution of the pH:

(A): In pure culture strains *V1, Listeria innocua* ATCC 33090 and in mixed culture strains *V1 + Listeria innocua* ATCC 33090.

(B): In pure culture strains *V1, Staphylococcus aureus* ATCC 43300and in mixed culturestrains *V1 + Staphylococcus aureus* ATCC 43300.

(C): In pure culturestrains *V1, Listeria ivanovii* ATCC 19119 and in mixed culturestrains *V1 + Listeria ivanovii* ATCC 19119.
Fig. 3: Kinetics of evolution of the Dornic acidity in pure culture
Kinetics of evolution of the Dornic acidity in pure culture for strains: *Leuconostoc mesenteroides* (V1) *Staphylococcus aureus* ATCC 43300 (Stap), *Listeria innocua* ATCC 33090 (LI) and *Listeria ivanovii* ATCC 19119 (LV).

Fig. 4: Kinetics of evolution of the Dornic acidity in mixed culture
Fig. 5: Growth rate in pure culture and in mixed culture

(A): In pure culture strains V1, *Listeria innocua* ATCC 33090 and in mixed culture strains V1+ *Listeria innocua* ATCC 33090.

(B): In pure culture strains V1, *Listeria ivanovii* ATCC 19119 and in mixed culture strains V1+ *Listeria ivanovii* ATCC 19119.

(C): In pure culture strains V1, *Staphylococcus aureus* ATCC 43300 and in mixed culture strains V1+ *Staphylococcus aureus* ATCC 43300.
Fig. 6: Results of Antibiotic resistance.

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