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The Isolation of lactic acid bacteria with a conservatory power

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ABSTRACT

Lactic acid bacteria are isolated from the following habitats: cow's milk, olives and pickles soaking liquid. Of 24 isolates selected lactic, four strains (BLN3, BLN4, and BLN8 BLN17) are selected for their potential characters in the biopreservation and fermentation. These isolates were subjected to morphological and biochemical identification tests including: Gram reaction, mobility, catalase test, growth at different temperatures, growth at different NaCl concentration[8], growth at different pH, CO_2 production from glucose. The four lactic strains are classified in the genus Lactobacillus. They are stored at 4 °C and -20 °C for use in industrial applications tests.

KEYWORDS

Lactic acid bacteria;
Habitats;
Biopreservation;
Lactobacillus.

INTRODUCTION

The chemical conservators used in food products for fighting pathogenic germs are supposed to be a discussed issue because of the allergies and risks that threat consumers' health[1]. Next to the using of the chemical conservations, there are other techniques used such as bio-conservation. We have applied this method through using lactic acid bacteria. These latter are known by their acidifying power. They produce organic acids. The lactic bacteria are used in industrial fermentation (transformation of milk, vegetables...). They are also used for their contribution to improve the texture, flavor, and the bio-preservation by lowering the pH as well as by secreting antagonists like Nisin which is considered as a food additive that has effects on pathogenic germs such as *Listeria monocytogenes*.[2,3] (Larsen., 1989 et Anon.,1990). Lactic acid bacteria collected are derived from cow's milk[4], olives and pickles soaking liquid[5]. The objective of this work is to isolate the strains with lactic biopreserving characters (low pH, high acidity).

MATERIALS AND METHOD

These samples are transported to the laboratory in sterile flasks of 500 ml. After their fermentation in a room temperature of 25° C of the laboratory for 72 hours, 1ml of the dilution 10^6, taken from every sample, is plated on to MRS agar plates and incubated in an anaerobic jar at 30°C for 24 hours. The enumeration of lactic acid bacteria is carried out on MRS medium which helps to rapid the development of such bacteria[6].

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Isolation and Purification

The isolation of lactic acid bacteria is realized on MRS medium following a series of dilution of the sample to 10^{-6}. After incubation at 30°C during 48 hours, the colonies apparently suspected of lactic acid bacteria with circular white, yellow -white or cream are removed and transplanted several times by the streaks method (Figure 2). From hundreds bacteria, only 24 lactic germs were retained.

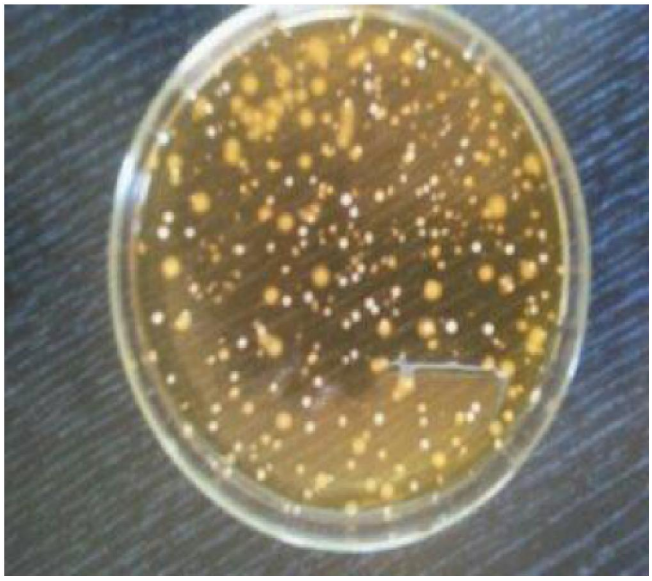

Figure1: Isolation

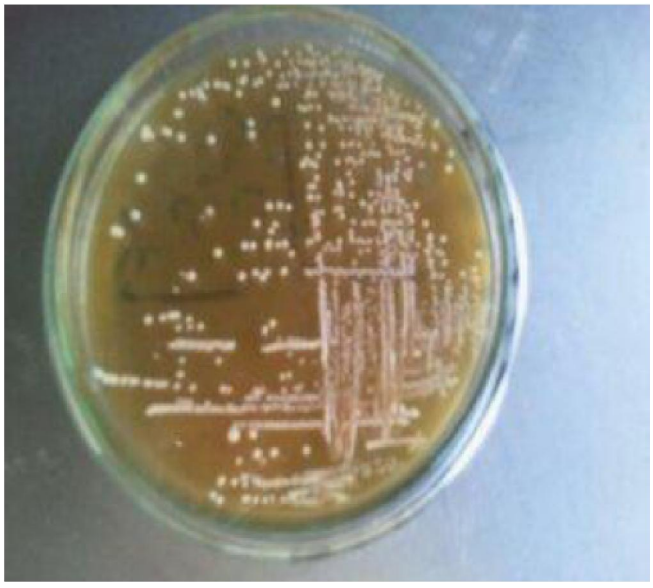

Figure 2 : Purification

After isolation, the colonies obtained are observed under a microscope to determine the mobility, and their morphological characteristics. Gram coloration is performed by conventional method. For the test of catalase: a colony of each lactic strain was diluted in a drop of hydrogen peroxide on a sterile blade, the formation of gas bubbles is an indicator of the presence of catalase [7].

After purification, the lactic bacteria were tested in their pH, acidity and optic density for searching bacteria that have acidifying power for the bioconservation. To do that, we have taken one colony from each box. This colony is inculcated in 3ml of MRS broth medium, and then incubated at 30°C. After 24 hours, this volume is transferred into a final volume of 30 ml. for measuring the initial and final parameters (pH*i*, Ac*i* and DO*i*) (pH*f*, Ac*f* and DO*f*) [8]. The biomass was measured by changes in optical density (OD) at 600 nm.

Identification

The Characterization of lactic acid bacteria is based on the determination of morphological, physiological and biochemical characteristics of these bacteria such as: catalase test, growth at different temperature, the reaction gram, mobility, growth at different concentration of NaCl, and growth in different pH. Also, the CO_2 production by using glucose.

Enumeration of lactic acid bacteria

For each strain lactic selected, 1ml of the solution of the 6th tube dilution is inoculated into a Petri dish followed by a flow of 15 ml MRS medium. The dishes were incubated at 30°C for 24 hours in an anaerobic jar followed by colonies counting[9].

Effect of temperature

The four lactic isolates are inoculated into tubes containing 5 ml of liquid MRS. Then; they are set to different incubation temperature for 24hrs. The lecture is made via the spectrophotometer at 600 nm[10].

Effect of pH

The four lactic strains are inoculated in 5 ml of MRS liquid whose pH is adjusted to the following values : 3, 5. 4. 5. 6. 8, 5 and 9. And they are incubated at 30°C for 24 hours. The lecture is performed via a spectrophotometer at 600 nm[11].

Effect of NaCl

After the 4 lactic acid bacteria were inoculated in a MRS medium at a different NaCl concentration; they were incubated for 24 hours at a suitable temperature. Results are revealed by measuring the biomass in a

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spectrophotometer at 600 nm[12].

Fermentation of glucose

A colony of each lactic strain was stitched vertically in a semi solid MRS medium at 8%, and then covered with agar at 15%. The tubes are placed in the incubator at 30°C for 24 hours. The lecture is done by the observation of tubes[13].

Conservation of lactic acid bacteria

Two copies of four selected lactic bacteria, which consist of the required conditions, are stocked in the refrigerator. The first copy is conservated at 4°C in inclined tubes. For the second one, the cells were collected from cultures, after centrifugation and suspended in micro-tubes that contain 20% of glycerol and 80% of the MRS broth; they were frozen at -20 °C[14].

RESULTS AND DUSCUSION

After their culture in a liquid MRS medium at a suitable temperature, their microscopic and biochemical analysis have revealed the results: All bacteria are gram+, catalase-, homofermentative, 10 strains were cocci and 14 strains were lactobacilli (see TABLE below).

The twenty-four lactic acid strains were isolated from three different habitats with the following percent-

Figure 3 : LAB conserved in inclined tubes of MRS agar

TABLE 1: Preliminary identification of 24 isolated LAB (Lactic Acid Bacteria)

Biotope	Isolats	Gram	Catalase	Fermentation type	Morphology	Genre
Cow's Milk	BLN1	+	_	Homo*	Cocci	streptococcus
	BLN2	+	_	Homo*	Bacille	lactobacillus
	BLN3	+	_	Homo*	Bacille	lactobacillus
	BLN4	+	_	Homo*	Bacille	lactobacillus
	BLN5	+	_	Homo*	Cocci	streptococcus
	BLN6	+	_	Homo*	Bacille	lactobacillus
	BLN7	+	_	Homo*	Bacille	lactobacillus
	BLN8	+	_	Homo*	Bacille	lactobacillus
pickles soaking liquid	BLN9	+	_	Homo*	Cocci	streptococcus
	BLN10	+	_	Homo*	Cocci	streptococcus
	BLN11	+	_	Homo*	Bacille	lactobacillus
	BLN12	+	_	Homo*	Bacille	L lactobacillus
	BLN13	+	_	Homo*	Cocci	streptococcus
	BLN14	+	_	Homo*	Bacille	lactobacillus
olives soaking liquid	BLN15	+	_	Homo*	Bacille	lactobacillus
	BLN16	+	_	Homo*	Cocci	streptococcus
	BLN17	+	_	Homo*	Bacille	lactobacillus
	BLN18	+	_	Homo*	Bacille	lactobacillus
	BLN19	+	_	Homo*	Bacille	streptococcus
	BLN20	+	_	Homo*	Cocci	streptococcus
	BLN21	+	_	Homo*	Bacille	lactobacillus
	BLN22	+	_	Homo*	Cocci	L lactobacillus
	BLN23	+	_	Homo*	Cocci	streptococcus
	BLN24	+	_	Homo*	Cocci	streptococcus

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ages: (33% of cow's milk, 25% of the pickle soaking liquid and 41% of the olives soaking liquid). The isolates which do not have the phenotypic characteristics of lactic acid bacteria (gram positive, catalase negative…) were rejected. Twenty-four isolates were selected finally. These isolates were divided into 14 Lactobacillus and 10 Streptococcus. The supplemented tests (pH, acidity and optical density) results are regrouped in the table below.

By comparing the values: of pH, acidity and optical density of 24 lactic isolates, it appears that the four bacterial strains (BLN3, BLN4, BLN8 and BLN17) are retained according to their great power to lower the pH.

TABLE 2 : Measuring the initial and final state of the following parameters: pH, acidity and DO of 24 isolated LAB

Isolats	pH*i*	pH*f*	D.O*i*	D.O*f*	Ac*i*	Ac*f*
BLN1	6,00	4,14	0,252	2,850	0,33	0,64
BLN2	6,04	4,20	0,264	2,471	0,33	0,52
BLN3	5,85	3,76	0,861	2,520	0,57	1,26
BLN4	5,78	3,77	0,948	2,650	0,46	1,25
BLN5	5,80	3,84	0,798	2,578	0,34	1,20
BLN6	6,10	4,20	0,298	2,430	0,50	0,98
BLN7	5,98	4,14	0,275	2,121	0,40	0,97
BLN8	6,00	3,70	0,868	2,491	0,33	1,29
BLN9	5,66	4,26	0,230	2,192	0,48	0,92
BLN10	6,08	4,12	0, 326	1,893	0,52	0,94
BLN11	5,97	4,15	0,255	2,072	0,46	1,07
BLN12	5,98	4,13	0,324	1,991	0,43	1,01
BLN13	5,80	4,14	0,278	1,795	0,36	0,94
BLN14	5,83	3,85	0,722	2,563	0,60	1,23
BLN15	5,74	3,81	0,820	2,711	0,62	1,22
BLN16	5,87	3,87	0,861	2,303	0,60	1,30
BLN17	5,72	3,77	0,820	3,031	0,65	1,27
BLN18	5,55	3,84	0,744	2,745	0,54	1,25
BLN19	5,62	3,85	0,685	2,309	0,56	1,24
BLN20	5,63	3,84	0,721	2,312	053	1,24
BLN21	6,00	4,24	0,241	1,571	0,43	0,78
BLN22	5, 97	3,95	0,852	2,177	0,53	1,26
BLN23	6, 13	4,16	0,756	1,452	0,42	1,20
BLN24	6,09	4,20	0,762	1,781	0,39	0,76

Enumeration of bacteria

Effect of temperature

Effect of pH

Effect of salinity

Enumeration of four selected lactic acid bacteria after seeding them on solid MRS medium revealed the dominance of the strain with BLN3 $9,5.10^7$[15]. And the results are displayed in the TABLE 3.

TABLE 3 : The biomass of bacteria

Lactic acid bacteria	Number of ufc/ml
BLN3	$9,5.10^7$
BLN4	$3,6.10^5$
BLN8	$5,5.10^7$
BLN17	$3,5.\ 10^6$

TABLE 4 : The biomass of bacteria

	Optical density at 600 nm			
Temperature	BLN3	BLN4	BLN8	BLN17
10°	2,12	1,43	1,39	1,12
20°	2,16	1,48	1,41	1,31
30°	2,66	2,25	2,50	2,45
35°	2,12	2,09	2,04	0,96
45°	0,01	0,00	0,01	0,01

TABLE 5 : The biomass of bacteria

LAB	Optical density at 600 nm					
	3	3,5	4	4,5	8,5	9
BLN3	0,03	2,36	2,75	2,80	0,02	0,02
BLN4	0,02	0,01	2,63	2,57	0,01	0,01
BLN8	0,01	0,01	1,98	2,09	0,01	0,01
BLN17	0,03	0,01	2,13	2,21	0,02	0,01

TABLE 6 : The biomass of bacteria

[NaCl]	Optical density at 600 nm			
	BLN3	BLN4	BLN8	BLN17
2 %	2,44	1,97	2,12	2,15
4,5 %	2,27	1,73	1,62	2,13
6 %	2,11	0,13	0,15	0,14

The study of the growth of four bacterial strains was assessed by measuring their biomass in a spectrophotometer at 600 nm. 30°C is an optimum temperature for growth of four lactic acid strains with a bacterial abundance for BLN3 strain. It is said that the bacteria are mesophilic types (TABLE 4).

The test of four Lactobacillus in different solutions concentration of pH reveals that only BLN3 has a high tolerance in a low pH for 24 hours after incubation in a solution with a pH of 3,5 (TABLE 5)[16].

According to the interpretation of the results in TABLE 6, it appears that a concentration of 6 % becomes unfavorable to the growth of lactic acid so that the concentration of 4, 5 % remains the maximum con-

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centration for the development of lactic acid bacteria in a salty medium. Except BLN3 strain that grows at 6 %.

CONCLUSION

Among hundred strains, twenty-four belong to the lactic acid bacteria. They are isolated from three habitats: milk, olives and pickles soaking liquid. The lecture on MRS macroscopic, microscopic and biochemical tests, which were realized on all strains, gave whitish milky colonies; cocci and bacilli, negative catalase and positive Gram. The homofermentative were the most dominant group. Then, we ended up with two kinds of lactic bacteria including Lactobacillus and Streptococcus. They represent 58% and 42%.

Hence, twenty four strains were cultured in MRS liquid medium to select those ones with interesting biotechnological characteristics. This test gave us four lactic strains: BLN3, BLN4, BLN8 and BLN17 They belong to the genus Lactobacillus. They grow at 30°C and pH = 4. For salt tolerance, the four strains showed their ability to replicate easily in concentration of NaCl 4.5 % except BLN3 that showed the exact conservatory characteristics (the growth of strains at 3, 5 pH, 6 % of NaCl and temperature of 10°C).

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