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Full Length Research Paper

Characterization of lactic acid bacteria isolated from Algerian arid zone raw goats' milk

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Diversity and density of lactic acid bacteria isolated from Algerian raw goats' milk in arid zones were studied by determination of morphological, cultural, physiological and biochemical characteristics. 206 lactic acid bacterial strains were isolated, with 115 of them belonging to lactic acid cocci and others to the genus, *Lactobacillus*. The representative species of the total cocci were *Lactococcus* sp. (76.16%), *Streptococcus thermophilus* (14.78%) and *Leuconostoc* sp. (8.6%). The dominating species is *Lactococcus lactis* subsp. *Lactis*. Lactobacilli species found in local raw goats' milk and their proportion were: *Lb. curvatus* (25.25%), *Lb. helviticus* (10.98%), *Lb. plantarum* (9.89%), *Lb. reuteri* (9.89%), *Lb. casei* (7.69%), *Lb. brevis* (5.49%), *Lb. bulgaricus* (5.49%) *Lb. paracasei* (4.39%) and *Lb. acidophilus* (2.19%).

Key words: Lactic acid bacteria, raw milk, goat, arid zones, identification.

INTRODUCTION

Interest in microorganisms as a component of biological diversity has been renewed in recent years (Alsopp et al., 1995). The interest in microorganisms occurring in foods is primarily due to the biotechnological potential of new bacterial species and strains (Leisner et al., 1999). Lactic acid bacteria (LAB) widely distributed in the nature and occuring naturally as indigenous microflora in raw milk are Gram-positive bacteria that play an important role in many food and feed fermentations. In this group are included representatives of the genus *Lactobacillus*, *Lactococcus*, *Pediococcus* and *Leuconostoc*. The lactic acid fermentation, which these bacteria carry out, has long been known and applied by humans for making different foodstuffs. For many centuries LAB have served to provide an effective form of natural preservation. In addition, they strongly determine the flavour, texture and, frequently, the nutritional value of food and feed products.

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In Algeria, dairy products are prepared from cow and goat's milk including cheese and curds (leben and rayeb). In these products, the species composition of lactic acid bacteria is more varing and inconsistent when compared with those of the trade products. In biotechnological aspect, the "wild" strains of the LABs are prospective bacteriocin producers (Niku-paavola et al., 1999; Park et al., 2003) and probiotics (Rinkinen, 2003)

The aim of this work was the isolation and taxonomic determination of a large number lactic acid bacteria from goat's raw milk in order to constitute an original collection of LAB strains and to use them as starter for different traditional fermented products such as leben and rayeb.

MATERIALS AND METHODS

Isolation of bacterial strains and culture conditions

The lactic acid bacteria were isolated form goat's milk in Algerian arid zone (Béchar, Saïda, Ghardaïa, Béni-Abbès, Tamanrasset and Tiaret region). The isolation was performed by the routine microbiological procedure and inoculation on a solid medium. Selective media for lactic acid bacteria used were MRS and M17

agar plates. Ten fold dilutions of milk samples were spread on the surface of these media. The cultivation was performed without shaking at the appropriate temperature (32 and 40°C) from one to five days. Once single colonies were obtained, eight colonies were randomly selected from high dilution in the MRS or M17 agar plates. Strains were grown in MRS broth at 25°C. Purity was checked by streaking on suitable agar medium. Strains were kept in MRS broth plus 20% glycerol at –20 °C and subcultured every six month. Working cultures were also kept on MRS agar slants at 4°C and subcultured every 4 weeks.

Physiological and biochemical tests

Each strain under examination was subcultured twice overnight in MRS broth. All strains were initially tested for Gram reaction, catalase production and spore formation (Harrigan and McCance, 1976). Cell morphology and colony characteristics on MRS agar were also examined, and a separation into phenotypic groups was undertaken. Only the Gram-positive, catalase-negative isolates (Sharpe, 1979) were further identified. Growth at different temperatures was observed in MRS broth after incubation for 5 days at 15°C, 37°C and 45°C. Supplemented test was performed for bacterial cocci and resistance at 60°C for 30 min was done in order to discard enterococcus bacteria. Hydrolysis of arginine was tested in M16BPC (Thomas, 1973; Thomas and Pritchard, 1987). Growth in the presence of 4 and 6.5% NaCl was performed in MRS broth for 5 days. Utilization of citrate was realised in Kempler and Mc Kay (1980) medium. Production of acetone from glucose was determined using Voges-Proskauer test (Samelis et al., 1994) .Production of dextrane from sucrose was done in MRS agar (Mayeux et al., 1962).

For performing the biochemical tests, an MRS-BCP broth medium (BCP 0.17 g/l) was used. The carbon source was added to the sterile basal medium as filter sterilized solution to a final concentration of 1%. Carbohydrates utilization was assessed at the 24 and 48th h. All strains were tested for fermentation of the following 14 sugars: L-Arabinose, Ribose, D-Xylose, Galactose, D-Fructose, Mannitol, Sorbitol, Cellobiose, Maltose, Lactose, Mélibiose, Saccharose, Tréhalose, and D-Raffinose. To ensure anaerobic conditions, two drops of sterile liquid paraffine were placed in each tube after inoculation. For further identification of the lactic acid bacteria, API 50 CH tests (bioMerieux) were also used.

RESULTS

All 206 isolates were Gram-positive and catalase-negative and nonspore-forming bacteria. The lactic isolates were further characterised as (1) mesophilic homofermentative cocci, 88 isolates (Table 1); (2) mesophilic heterofermentative cocci; 10 isolates; (3) thermophilic homofermentative cocci; 17 isolates.

In group 1, on the basis of arginine hydrolysis we distinguish three subgroups. The first subgroup represented by ADH+ (positive arginine hydrolysis) contain 39 species with positive acetone production and citrate utilization. These were identified as *Lactococcus lactis* subsp*. lactis biovar diacetylactis* (44.32%). The second subgroup is represented by the remainder of the ADH+ species (35 species). Those strains were identified as *Lactococcus lactis* subsp*. lactis* (39.77%). The last subgroup is represented by 14 strains ADH- and belong to *Lactococcus lactis* subsp. *cremoris*.

For the group 2, all strains were closely related to Leuconostoc bacteria and divided in two subgroups. The first one represented by two strains (20%) presented a reduced fermentative profile; do not hydrolyse arginine and inability to produce dextrane production, were identified as *Leuconostoc mesenteroïdes* subsp. c*remoris.* Among the isolates (80%) in the second subgroup, the dominant species is *Leuconostoc mesenteroïdes* subsp. *Mesenteroïdes*.

All strains of group 3 promoted growth at thermophilic conditions and do not grow in Sherman medium, and so they belonged to *Streptococcus thermophilus*.

Lactobacilli bacteria (91 isolates) was represented by 3 groups; (1), mesophilic homofermentative lactobacilli, 53 isolates; (2), thermophilic homofermentative lactobacilli, 20 isolates and (3) mesophilic heterofermentative lactobacilli, 18 isolates. Group 1 is represented by *Lb. plantarum* (9 isolates, 9.89%), *Lb. casei* (7 isolates, 7.69%), *Lb. curvatus* (23 isolates, 25.27%), *Lb. rhamnosus* (10 isolates, 10.98%) and *Lb. paracasei* (4 isolates, 4.39%). Group 2 include *Lb. acidophilus* (2 isolates, 2.19%); *Lb. helviticus* (10 isolates, 10.98%), *Lb. delbrueckii* ssp. *bulgaricus* (5 isolates, 5.49%) and *Lb. delbrueckii* ssp. *lactis* (3 isolates, 3.29%). Group 3 contain *Lb. brevis* (5 isolates, 5.49%), *Lb. reuteri* (9 isolates, 9.89%) and *Lb. fermentum* (4 isolates, 4.39%)

DISCUSSION

All 206 isolates were Gram-positive. In the lactobacilli isolates, especially for mesophilic homofermentative lactobacilli group, we noted that it was represented by five species. On the basis of melibiose fermentation and arginine hydrolysis (Schillinger and Lucke, 1987; Montel et al., 1991), 23 isolates were identified as *Lb. curvatus*. The above results are in agreement with those obtained by other workers where such mesophilic lactobacilli have been found to dominate in vacuum packed and fermented meats (Kitchell and shaw, 1975; Hitchener et al., 1982). It is well established that such isolates are currently classified to *Lb. curvatus* and *Lb. sake*. Seven isolates were identified as *Lb. casei* and 4 as *Lb. paracasei* according to Collins et al. (1989) whom created the new species *paracasei* to include all genetically distinct strains previously regarded as subspecies of *Lb. casei*. Nine isolates are capable of fermenting all common sugars (Table 1). Colonies were easily distinguished from all other groups since they were larger, more convex and shiny or cheesy white. A supplementation test mannose and melizitose fermentation permitted the identification of 5 isolates as *Lb. brevis*; the use of the second carbohydrate is to differenciate *Lb. buchneri* from the *Lb. brevis* species (Morishita and shiromizu, 1986; Samelis et al., 1994). Those species are commonly met in the later phase of the maturing of the cheese, together with *Lb. casei*, *Lb. brevis* and *Lb. buchneri* (Hammes et al., 1999). Olarte

Table 1. Morphological, cultural, physiological and biochemical characteristics of the isolated strains.

		cocci						lactobacilli		
Group		**1**			**2**		**3**	**1**	**2**	**3**
No. of isolates		35	39	14	8	2	17	53	20	18
Gram stain reaction		G+	G+	G+	G+	G+	G+	G+	G+	G+
Spores formation		-	-	-	-	-	-	-	-	-
Catalase activity		-	-	-	-	-	-	-	-	-
CO_2 from glucose			-			+	-	-	-	+
NH_3 from arginine		+	+	-	-	-	6	-	-	10
Growth at temperature (°C)	15	+	+	+	+	+	-	-	-	-
	37	+	+	+	+	+	+	+	+	+
	45	-	-	-	-	-	+	-	+	-
Growth in a medium with NaCl (%)	4	+	+	-	+	-	-	+	+	+
	6.5	-	-	-	+	-	-	+	+	+
Production of										
Dextrane from sucrose		-	-	-	+	-	-	-	-	-
Acetoine from glucose		+	+	-	-	+	-	-	-	-
Sugar Fermentation										
Arabinose		11	13	4	+	-	5	15	2	10
Ribose		33	+	+	+	+	5	47	+	13
Xylose		2	3	-	+	-	-	30	3	-
Galactose		34	37	+	+	1	5	50	14	12
Fructose		+	30	+	+	-	7	51	18	+
Mannitol		31	29	13	+	-	-	44	4	16
Sorbitol		6	11	-	-	-	-	35	6	7
Cellobiose		33	21	12	+	-	3	+	4	+
Maltose		+	31	11	+	1	2	49	11	13
Lactose		33	+	13	+	+	+	52	18	12
Melibiose		25	35	2	5	+	+	21	1	7
Saccharose		23	22	6	+	-	16	33	2	16
Trehalose		32	+	+	+	+	+	27	17	7
Raffinose		1	1	-	+	-	-	8	-	-

Positive reaction (+), negative reaction (-), The number designate the amount of positive strains.

(2000) noted that the presence of *Lb. plantarum* in the cheese (Cameros) from goat's milk decreased the number of the enterobacteria and fecal coliforms in the final product. For the last group, thermophilic lactobacilli obligate homofermentative, we identified 10 isolates. This species is included in starter cultures during the production of the cheese Gruyere, Gorgonzola, Mozarella (Hammes et al., 1999).

In cocci groups, 8 isolates were positively identified as *Leuconostoc mesenteroides* subsp. *mesenteroides* (Garvie, 1986; Schillinger et al., 1989). The rest of strains in this group are identified as *Leuconostoc mesenteroides* subsp *cremoris* . The lower numbers of leuconostocs is probably due o their inability to compete with other LAB in mixed cultures (Teuber and Geis, 1981; Togo et al., 2002)

The identified isolates will undergo tests for lactic acid production and selected for further tests (production of bacteriocins and volatile compounds) to assess their potential as starter culture to preparing traditional fermented milk from raw goat milk in arid zones. The LAB starter can contribute to reducing spoilage problem encountered in this domestic fermentation.

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