Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2017, 9 [6]:243-252
[http://scholarsresearchlibrary.com/archive.html]

Scholars Research Library
ISSN 0975-5071
USA CODEN: DPLEB4

Characterization, Identification of the producing bacteriocin lactic bacteria isolated from Algerian Human Breast Milk

KHODJA B[1], BENALI M[1], LARBI DAOUADJI K[1], REFFAS FZI[1], GHANEM M[1], BENAL M[1], ABBOUNI B[1]

[1]*Laboratory for Molecular Microbiology Proteomics and Health, Department of Biology,*
[2]*Laboratory for Biotoxicology, Faculty of Natural Sciences and Life, University Djillali Liabès of Sidi Bel Abbès,, Algeria.*

****Corresponding Author:** Abbouni B, Laboratory for Molecular Microbiology Proteomics and Health, Department of Biology, Faculty of Natural Sciences and Life, University Djillali Liabès of Sidi Bel Abbès, 22000, Algeria. Email: abbounibouziane@yahoo.de*

ABSTRACT

Lactic bacteria form a important group for human health due to their production of some antimicrobial substances, which are capable to inhibit the proliferation of wide rang pathogenic bacteria. Recently, the used isolated, selected lactic acid bacterial strains from diverse ecosystems in food bio-preservation was considerably augmented the duration of bio-preservation.

The main aim of the present work was the isolation and the screening of large range of lactic acid bacteria from Algerian human milk and the characterization of the molecules responsible for antimicrobial activity. The primary screening indicated the isolation 13 lactic acid bacterial strains from Algerian human breast milk, where the identification has been achieved by the study of phenotypical, physiological and biochemical characteristics. On others hand, the evaluation of the antagonistic activity of the isolated, selected lactic acid bacteria against some pathogenic bacteria strains such as Staphylococcus aureus UT 602, S. aureus CECT 86T, S. aureus ATCC 25923, S. aureus ATCC 43300, E. coli ATCC 25922 has been achieved by the using agar well diffusion method and the study of the E. coli ATCC 25922 bacterial growth in the absence and in the presence of the supernatant of the cultivated selected lactic acid bacteria (LbC3). Furthermore, the identification of the bacteriocin producing selected lactic acid bacteria (LbC3) by the using of 16S rRNA gene sequence analysis indicated their belonging to the genus Lactobacillus brevis.

The obtained results indicated that the highest amount of bacteriocin was yielded at 30°C and pH-value of 6. Hence, the study of bacterial growth of E. coli ATCC 25922 in the absence and in the presence of the supernatant L. brevis (LbC3) indicated a considerable biomass reduction accompanied with unbalanced growth after adding of the supernatant. The isolated, selected antagonistic L. brevis has manifested high level of resistance toward to vancomycin, tobramycine, nalidixique acid, nitroxolin and oxacilin and sensible against penicillin, pristinamycin, chloramphenicol.

Key Words: *Antagonistic activity, Lactic bacteria, 16S rRNA gene, identification, human breast milk.*

INTRODUCTION

Human breast milk is undoubtedly the best food during the first weeks or the first months of the infant's life, acts as a transport medium for many essential substances from mother to newborn. Additionally, the breast milk remains special its richness by several bacterial groups predominated by *Staphylococcus*, *Streptococcus*, *Micrococcus*, *Lactobacillus*, *Enterococcus*,

The lactic acid bacteria present in breast milk and feces of the newborn consist a rich and important ecological niche for the isolation and the screening of lactic strains [1].

Lactic acid bacteria (LAB) comprise a wide range of genera and include a considerable number of species. These bacteria are the component of the starters used in fermentation, especially for dairy products, and some of them are also natural components of the gastrointestinal microflora. *Lactobacillus* is one of the most important genera of LAB [2], comprise à large and diverse group of gram positive, non-spore forming, catalase negative rod bacteria, able to produce lactic acid as the mean end-product of the fermentation of carbohydrates [3].

While the beneficial effect of some microorganisms such as the lactic acid bacteria by the digestion of nutritional substances, prevent the proliferation of pathogenic microorganisms due to certain inhibitory substances, which they secret during digestion.

These inhibitory substances include metabolites like organic acids, diacetyl, hydrogenperoxide, acetoin, 2,3-butanediol, acetaldehyde, benzoate, bacteriolytic enzymes, bacteriocin, reuterin, etc. display antagonistic activity towards many pathogenic microorganisms [4]. Few works have been focused to selected LAB strains from Algerian human breast milk with characteristics bio preservative related to safety and probiotic properties. The main aim of the present work was the isolation and the screening of large range of lactic acid bacteria from Algerian human milk and the characterization of the molecules responsible for antimicrobial activity.

MATERIAL AND METHODS

Samples

The human breast milk samples were voluntarily collected by mothers in good health from the region of North-West of Algeria (Sidi Bel Abbès, Oran, Tlemcen), with a total of 24 women.

Where, the human breast milk samples were collected in the period from the first day and the last day of the month in a sterile tube, by the using of the sterile gloves and the nipples were cleaned with sterile water and soap, wiped with 70 % ethanol. The first drops were discarded and the samples were transported immediately under refrigeration at 4°C until the delivery to the laboratory for further analysis.

Isolation of Lactic Bacteria

A volume of 1 ml of each breast milk sample was inoculated into a volume of 9 ml Man Rogosa and sharpe (MRS) culture medium. The prepared serial dilution were plated into MRS solid culture medium, supplemented with 0.25 % (w/v) L cysteine, incubated under anaerobic conditions at temperature 37°C for 48 hours [5]. The isolated lactic bacteria strains were stored in skimmed milk in the presence of 30% (v/v) glycerol at -20°C. The cultivated lactic bacteria were kept on MRS solid culture medium or on slant at 4°C and streaked every 4 weeks [6, 7].

Phenotypic characterization of isolates

The phenotypic identification of the isolated, selected producing bacteriocin lactic bacteria was carried out in two main steps. The first step was focused on the determination of the Gram coloration and the catalase reaction, where the second step was essentially based on the macroscopic and microscopic observation [8], followed by further assay such as gaz production from glucose, containing in MRS culture medium, explored by the introduction of the bell Durham, bacterial growth at different temperature (15, 45°C) and pH value in the range (5.4-6.5), and the salt tolerance to a concentration between (3,5-6,5%).

Additionally, further biochemical identification was carried out by the using API 50 CH system (BioMerieux, France), according to the manufacturer's instructions and the test of hemolytic activity.

Antimicrobial activity Assay

The antagonistic activity of the isolated, selected producing bacteriocin lactic baceria against some pathogenic bacteria such as *Staphylococcus aureus* UT 602, *S. aureus* CECT 86T, *S. aureus* ATCC 25923, *S. aureus* ATCC 43300 and *E. coli* ATCC 25922 was assayed by the using of dual agar overlay method. For this purpose, the isolated, selected producing bacteriocin lactic baceria was inoculated in spot on MRS solid culture medium plates, incubated at temperature of 30°C for 24 hours, overlaid with a volume of 5 ml of MH soft agar, which was seeded with a volume of 0,1 ml culture of pathogenic bacteria and further incubated under aerobic condition at 30°C for 24 hours and the obtained diameter of inhibition zone was measured [9].

Well diffusion assay

The antagonistic activity of the isolated, selected producing bacteriocin lactic bacteria against all investigated pathogenic bacteria was determined by well diffusion method according to Barefoot *et al*. [10]. For this assay, the isolated, selected producing bacteriocin lactic bacteria cultivated on (MRS) culture medium, incubated at temperature of 30°C for 18 hours, where
the supernatant was harvested by centrifugation at 15000 g for 10 minutes and stored at 4°C and the pathogenic bacteria such as *Staphylococcus aureus* UT 602, *S. aureus* CECT 86T, *S. aureus* ATCC 25923, *S. aureus* ATCC 43300 and *E. coli* ATCC 25922 was inoculated on the MRS solid culture medium. Furthermore, a volume of 100 µl of each harvested supernatant of the of the isolated, selected producing bacteriocin lactic bacteria was introduced in well. After that, plates were kept at 4°C for 2 hours and then incubated at 37°C for 24 hours. The antagonistic activity was determined by measuring the diameter of the formed diameter of the zone of inhibition around the wells.

Characterization of the produced bacteriocin

Effect of pH value

In order to explore the effect of the pH value on the antagonistic activity of the harvested supernatant of the isolated, selected producing bacteriocin *Lactobacillus brevis* (LbC3) against *E. coli* ATCC 25922, the inoculated MRS liquid culture medium with the isolated, selected producing bacteriocin *Lactobacillus brevis* (LbC3) was adjusted at different initial pH levels (4.5, 5, 5.5, 6, 6.5, 7 and 7,5), incubated without agitation, at temperature 30°C for 24 hours and the antagonistic activity was assayed by measuring the formed diameter of the zone of inhibition around the wells.

Effect of temperature

In order to investigate the effect of temperature on the antagonistic activity of the harvested supernatant of the isolated, selected producing bacteriocin *Lactobacillus brevis* (LbC3) against *E. coli* ATCC 25922, the inoculated MRS liquid culture medium with the isolated, selected producing bacteriocin *Lactobacillus brevis* (LbC3) was incubated at different temperature (25, 30, 35, 40, 45°C) for 24 hours and the antagonistic activity was assayed by measuring the formed diameter of the zone of inhibition around the wells.

Bacterial growth *of E. coli* ATCC 25922 by the adding of the produced bacteriocins

The exploration of the mode of action of the produced bacteriocins by *Lactobacillus brevis* (LbC3) against *E. coli* ATCC 25922 has been investigated by the study of the viability of the treated cells, where the bacterial growth of *E. coli* ATCC 25922 in the absence (control) and in the presence of the produced bacteriocins by *Lactobacillus brevis* (LbC3) in the supernatant
has been assayed. For this purpose, *E. coli* ATCC25922 was inoculated in the MRS culture medium with initial optical density of 0.5 at 600 nm according the protocol described by Abbouni and coworkers [11, 12]. A volume of 1ml of the recovered supernatant of *L. brevis* (LbC3) was added 6 hours after the bacterial growth. The bacterial growth was followed by the measurement of the optical density at wave length at 600 nm. A volume of 1 ml of the harvested produced bacteriocins by *Lactobacillus brevis* (LbC3) was added 6 hours after the onset of the bacterial growth.

Display of crude bacteriocin production

In order to explore the production of crude bacteriocin by the isolated, selected producing bacteriocin strains with a highest antagonistic activity was inoculated overnight in the MRS culture medium, incubated at 37°C for 24 hours and harvested by centrifugation at 5000 g for 10 minutes at 4°C. The recuperated supernatant of the isolated, selected producing bacteriocin strains was investigated for the study of the thermo-stability of the produced bacteriocin, which was tested by heating at 100°C for 0, 15, 20, 30 min respectively. After the treatment, the samples were rapidly cooled and the bacteriocin activity was assayed according to the described method by Labioui and co-workers [17].

Display of antibiotic resistance

The antibiotic resistance was displayed by the using of the disk diffusion method, modified according to the described method by Charteris *et al*. [14], where the isolated, selected producing bacteriocin bacterial strains were inoculated in the MRS liquid culture medium, incubated at temperature of 37°C for 24 hours. After that the concentration of the inoculums was standardized to 0.5 McFarland standard, which was equivalent to the bacterial density of 10^8 UFC/ml. Furthermore, a volume of 5 ml of the maintained MRS semi solid culture medium at temperature of 45°C was inoculated with a volume of 1.25 ml of the standardized inoculums and a further 8 ml of the inoculated MRS culture medium was poured on top of 15 ml MRS Petri plates. The followed antibiotic discs were used: tobramycine (TOB 10 µg), penicilin (P6 µg/ 10 IU), erythromycin (E 15 µg), nitroxolin (NI 5 µg), vancomycin (VA 30 µg), chloramphenicol (C 3µg), pristinamycin (PT 15µg), oxacillin (OX 5µg), spiramycin (SP100 µg), nalidixique acid (NA 30 µg) were placed and pressed on top of the agar plate, incubated at temperature of 37°C for 24 hours and the obtained diameter of the inhibition zone was measured and compared with standard given by the Clinical and Laboratory Standards Institute (CLSI) for antimicrobial susceptibility testing (Clinical and Laboratory Standards Institute 2012) [15].

Amplification of 16S rRNA and sequencing

The gene-encoding 16S rRNA of the isolated, selected producing bacteriocin Lactic bacteria was amplified with the polymerase chain reaction (PCR) by the using universal primers fD1 (5´-AGAGTTTGATCCTGGCTCAG-3´) and rD1(5´AAGGAGGTGATCCAGCC-3´).The used amplification conditions were the initial denaturation step for 3 min at 94°C, followed by 35 cycles of 94°C for 45 sec (denaturation), annealing at 53°C for 1 min and final elongation at 72°C for 2 min [16]. The amplified polymerase chain reaction (PCR) products (5 µl) were separated by electrophoresis in 1,2 % agarose gels stained with ethidium bromide, sequenced, analyzed by the using of basic local alignment search tool (BLAST) at National Center for Biotechnology Information, USA (NCBI) database and the closest match of known phylogenetic affiliation was used to assign the isolated strains to specific taxonomic groups identified based on the similarity score.

RESULTS AND DISCUSSION

Isolation and identification of Lactic bacteria

13 lactic bacteria were isolated from 24 human breast milk Algerian, by the dilution of a volume of 10 ml samples of milk in 90 ml saline solution, and the suitable serial dilutions were placed on the MRS Agar (Fluka, France), incubated at 30°C for 48 hours. The typical colonies were purified and identified according to physiological and biochemical characteristics, where the all the isolates were appeared a Gram positive, cocci or rod shaped, catalase negative, non motile and non- hemolytic. Furthermore, the use of differentiation criteria indicated the membership of the 13 isolated bacterial strains to lactic acid bacteria. Moreover, they are divided into 3 groups: *L. plantarum* was the most frequently isolated strain (30.76%), followed by *L. brevis* (15.38 %).

Generally, the obtained results in the present work were similar to the observation reported by Ozgun *et al* [5] and Abrahamsson *et al* [17], with a minor percentage the *Enterococcus faecium* (7.69%). Albesharat and co-workers [18] has reported that the breast milk has constituted a excellent source of lactic acid bacteria and appeared endogenous origin and devoided from breast skin. Furthermore, a little is known about the bacterial composition of human colostrums [19], although few studies like that of Martín *et al*. [20], reported that human breast milk provided a rich source of commensal lactic acid bacteria (LAB) to the infant during breast feeding and stimulates abundant growth and colonization of these bacteria at mucosal surfaces in the infant

gastrointestinal tract. The hypothesis was the vertical transfer of intestinal LAB from the mother's gut to her milk and through the milk to the infant's gut [18], is confirmed by the above results.

Antibacterial activity

The obtained results showed that 69.23 % of the tested, isolated producing bacteriocin *Lactobacillus brevis* has manifested the absence of the antibacterial activity, while 30,76% of bacterial strains have revealed the a excellent activity against some pathogenic bacteria. Recently, Jara and Saris and co-workers have presented a similar results [1, 21].

The spectra of inhibition varied considerably within the species such as *L brevis*, *L plantarum*, *E faecium*, where *L brevis*, *E faecium* (LbC3, LbL15) have manifested an excellent antagonistic activity against the investigated pathogenic bacteria (*Staphylococcus aureus* UT 602, *S. aureus* CECT 86T, *S. aureus* ATCC 25923, *S. aureus* ATCC 43300, *E. coli* ATCC 25922). However, *L. plantarum* (LbL4) showed a excellent antibacterial activity against (*S. aureus* CECT 86T) (Figure 1). Ibhanesebhor and co-worker [22] have reported the presence of antibacterial activity of human colostrums against *S. aureus* and coliform organisms, where has been explained by the presence of the high immunoglobulin's (Igs) in the colostrums [23]. Moreover, Also,Abd El-Salam and Al Rubayyi have suggested that the breast milk excreted a important bactericidal substances against such pathogenic bacteria (*E. coli* and *S. aureus)* [24].

Characterization of the inhibitory agent

The antagonistic activity of the isolated *Lactobacillus brevis* can induced by the different parameters such as the production of bacteriocins the production of organic acids, hydrogen peroxide, phages and/or bacteriocins [22]. In order to characterize the nature of the molecules responsible for antagonistic activity of the isolated producing bacteriocin *Lactobacillus brevis* (LbL4, LbC3, LbC11, LbL15), the effect of the produced organic acids was minimized by the adjustment of pH value of the culture medium MRS agar medium 6,5.

The obtained results indicated that the diameter of inhibition zones was considerably reduced in the neutralized MRS culture medium compared to early used culture medium. Furthermore, the used hydrogen peroxide is known as an important factor for antimicrobial activity of lactic acid bacteria, which were catalase negative and are capable to accumulate the hydrogen peroxide under aerobic or in microaerobic conditions [9] (Figure1).

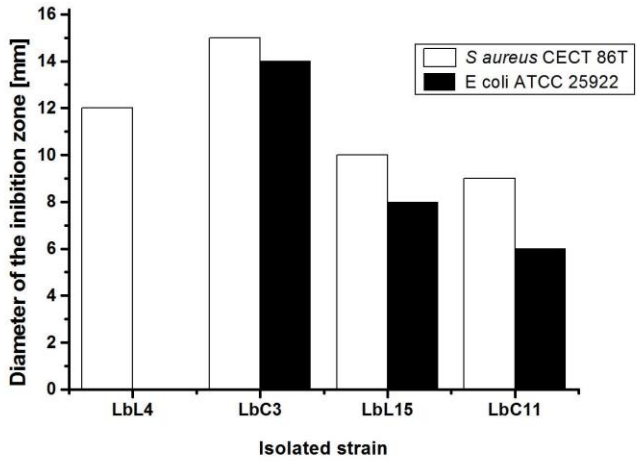

Figure 1. Illustration of the produced diameter of zone inhibition by *S. aureus* CECT86T and *E.coli* ATCC 25922 in the presence the isolated selected antagonistic lactic acid bacteria.

The exploration of the molecules responsible for antibacterial activity indicated that the inhibition could be affected by several factors such as the acidity, which was observed by (LbL15, LbL11), the effect of hydrogen Peroxide by (LbL4). Furthermore, the presence the antagonist activity

after the removal of mainly parameters such as the effect of organic acid and the hydrogen peroxide by isolated *Lactobacillus brevis* LbC3 has explored the presence of the antibacterial substances such as bacteriocins.

Production of crude bacteriocin

The antagonistic activity of the isolated, producing bacteriocin *Lactobacillus brevis* (LbC3) was stable after heat treatment at a temperature of 100°C for (15, 20, 30) min respectively (Figure 2).

The antagonistic activity of *L. brevis* (LbC3) indicated that the produced bacteriocin was responsible for the inhibition of the proliferation of the tested pathogenic bacteria. Therefore, the produced bacteriocin can find a wide use for food preservatives for directing or preventing the development of specific bacterial species in fermented dairy products [25]. Furthermore, the produced bacteriocins can be incorporated directly into fermented foods by the using of a bacteriocin producer as a starter or adjunct culture [26].

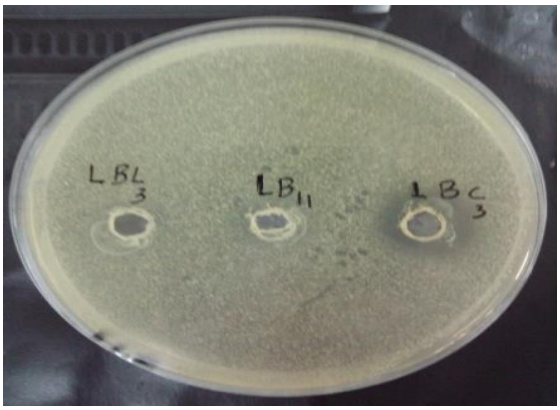

Figure 2. The effect of the produced bacteriocin in the supernatant of LbC3 against bacterial growth of *Staphylococcus aureus* CECT 86T.

Characterization of the selected antagonistic activity by PCR amplificacation of 16S rRNA and sequencing

The 16S rDNA gene of the isolated, selected *Lactobacillus brevis* (LbC3) was amplified with success and visualized by the using of agarose gel electrophoresis (Figure 3). The amplified obtained product revealed the presence of bands of 1000 bp, which was the size of the target fragment. Based on 16S rRNA gene analysis, the isolated, selected *Lactobacillus brevis* (LbC3) were phylogenetically characterized and identified by the using of NCBI database with BLAST, which confirmed their memberships to Lactobacillaceae family **(Table1)**. Furthermore, the use of the homology search of 16S rDNA nucleotide sequence indicated the presence of high identity to the recorded *Lactobacillus brevis* strains in the NCBI data base (Table 1).

Table 1: Molecular analysis of the isolated, selected producing bacteriocin (LbC3) from Algerian human breast milk.

Code	**Strain**	**Gen Bank** **Accession No**	**%** **Similarity**
LbC3	*Lactobacillus brevis*	CP018071.1	99%

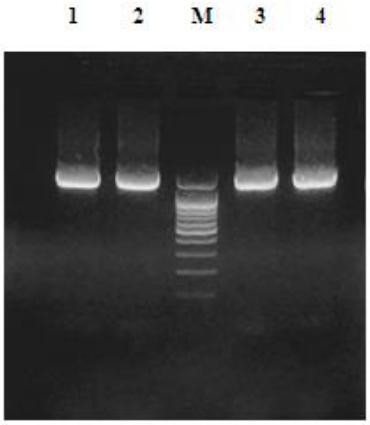

Figure 3: Agarose gel electrophoresis of the amplified 16S rDNA gene of the isolated, selected producing bacteriocin (1:LbC15, 2:LbC3, 3:LbC11, 4:LbL4, M: molecular weight).

Effect of pH value on bacteriocin production

The exploration of the effect of the pH-value on the antagonistic activity of the selected producing bacteriocin *Lactobacillus brevis* (LbC3) against *E. coli* ATCC 25922 was evaluated by the measurement of the diameter of the inhibition zone. The obtained results showed that the highest antagonistic activity was observed at pH value of pH 6.0, with diameter of the inhibition zone 15 mm (Figure 4). Mahrous and colleagues [27] has reported that a similar antagonistic activity has been yielded at pH value of pH 6.0 by *L. acidophius*, and the bacteriocin production was considerably influenced by the culture conditions and composition of the growth medium [28].

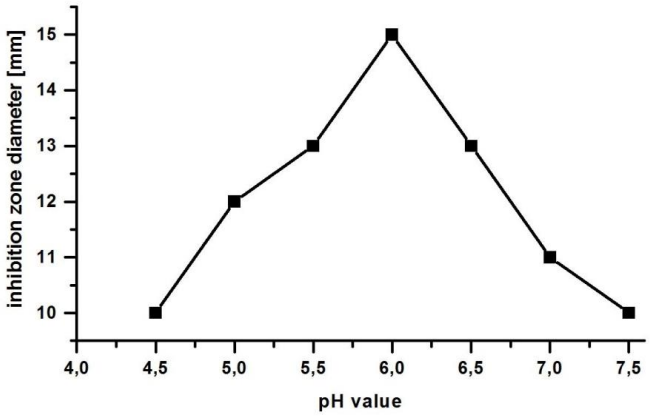

Figure 4: Effect of pH on the antagonistic activity in the presence of the supernatant of *Lactobacillus brevis* (LbC3) against *E. coli* ATCC 25922.

Effect of temperature on bacteriocin production

The characterization of the effect of the temperature on the antagonistic activity of the selected producing bacteriocin *Lactobacillus brevis* (LbC3) against *E. coli* ATCC 25922 was evaluated by the measurement of the produced diameter of the inhibition zone.

The obtained results indicated that the highest antagonistic activity of the selected producing bacteriocin *Lactobacillus brevis* (LbC3) was showed at temperature 30°C, with a diameter of inhibition zone of 15 mm (Figure 5).

Lim and co-workers has reported that the highest antagonistic activity by the selected producing bacteriocin *Lactobacillus Plantarum KC21* was yielded at 30°C and drastically decreased at 45°C [29]. Furthermore, Messens and colleagues has suggested that the use of the high temperature of incubation, induced the proteases activation in response to heat shock [30].

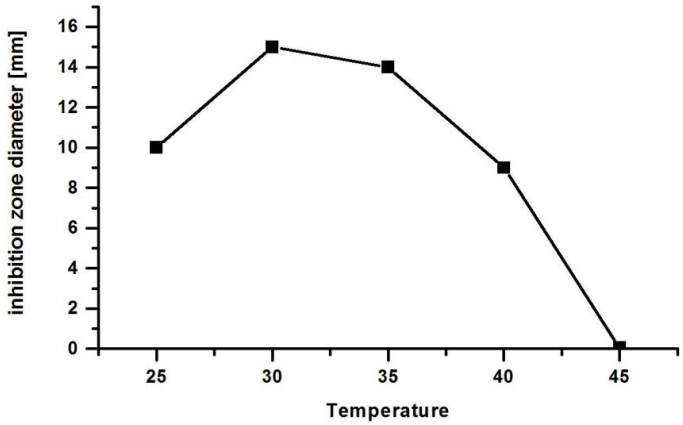

Figure 5: Effect of temperature on the antagonistic activity in the presence of the supernatant of *Lactobacillus brevis* (LbC3) against *E.coli* ATCC 25922.

Study of *E. coli* ATCC 25922 growth in the presence of bacteriocin

In order to explore the antagonistic activity of the produced bacteriocin of *Lactobacillus brevis* (LbC3) against *E. coli* ATCC25922. The bacterial growth of *E. coli* ATCC 25922 in the absence (control) and in the presence of the supernatant of *L. brevis* (LbC3) have been investigated. For this purpose, *E. coli* ATCC25922 was inoculated in the MRS culture medium with initial optical density of 0.5 at 630 nm according the protocol described by Abbouni and coworkers [11, 12]. A volume of 1 ml of the recovered of the produced bacteriocins by *L. brevis* (LbC3) was added 6 hours after the onset of the bacterial growth.

The obtained results (Figure 6) showed a considerable inhibition of the growth *E. coli* ATCC 25922, after the adding a volume 1 ml of the produced bacteriocins *Lactobacillus brevis* (LbC3), to the culture medium of the above tested strain during early exponential growth phase, which was explained the absence of the phenomenon of bacterial lysis of *E. coli* ATCC25922 and bacterial lysis in the presence of the produced bacteriocin by *Lactobacillus brevis* (LbC3), which has induced unbalanced growth, and more over the arrest of the cell cycle *E. coli* ATCC 25922, in comparison with the untreated biomass (balanced growth). This is in agreement with previous reports by the several researchers [31, 32].

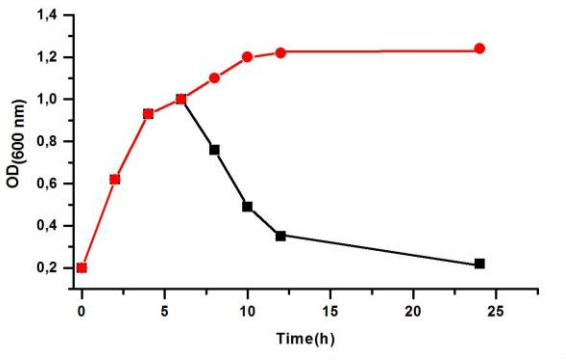

Figure 6: Bacterial growth of *E.coli* ATCC25922 in the absence and the presence of the supernatant of *L. brevis* (LbC3), inoculated in the MRS liquid culture medium, incubated at temperature of 30°C for 24 hours.

Antibiotic resistance

In order to explore the antibiotics resistance, the isolated *Lactobacillus brevis* from human breast milk was screened for their antibiotic sensitivity profile by the using of disc diffusion soft agar overlay method. For this purpose, the susceptibility of the isolated *Lactobacillus brevis* to antibiotics was evaluated as either as R, IR or S. by the measurement of the diameter of inhibition zone. The obtained results indicated the sensitivity the isolated *Lactobacillus brevis* to penicillins, β-lactams.

The investigation of the cell wall synthesis inhibitors such as oxacilin for inhibition of bacterial growth of the isolated *Lactobacillus brevis* has manifested a excellent resistance.

Furthermore, the isolated *Lactobacillus brevis* has further manifested a important resistance against nalidixic acid, nitroxolin, tobramicin and vancomycin. Usually, *Lactobacilli* are resistant to the most inhibitors, implicated in the inhibition synthesis of nucleic acid such as nalidixic acid and fluoroquinolone metronidazole and have also manifested intrinsic resistance against aminoglycosides, glycopeptides [33]. Furthermore, the resistance of many *Lactobacillus* species toward glycopeptides (vancomycin) has been often described as intrinsic [34].

The obtained results indicated that the isolated *Lactobacillus brevis* from human breast milk has manifested a important sensitivity against chloramphenicol, erythromycin (Table 2). Whereas, in previously study reported by Rojo-Bezares and co-workers [35] has manifested a similar effect.

Table 2. Illustration of the produced diameters of the inhibition zone by *L. brevis* in the presence of 10 several antibiotics, by the using of disc diffusion soft agar overlay method.

Strain	**Antibiotics**									
	P	OX	SP	E	PT	TOB	VA	C	NA	NI
L.brevis	30 S	0 R	31 S	25 S	33 S	10 R	0 R	27 S	0 R	0 R

Note: S- sensitive strain; R: resistant strain; P: penicillin; OX: oxacillin; SP: spiramicin; E: erythromycin; PT: pristinamycin; GN: gentamicin; TOB: tobramicin; VA: vancomycin; C: chloramphenicol; NI: nitroxolin; NA: nalidixic acid.

CONCLUSION

This work has allowed us to detect *L. brevis* (LbC3) isolated from breast milk of Algerian mothers. *L. brevis* is found in the initial flow of human milk. It is found in a minority of nursing mothers, capable of inhibiting reference pathogenic strains tested and led to an opportunity to develop a local dairy starter having sought preventive and technological characteristics.

Acknowledgement

We are thankful to the department head of the bacteriology laboratory of hospital of University of Sidi Bel Abbès Mr. Harrachi Abdelkader for providing us with the bacterial strains. We would like to thank Prof. Dr. HARIR Noria, for your encouragement, your support and your useful discussion. Without the kind cooperation of the above mentioned people the work could not have been possible.

REFERENCES

[1] Jara, S., Sanchez, M., Vera, R., et al. E Castro. *Anaerobe*, **2011.** 17: 474-477.

[2] Coeuret, V., Dubernet, S., Bernardeau, M., et al. Lait, **2003.** 83:269-306.

[3] Pelinescu, DR., Sasarman, E., Chifiriuc, MC., et al. *Romanian Biotechnology Letter*, **2009.** 14: 4225-4233.

[4] De Vuyst, L., Vandamme, EJ., 1st ed. Blackie Academic and Professional, Chapman and Hall, *Glasgow*, **1994.** 91-142.

[5] Ozgun, D., CingilliVural, H., *Journal of Medical Genetics and Genomics*, **2011.** 3(3):46-49.

[6] Samelis, J., Maurogenakis, F., Metaxopoulos, J., Int. *J. Food. Microbiol*, **1994.** 23:179-196.

[7] Herrero, M., Mayo, B., Gonzalez, B., et al., *J. Appl. Bacteriol*, **1996.** 81: 565-570.

[8] De Roissart, HB., Tome 3. Ed. Technique et documentation Lavoisier.Paris, **1986**. 343-408.

[9] Fleming, HP., Erchells, JL., Caslilow, RN., *Appl. Environ. Microbiol*, **1975.** 30:1040-1042.

[10] Barefoot, SF., Klaenhammer, TR., *App. Environ. Microbiol*, **1983.** 45: 1808-1815.

[11] Abbouni, B., Elhariry, HM., Auling, G ., *Archives of Microbiology*, **2004**. 182: 19-125.

[12] Abbouni, B., Elhariry, HM., Auling, G., *Biotechnology Letters*, **2003.** 25: 143-147.

[13] Labioui, H., El Moualdi, L., El Yachchioui, M., et al. *Bull.Soc. Bordeaux*, **2005**. 144: 237-250.

[14] Charteris, WP., Kelly, PM., Morelli, L., et al.. *J. Food. Prot*, **1998**. 61: 1636-1643.

[15] Clinical and Laboratory standards institute. CLSI Document M100-S22, Clinical Laboratory Standard Institute, Wayne., **2012**, PA. ISBN 1-56238-785-5, (Print); ISBN 1-56238-786-3 (Electronic).

[16] Weisburg, WG., Barns, SM., Pelletier, DA., et al. *Journal of bacteriology*, **1991.** 173(2): 697-703.

[17] Abrahamsson, TR., Sinkiewicz, G., Jakobsson, T., et al. *Journal of Pediatric Gastroenterology and Nutrition*, **2009.** 49: 349-354.

[18] Albesharat, RM., Ehrmann, A., Korakli, M., et al. *Systematic and Applied Microbiology*, **2011.** 34: 148-155.

[19] Jiménese, E., Delagdo, S., Fernandez, L., et al. *Research in Microbiology*, **2008.** 159: 595-601.

[20] Martín, V., Maldonado, A., Fernández, L., et al. *Breastfeeding Med*, **2010.** 5(4):153-158.

[21] Heikkila, MP., Saris, PEJ., *Journal of Applied Microbiology*, **2003.** 95: 471-478.

[22] Ibhanesebhor, SE., Otobo, ES., *Journal of Tropical Pediatrics*, **1996.** 42: 27-33.

[23] Langer, P., *Journal of Mammalogy*, **2009.** 90: 332-341.

[24] Abd El-Salam, BA., Al Rubayyi, KA., *Egyptian Journal OF Dairy Science*, **2012.** 40:7-14.

[25] Cotter, PD., Hill, C., Ross, RP., *Nature Reviews. Microbiology*, **2005.** 3(10): 777-788.

[26] Deegan, LH., Cotter, PD., Hill, C., et al. *International Dairy Journal*, **2006.**16:1058-1071.

[27] Mahrous, H., Mohamed, A., El-Mongy, MA., et al. *Food and Nutrition Science*, **2013.** 4: 342-356.

[28] Tagg, JR., Dajani, AS., Wannamaker, LW., *Bacteriological Re-views*,**1976**. 40(3):722-756.

[29] Lim, SM., *Foud Sci. Biotechnol*, **2010.** 19: 793-802.

[30] Messens, W., Verluyten, J., Leroy, F., et al. *Int. J. Food Microbiol*, **2003.** 81: 41-52.

[31] Mekri, M., Abbouni, B., Tifrit, A., et al. **2015.** 15(5): 485-492.

[32] Hadjazi, D., Larbi Daouadji, K., Reffas, FZI., et al.. *Global Journal of Biotechnology& Biochemistry*, **2015.** 10 (2):100-105.

[33] Zhou, JS., Pillidge, CJ., Gopal, PK., et al.. *Int J Food Microbiol*, **2005.** 98: 211-217.

[34] Ammor, MS., Florez, AB., Mayo, B., *Food Microbiol*, **2007.** 24: 559-570.

[35] Rojo-Bezares, B., Saenz, B., Poeta, P., et al. *Int J Food Microbiol*, **2006.** 111(3):234-240.