African Journal of Microbiology Research Vol. 6(13), pp. 3192-3201, 9 April, 2012
Available online at http://www.academicjournals.org/AJMR
DOI: 10.5897/AJMR11.1542
ISSN 1996-0808 ©2012 Academic Journals

Full Length Research Paper

Bioprospecting of *Leuconostoc mesenteroides* strains isolated from Algerian raw camel and goat milk for technological properties useful as adjunct starters

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Accepted 9 February, 2012

Leuconostoc **species are lactic acid bacteria widely used in milk fermentation. Based on morphological, physiological and biochemical analysis, 18 strains of *Leuconostoc mesenteroides* were isolated and identified from 10 samples of goat's milk and camel's milk. Strains were identified as follows 09 strains of *L. mesenteroides* subsp. *mesenteroides* and 09 strains of *L. mesenteroides* subsp. dextranicum. The results of technological tests of the strains showed that strains produced dextran, carbon dioxide, and resist to 55°C for 15 min, which promote their industrial use. The growth kinetic, acidification evolution and carbon dioxide production of *L. mesenteroides* subsp. *dextranicum* strain in skim milk at 30°C were slightly higher than *L. mesenteroides* subsp. *mesenteroides*. Addition of yeast extract to skim milk stimulates the development of *L. mesenteroides* subsp. *mesenteroides* by increasing its growth, acidification activity and evolved CO_2. Susceptibility to antibiotics was also evaluated on 33 antibiotics and strains of *L. mesenteroides* showed resistance to 42.4% of antibiotics used.**

Key words: Technological, *Leuconostoc,* milk, growth kinetic, carbon dioxide, yeast extract, antibiotics.

INTRODUCTION

Lactic acid bacteria are a heterogeneous group of microorganisms that produce lactic acid as the main product of metabolism (Carr et al., 2002). They colonize many food products such as dairy products, meat, vegetables and cereals; they are part of the intestinal and vaginal flora (Dortu et al., 2009; Mayo et al., 2010). *Leuconostoc* species are widely used as starter cultures and play an important role in food preservation, microbiological stability and production of aroma compounds in various food products. Indeed, lactic acid bacteria produce many metabolites with antimicrobial properties such as organic acids, carbon dioxide and diacetyl. They play an important role in hygiene by lowering the pH and excreting a variety of inhibitory compounds that inhibit the development of undesirable bacteria (Hugenholtz et al., 2002; Guessas et al., 2005; Dortu et al., 2009). Lactic acid bacteria are Gram-positive, catalase-negative, immobile and non-sporulating; they grow in anaerobic conditions but they can be aerotolerant (Mayo et al., 2010).

Currently, the lactic acid bacteria include thirteen different bacterial genera: *Lactobacillus, Leuconostoc, Lactococcus, Streptococcus, Enterococcus, Pediococcus, Bifidobacterium Carnobacterium, Oenococcus, Weissella, Aerococcus, Tetragenococcus* and *Vagococcus* (Dortu et al., 2009).

Among the lactic acid bacteria commonly used in dairy industry, heterofermentative bacteria of the genus

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Leuconostoc, which produce from lactose the lactic acid, acetate or ethanol and carbon dioxide. These bacteria are considered as essential technological auxiliaries in the formation of openings in the blue cheese such as Roquefort, due to the production of carbon dioxide that is formed from two distinct substrates: lactose and citric acid. The stability of these openings depends on the kinetics of CO_2 production by *Leuconostoc* facilitating the growth, development and correct installation of *Penicillium roqueforti* (Pinchon, 1989; Kihal et al., 1996; Kihal, 1996; Kihal et al., 2009). The *Leuconostoc* are involved in the fermentation, the improvement of animal feed and the improvement of the flavor of food flavor by the production of aromatic compounds and dextran (Hemme et al., 2004)

The objective of this work was to the study of microbiological and technological characteristics of *L. mesenteroides* species isolated from goat milk and camel milk in order to select efficient strains that can be used in dairy industry.

MATERIALS AND METHODS

Sampling

Raw goat and camel milk samples were collected from Oran and Becher regions between September and December, 2009. The Danish Roquefort cheese “Rosenberg” manufactured in June, 2009 was also used for isolate the reference strains.

Pathogenic bacteria

The test strains used were pathogenic *Staphylococcus aureus* (ATCC 25923) and *Escherichia coli* (ATCC 25922), which were collected from the collection of applied microbiology laboratory at the University of Oran, Algeria.

Isolation, purification and growth conditions of bacterial strains

In order to select the *Leuconostoc* species, the strains were been isolated on MRS agar at pH 6,5 supplemented with 30 µg/ml of Vancomycin (Mathot et al., 1994) and were incubated at 30°C for 48 h. MSE agar was also used to detect the *Leuconostoc* strains producing dextran (Mayeux et al., 1962). The cultures were then purified on MRS and MSE agar medium by stries. Among 40 isolates, only 36 were Gram-positive and catalase-negative, isolated from raw milks and Danish Roquefort cheese. Roquefort cheese is used to isolate *leuconotocs* species which are used as reference strains (Ghazi et al., 2009).

The strains were stored in sterile skim milk with 30% (v/v) glycerol at -20°C. Working cultures were also kept on MRS agar slant at 4°C and streaked every 4 weeks (Badis et al., 2004).

Identification of strains

On the basis of morphological critera, 18 representative strains were selected for this study. They were tested for morphological aspect, proteolytic activity on PCA supplemented with 2% of skim milk, production of CO_2 from glucose, hydrolysis of arginine on M16BCP, Growth at different temperature 4°C, 15°C, 37°C and 45°C, themotolerance at 63.5°C for 30 min and 55°C for 15 min (Guiraud, 1998), growth at 3 and 6.5% NaCl, growth at pH 4 and pH 6.5.

The production of dextran was detected on MSE agar (10% sucrose) (Mayeux et al., 1962). The hydrolysis of aesculin was studied on Agar with 0.5% aesculin (w/v) (Lelliott et al., 1987). The citrate Utilization, in the presence of glucose, was performed on KMK agar (Kempler et al., 1980). The production of acetoin from glucose was determined using the Voges-Proskauer test on Clark and Lubs broth (Samelis et al., 1994; Guiraud, 1998).

All strains were tested for the production of acids from fermentation of carbohydrates on MRS-BCP broth. The carbon source was added to the broth as sterile solution with a final concentration of 3% (w/v). The sugar used were: glucose, lactose, galactose, fructose, L(-) arabinose, D(+) xylose, maltose, D(-) mannitol, aesculin, sucrose, L(+) rhamnose, sorbitol. The use of carbohydrates was evaluated after 24 and 48 h of incubation (Badis et al., 2004; Guessas et al., 2004).

Growth kinetics of *Leuconostoc mesenteroides* in skim milk

To study the growth kinetics of *L. mesenteroides*, strains C1 LMA, C3 LMA and R2 LMA were selected and inoculated in skim milk which was prepared from skim milk powder reconstituted to 10%, sterilized at 110°C for 10 min, cooled to 20°C (Kihal et al., 2009). The cultures were divided in tubes and incubated at 30°C for 24 h. Every two hours, the samples were aseptically withdrawn from tubes to determine the pH, titrable acidity, the growth rate and evolved CO_2.

Determination of pH and titrable acidity

Measurement of pH was carried out by pH-meter. The total acidity was determined by titrating 10 ml of culture with the basic solution 0.1 N NaOH using the pH indicator phenolphthalein. The acidity was expressed as mM of lactic acid (Accolas et al., 1977).

Growth kinetics

Cultures samples were collected aseptically at 0 and every 2 h. Cultures samples of1 ml was submitted to decimal dilutions in sterile physiological solution and Agar plate was performed to assess cell count. *L. mesenteroides* strains were enumerated in MRS according to the method of Mathot et al (1994). Plates were incubated at 30°C for 48 h. Only the plates containing between 25 and 250 colonies were selected. The generation time and growth rate were calculated in the logarithmic phase of growth (Kihal et al., 2009).

CO_2 production

Production of carbon dioxide was measured by a technique presented by Kihal (1996); the principle of this method is based on the pressure created by CO_2 production by the culture in tubes. Evolved CO2 was trapped and was measured by displacement of acidifying water in the burette (Figure 1). The total amount of CO_2 produced was released by acidifying with 2 M HCl.

After incubation at 30°C, the tube contained 10 ml of culture was connected to graduated burette for measurement. 1ml of 2 M HCl was tipped in using a syringe to release any dissolved CO_2. After sharking with vortex for 2 min, the experiment terminated.

To control the measurement system, a chemical reaction was performed using a solution of Na_2CO_3, the principle is the following:

$$Na_2CO_3 + 2\ HCl \longrightarrow 2\ NaCl + CO_2 + H_2O$$

The volume of CO_2 regenerated from 10 ml of 50 mM Na_2CO_3 was

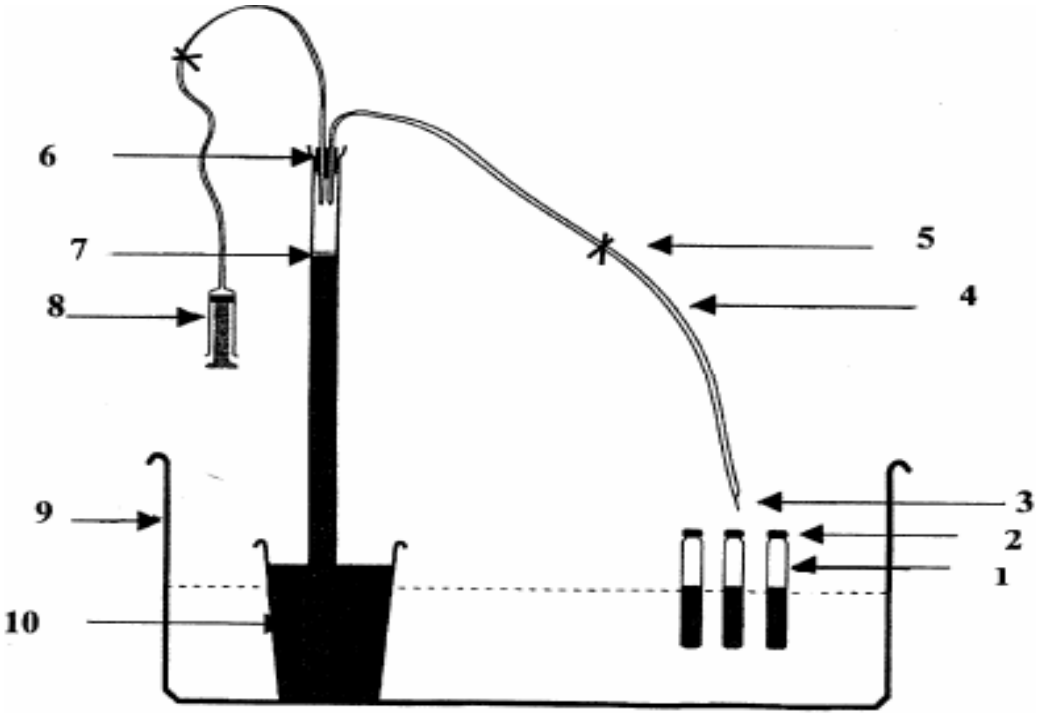

Figure 1. Principal measurement of evolved CO_2 by *L. mesenteroides* by displacement of acidifying liquid in graduated burette in constant pressure. Tube (1), silicon and rubber stopper (2), needle (3), tubing rubber (4), pliers (5), thin rubber stopper (6), graduated burette (7), syringe (8), water bath (9) and acidifying water (10).

11.2 ml of CO_2 when it reacts with 1 ml of 2 M HCl (Kihal et al., 2009).

Effect of yeast extract on growth *of Leuconostoc mesenteroides*

The effect of yeast extract on the development of *L. mesenteroides* strains was investigated on reconstituted skim milk (10%) using the following concentrations 0.1, 0.3 and 0.5% yeast extract to evaluate their growth, lactic acid production and evolved carbon dioxide by *L. mesenteroides* strains in pure culture. Inoculation of reconstituted skim milk without yeast extract by *Leuconostoc* is used as a control.

The same methodology was followed to measure the pH, the titrable acidity, the amount of CO_2 produced and the growth rate which was calculated by counting on MRS agar.

Antibiogram

The susceptibility of *L. mesenteroides* strains to antibiotics was determined by a diffusion method, proposed by Antibiogram Committee of the French Microbiology Society, on agar (MRS) (Bauer et al., 1966; Dalache et al., 2003; Ruiz-Moyano et al., 2009). Two reference strains, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 were included in the trials (Zhou et al., 2005). The diameters of the inhibition zone observed around the colonies classified bacteria as chemically sensitive (S), intermediate (I) or resistant (R) to a given antibiotic. They are compared with the critical diameters for the various classes of antibiotics proposed by ACFMS (2009).

Antibiotics tested are as follows : amikacin, amoxicillin + clavulanic acid, ampicillin, bacitracin, cefsulodin, cefazolin, cefotaxime, cefoxitin, ceftazidime, cephalothin, ciprofloxacin, clindamycin, colistin, erythromycin, fusidic acid, imipenem, lincomycin, nalidixic acid, netilmicin, nitrofurantoin, ofloxacin, oxacillin, pefloxacin, penicillin, piperacillin, pristinamycin, rifampin, spiramycin, tetracycline, ticarcillin, tobramycin, trimethoprim-sulfamethoxazole (cotrimoxazole), Vancomycin. These discs of antibiotics come from Bio-Rad (Marnes la Coquette, France), except VA come from Oxoid (Basingstoke, Hampshire, England).

RESULTS AND DISCUSSION

The study of macroscopic aspect of 18 strains on MRS agar revealed the small, round, white and lenticular colonies, while on MSE agar, the colonies are transparent, glutinous with gelatinous aspect. The microscopic observation showed that the cells are Gram-positive with an ovoid shape associated in pairs and short chains. All strains are catalase-negative, able to produce CO_2 from glucose, unable to hydrolyze arginine, therefore these strains are considered as *Leuconostoc* strains (Garvie, 1986; Badis et al., 2005; Ogier et al., 2008; Ghazi et al., 2009). The strains isolated and purified have been able to grow at 15 and 37°C but not at 4 and 45°C, confirming that they are mesophilic bacteria. Five strains were resistant to a concentration of 3% NaCl, while no strain was pushed at 6.5% NaCl. The isolates are able to grow at pH 6.5 and not at pH 4. They are non-proteolytic, the thermotolerance was tested at two temperatures: 63.5°C for 30 min at which strains could not resist, but

they were able to grow at 55°C for 15 min (Guiraud, 1998).

Production of dextran on sucrose medium (MSE) is an important character to differentiate between the *Leuconostoc* species. 18 isolates are capable of hydrolyzing sucrose and produce dextran (Badis et al., 2005; Carr et al., 2002; Ogier et al., 2008).

The results of use of carbohydrates (Table 1) show that this strains are divided into two groups, the first contains nine strains that are "Glu^+ Lac^+, Gal^+ Suc^+, Frc^+, Mal^+, Xyl^+, Mnt^-, Rha^-, Ara^+ $Sorb^+$ Esc^+ " and the second contains the nine remaining strains that are " Glu^+ Lac^+, Gal^+ Suc^+ Frc^+, Mal^+, Xyl^+, Mnt^-, Rha^-, Ara^-, $Sorb^-$, Esc^- ". The arabinose sugar is a key for the differentiation between *L. mesenteroides* subspecies.

The identification by microbiological, physiological and biochemical tests revealed that five strains isolated from Roquefort cheese and four strains isolated from goat milk, belong to the subspecies *L. mesenteroides* subsp. *mesenteroides* while six strains isolated from camel milk and the three strains isolated from goat milk belong to *L. mesenteroides* subsp. *dextranicum,* and that according to the work of Kihal (1996), Carr et al. (2002), Bjorkroth et al. (2006) and Ghazi et al. (2009).

The strains present a variable character about the citrate utilization, 13 strains from 18 are able to form blue colonies on KMK agar, reflecting their ability to use the precursor of aromatic compounds which is taken as an important character in the selection of technological interest of species. This variability according to Bellengier et al. (1994) and Kihal et al. (1996), may be due to the loss of plasmids encoding the genes responsible for the degradation of citrate. All strains are unable to produce acetoin from glucose, which corresponds to the characteristics of two sub-species *L. mesenteroides* subsp. *mesenteroides* and *L. mesenteroides* subsp. *dextranicum* (Badis et al., 2005). Hydrolysis of aesculin, studied on agar with aesculin, showed the same result found in MRS-BCP broth.

The growth kinetic of *L. mesenteroides* was investigated on skim milk using the reference strain. The proportions inoculated at the beginning are as follows: 25 10^6 cfu /ml, 21 10^6 cfu/ml, 12 10^6 cfu/ml of strains C3 LMA, C1 LMA, R2 LMA, respectivily. The pH, titrable acidity, the amount of CO_2 produced and the growth rate were measured at a regular time interval. Figure 2 (A, B) showed that *L. mesenteroides* subsp. *dextranicum* has a higher rate of acidification compared to that of *L. mesenteroides* subsp. *mesenteroides* after 8 h of incubation. The rate of decrease in pH of strains C3 LMA, C1 LMA is 0.05 upH/h and 0.036 upH/h, respectively. The rate of CO_2 produced by *L mesenteroides* subsp. *dextranicum* is higher than that produced by *L. mesenteroides* subsp. *mesenteroides* with 0.73 mM/h for C3 LMA, 0.63 mM/h for C1 LMA and 0.66 mM/h for R2 LMA. The kinetics of production of carbon dioxide by *L. mesenteroides* subsp. *mesenteroides* is almost identical with the reference strain. A coefficient of correlation between pH and the CO_2 produced by this subspecies is equal to 0.95. A close relationship was noted between CO_2 production and titrable acidity of the two subspecies. The rate of growth of *L. mesenteroides* subsp. *dextranicum* is higher than that of *L. mesenteroides* subsp. *mesenteroides*, a high correlation was observed between bacterial growth and CO_2 production, which proves that the method used to measure the carbon dioxide, is an indirect way to follow the bacteria growth. These results show that *L. mesenteroides* subsp. *dextranicum* has a rate of CO_2 production, a growth rate, an acidification rate higher than *L. mesenteroides* subsp. *mesenteroides*. Similar observations were obtained by Kihal (1996), Kihal et al. (2006) and Kihal et al. (2009).

The effect of yeast extract on the growth of *L. mesenteroides*

The rate of decrease in pH in the presence of 0.1% yeast extract was 0.08 upH/h with a decrease in pH from 6.4 to 5 after 24 h of incubation, while the rate in the presence of 0.3 and 0.5% yeast extract were 0.098 and 0.095 upH/h with final values of 4.6 and 4.7, respectively. The control shown in Figure 3 (A), has a rate of 0.042 upH/h with a final value of 5.4, indicating that the addition of 0,3% yeast extract increases the rate of decrease in pH. The rate of carbon dioxide production in the presence of 0.3% yeast extract was 3.9 mM/h with a final volume of 53 mM, the control had a speed of 0.79 mM/h with a final volume of 25 mM. The growth rate in the presence of 0.3% yeast extract was 1.10 h^{-1} with a generation time of 54 min, by cons it was 0.69 h^{-1} in the control with a generation time of 86 min. The acidity shown in Figure 3 (B) also increased when yeast extract was added. A close correlation was found between CO_2 production and titrable acidity with a coefficient of correlation in skim milk supplemented with 0.3% yeast extract was equal to 0.98. From the results of the kinetics obtained, it was noted that growth in the presence of 0.3% yeast extract gives a better acceleration of growth of *Leuconostoc* species and better acidification of milk and this is reflected by the quantity of acids and carbon dioxide produced during the exponential phase (Table 2). So, the growth of *L. mesenteroides* subsp. *mesenteroides* is influenced by the addition of this factor because the main desired property in lactic acid bacteria, used as starter cultures in food industry, is their ability to acidify and to grow steadily. In milk, they must find a number of nutrients needed for their growth and, in particular, amino acids and vitamins, which may be provided by yeast extract. These results are consistent with the work reported by Accolas et al. (1980), Desmazaud (1983), Garault et al. (2001) and Kihal et al. (2006).

The sensitivity of two subspecies *L. mesenteroides*

Table 1. physiological and biochemical tests of *Leuconostoc* strains obtained from raw goat milk, camel milk and Roquefort cheese.

Parameter	Samples																	
	Goat milk strains							Roquefort cheese strains					Camel milk strains					
	C1LMA	C2 LMA	C3 LMA	C4 LMA	C5 LMA	C6 LMA	C7 LMA	R2 LMA	R3 LMA	R7 LMA	R8 LMA	R9 LMA	CH5 LMA	CH6 LMA	CH7 LMA	CH9 LMA	CH10LMA	CH11LMA
Hydrolysis of arginine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gas production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Dextran production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrate utilization	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+
Hydrolysis of aesculin	+	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-
Acetoin production	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3% NaCl	-	+	+	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-
6,5% NaCl	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T° 4°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
T° 15°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
T° 37°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
T° 45°C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
63,5°/30min	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
55°C/15min	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
pH 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
pH 6,5	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acid production from: Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Galactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fructose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arabinose	+	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-
Xylose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Maltose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Aesculin	+	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Rhamnose	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Sobitol	+	-	-	+	-	+	+	+	+	+	+	+	-	-	-	-	-	-

Symbols: +, positive reaction, -, negative reaction.

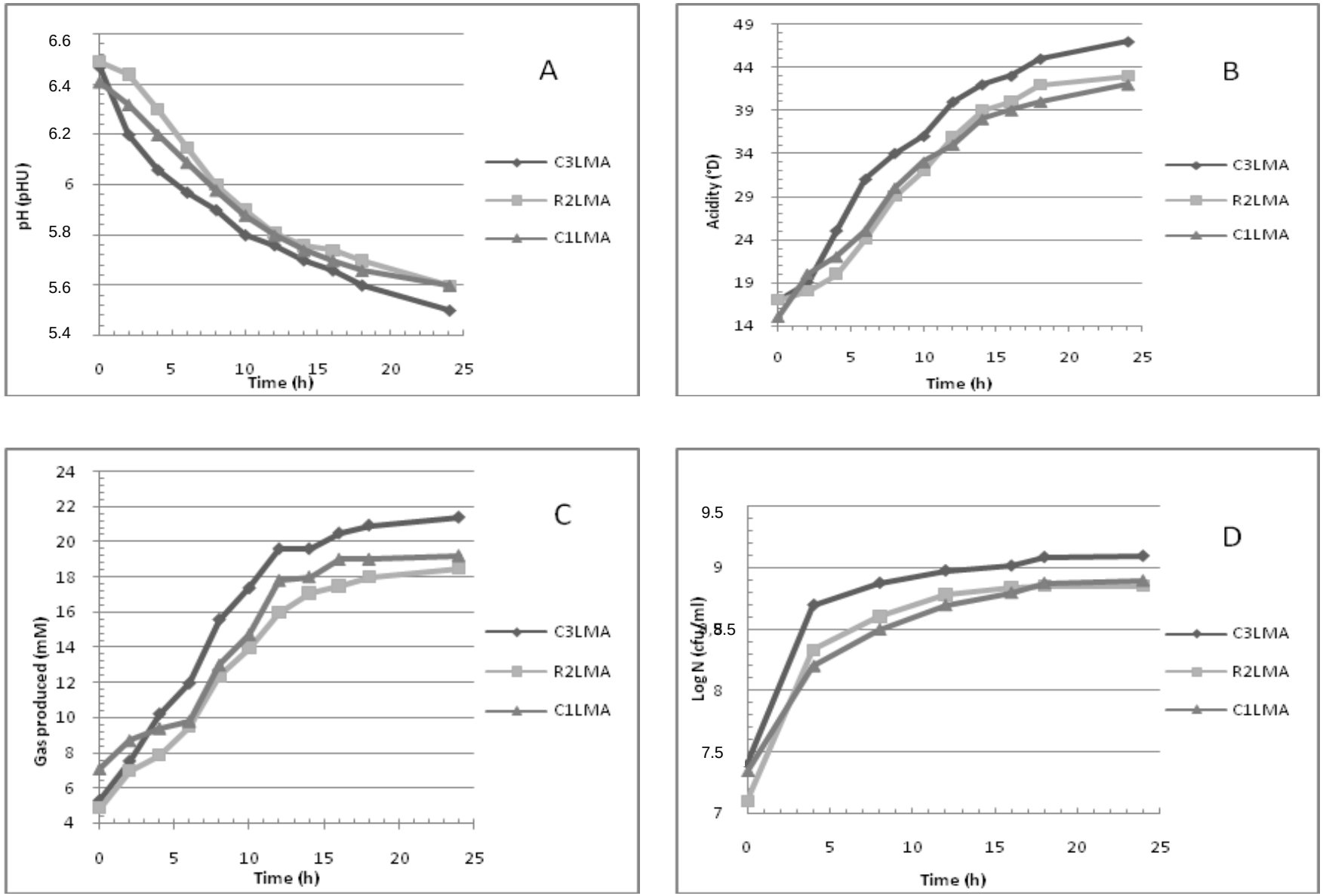

Figure 2. Kinetics of growth of *Leuconostoc* strains, decrease in pH (A), evolution of titrable acidity (B), CO_2 production (C), bacterial growth (D) of strains: C3 LMA: *Leuconostoc mesenteroides* subsp. *dextranicum,* C1 LMA*: Leuconostoc mesenteroide*s subsp. *mesenteroides*, R2 LMA: *Leuconostoc mesenteroides* subsp. *mesenteroides* in milk medium without yeast extract.

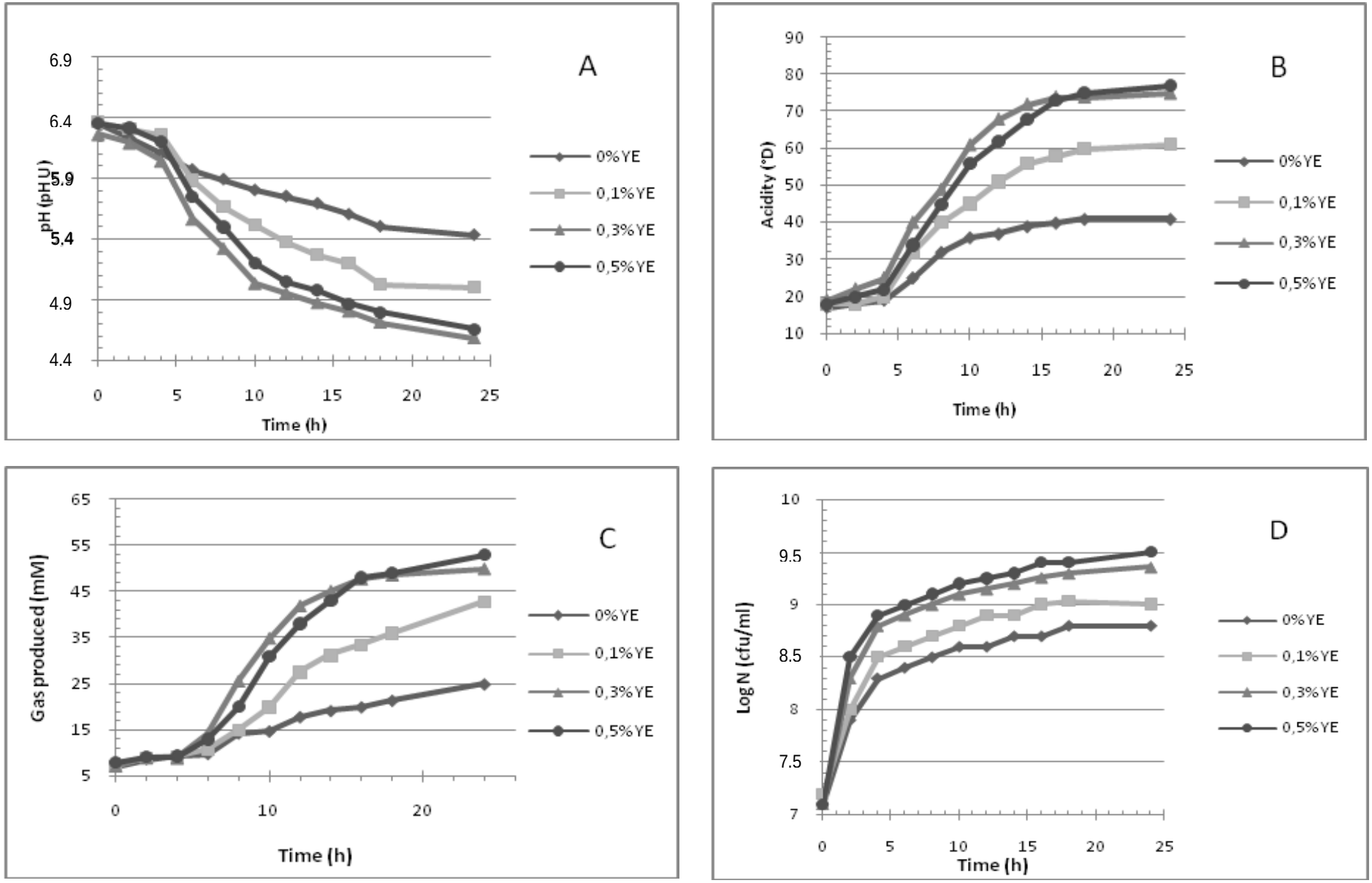

Figure 3. Kinetics of growth of *Leuconostoc* strains, decrease in pH (A), evolution of titrable acidity (B), CO_2 production (C), bacterial growth (D) of *L. mesenteroides* subsp. *mesenteroides* (strain C7 LMA) in skim milk at 0%, 0.1%, 0.3% and 0.5% yeast extract.

Table 2. Test of antibiotics susceptibility of *Leuconostoc mesenteroides* species isolated from goat and camel milk collected in Algeria.

Antibiotics	Disk load (µg)	Symbol	C7LMA		C3LMA		R2LMA		CH11LMA	
Amikacin	30	AN	16	R	14	R	20	I	18	I
Amoxycillin + Clavulanic Acid	20 + 10	AMC	29	S	28	S	17	R	24	S
Ampicillin	10	AM	0	R	28	S	27	S	24	S
Bacitracin	0,02 à 0,04 IU	BAC	14	R	14	R	14	R	14	R
Cefazolin	30	CZ	18	R	15	R	18	R	13	R
Cefotaxim	30	CTX	19	I	23	S	25	S	19	I
Cefoxitin	30	FOX	22	S	23	S	16	I	18	I
Cefsulodin	30	CFS	12	R	12	R	11	R	10	R
Ceftazidim	30	CAZ	10	R	9	R	0	R	0	R
Cephalothin	30	CF	24	S	27	S	32	S	28	S
Ciprofloxacin	5	CIP	12	R	14	R	19	R	14	R
Clindamycin	2	CM	25	S	22	S	34	S	26	S
Colistin	50	CS 50	9	R	10	R	13	R	12	R
Erythromycin	15	E	26	S	25	S	31	S	25	S
Fusidic Acid	10	FA	17	I	16	I	18	I	18	I
Imipenem	10	IPM	27	S	24	S	24	S	23	S
Lincomycin	15	L	29	S	27	S	36	S	27	S
Nalidixic Acid	30	NA	0	R	0	R	0	R	0	R
Netilmicin	30	NET	18	R	18	R	20	R	20	R
Nitrofurantoin	300	FT	0	R	28	S	27	S	25	S
Ofloxacin	5	OFX	13	R	16	R	21	R	14	R
Oxacillin	1	OX1	0	R	0	R	0	R	0	R
Pefloxacin	5 µg	PEF	0	R	0	R	14	R	10	R
Penicillin	6 µg / 10 IU	P	29	S	29	S	36	S	29	S
Piperacillin	75	PIP 75	22	S	26	S	28	S	25	S
Pristinamycin	15	PT	31	S	25	S	40	S	27	S
Rifampin	30	RA 30	31	S	34	S	40	S	30	S
Spiramycin	100	SP	20	I	20	I	26	S	18	I
Tetracyclin	30	TE	28	S	30	S	40	S	26	S
Ticarcillin	75	TIC	30	S	30	S	40	S	33	S
Tobramycin	10	TM	12	R	12	R	16	R	14	R
Trimethoprim-Sulfamethoxazole (co-trimoxazole)	1.25 + 23.75	SXT	0	R	0	R	0	R	0	R
Vancomycin	30	VA	0	R	0	R	0	R	0	R

subsp. *mesenteroides and L. mesenteroides* subsp. *dextranicum* were conducted using 33 antibiotics with different doses On MRS agar. The four strains tested are almost sensitive to cephalothin, clindamycin, erythromycin, imipenem, lincomycin, penicillin, piperacillin, pristinamycin, rifampin, tetracyclin, ticarcilline, which is similar to the work of Milliere et al. (1989); Kihal. (1996), while some strains possess an intermediate profile. *L. mesenteroides* subsp. *mesenteroides* (C_7 LMA) is resistant to ampicillin and nitrofurantion unlike other strains, which are sensitive. The observed results indicate that the subspecies are resistant to vancomycin.

According to Hemme et al. (2004), the resistance of *L. mesenteroides* subsp. *mesenteroides to* vancomycin is usually an intrinsic characteristic, which is related to the presence of pentapeptides with D-lactate related to C-terminus instead of D-alanine in the peptidoglycan composition, which prevents the penetration of the antibiotic and consequently cell lysis. In addition to vancomycin, the strains have a polyresistance to 13 antibiotics: nalidixic acid, oxacillin, trimethoprim/ sulfamethoxazole, bacitracin, cefazolin, cefsulodin, ceftazidime, ciprofloxacin, colistin, tobramicine, pefloxacin, oflaxacine, netilmicin. According to Dalache et al. (2003), this polyresistance is commonly attributed to plasmids or transposons in many bacterial species. For all antibiotics tested, there was no great difference between the strains studied. The results of antibiogram are similar to those found by Milliere et al. (1989); Swenson et al. (1990); Philippon et al. (2008).

Conclusion

Given their resistant character to vancomycin, the strains of *Leuconostoc* were isolated and selected from goat milk, camel milk and Roquefort cheese using MSRV and MSE. Microbiological, biochemical and physiological tests isolates have identified two sub-species *L. mesenteroides* subsp. *mesenteroides* and *L. mesenteroides* subsp. *dextranicum*. The strains present very important technological characteristics such as production of dextran, production of CO_2 and thermotolerance at 55°C for 15 min. The study of the kinetics of growth, production of CO_2, decrease in pH and titrable acidity on skim milk of two subspecies found that *L. mesenteroides* subsp. *dextranicum* has a growth rate, acidifying activity and a CO_2 production, higher than those of *L. mesenteroides* subsp. *mesenteroides*. Monitoring the same parameters for 24 h in the kinetics of *L. mesenteroides* subsp. *mesenteroides* in milk supplemented with different concentrations of yeast extract demonstrated that the addition of 0.3% of this component gives a great stimulation of bacterial development, gas and acids production. The antibiogram showed that in addition to Vancomycin, the strains have a polyresistance to 13 antibiotics from 33 antibiotics used.

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