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Antibacterial effects of supernatant of lactic acid bacteria isolated from different Dough's in Gorgan city in north of Iran

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Abstract

This study was conducted with the aim of isolation of Lactic Acid Bacteria (LAB) from dough and their effects on the main pathogenic bacteria in intestine. Morphological, cultural and biochemical characteristics were employed to identify lactic acid bacteria, isolated from dough in different areas in Gorgan city, Iran. From 13 traditional dough and 2 industrial dough samples a total of 35 isolates were isolated, 32 isolates from local dough and 3 isolates from industrial dough. The results showed that *Lactobacillus casei* has the highest frequency. Also this species showed antagonistic activity against pathogens including *Escherichia coli, Staphylococcus aureus, Bacillus cereus* and *Citrobacter freundii*, with an inhibition zone diameter of 17 mm.

Introduction

Interest in microorganisms occurring in foods is primarily due to the biotechnological potential of new bacterial species and strains [1]. Lactic acid bacteria (LAB) are widely distributed in nature and occur naturally as indigenous microflora in raw milk, drinking yoghurt, etc. Lactic acid bacteria are a group of gram-positive bacteria including the genera *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus,* and *Streptococcus* and play an important role in many food fermentation processes. The lactic acid fermentation has long been known and applied by humans for making different food stuffs. In addition, they strongly determine the flavour, texture and frequently, the nutritional value of food and feed products [2-4]. In fermented foods, LAB display numerous antimicrobial activities. This is mainly due to the production of organic acids and other compounds, such as bacteriocins and antifungal peptides [5]. Several studies have shown the inhibitory activities of numbers of LAB such as *Lactobacillus brevis* isolated from Turkish dairy products [6] and *Lactobacillus acidophilus* isolated from Iranian yoghurt against *Staphylococcus aureus* [7].

According to reports, it appears that Middle East is the origin of fermented dairy products mainly yoghurt [8]. In Iran, a number of traditional dairy products are consumed of which yoghurt, well known, as Mast is one of the most popular fermented milk products. While, traditionally made sour buttermilk especially made from ewe milk is more common in rural areas of the country [9].

It is a well-established fact that the composition of LAB in these traditional dairy products is varied and inconstant. In Iran, there are different kinds of traditional dairy products which are produced from cow, camel, sheep and goat milk such as drinking yoghurt, yoghurt, kashk, gharaghooroot, cheese, etc. In comparison with the commercial species, composition of lactic acid bacteria is more varied and inconstant in these products. The main objective of present study was to investigate LAB of traditional dough, which might provide important information regarding its probiotic potential and its utilization in the future.

Material and methods

Dough samples

During the spring of 2014, a total of 15 dough (13 traditional and 2 industrial dough samples) were collected from Gorgan city, Iran. The samples were collected in sterile universal tubes and kept cool until they could be taken to the laboratory, where they were kept at 4°C for further use.

Isolation of lactic acid bacteria

The samples were aseptically weighted and homogenazied. From each sample, a 1:10 dilution was subsequently made using peptone water followed by making a 10 fold serial dilution. 1 ml from each dilution was then subcultured, in duplicate, into the Plate Count Agar and MRS agars (Merck, Germany) used for isolating LAB [10] and were incubated anaerobically using the Gas Pack system (Merck Anaerocult type A) at 37°C for 2 days.

Identification of the bacterial strains

All strains were initially tested for gram reaction, catalase production and spore formation [11]. Colonies were characterized on MRS Broth and M17 Broth. Strains with gram positive and catalase negative reactions were finally used for further identification. Growth at different temperatures (10°C and 45°C) for 2 days, growth in the

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presence of 6.5% NaCl, were considered to identify the strains.

All strains were also tested for fermentation of L-arabinose, D-xylose, galactose, D-fructose, sorbitol, lactose, maltose, mannitol, raffinose, sucrose, rhamnose and mannose [3].

Staphylococcus aureus (PTCC 1431), *Escherichia coli* (PTCC 1399), *Bacillus cereus* (ATCC 1252) and *Citrobacter freundii* (1600) were used as indicator culture. As mentioned earlier, the culture broths of both the producer and indicator strains were adjusted to McFarland Index 0.5 (1.5×10^8) to use. The growth of Lactic acid bacteria strains isolated from dough samples at 37°C was visually confirmed by the changes in turbidity of Muller Hinton Agar or BHI after 24h of incubation.

The surface of Muller Hinton Agar plates were evenly streaked with selected indicator strains, with a sterile cotton swab. The culture broth of the producer strains (100 ul) were poured into the wells (7 mm) made in these agar plates with a sterile borer. All plates were stored for 2h at 4°C prior to incubation at 37°C for 24h. The antimicrobial activity was recorded as appearance of clear zone around the wells and the zone diameter (Resistance: 7 mm>zone, Inter mediate: 8-9 mm<zone, Sensitive: 10 mm<zone) measured in millimeter. All tests were run in duplicate.

Results and discussion

During the spring of 2014, a total of 15 dough samples (13 traditional and 2 industrial dough samples) were collected from Gorgan city (Table 1).

Microbial count

The results showed that the average number of microbial count in Plate count agar is the maximum amount in Cow samples ($6/8 \times 10^8$ CFU/ml) and minimum amount in industrial samples ($2/2 \times 10^8$ CFU/ml) (Table 2). Kiai *et al.* 2006 have reported in previous studies, the microbial count in traditional dough in Golestan province between CFU/ml $7/4 \times 10^9$ and $3/92 \times 10^9$ CFU/ml [12].

Also average number of microbial count in MRS Agar is the maximum amount in water buffalo samples ($6/7 \times 10^8$ CFU/ml) and minimum amount in cow samples ($1/8 \times 10^8$ CFU/ml) (Table 3), that has been reported in previous studies on local yogurts, between CFU/ml $3/6 \times 10^9$ and $7/2 \times 10^9$ CFU/ml [12]. The daily intake of 10^8 to 10^9 live bacteria, is the minimum acceptable value, therefore daily intake of 100 grams of probiotic product containing up to 1×10^6 - 5×10^8 Cfu live bacteria per gram of product, can provide the optimum desired [13].

Table 1. Type and number of dough samples collected from the Gorgan city.

NO.	Type	Number of samples
1	Cow	3
2	Sheep	3
3	Water buffalo	2
4	Camel	4
5	Goat	1
6	Industrial	2

Table 2. Average counts of lactic acid bacteria in dough samples in Plate count agar in Gorgan city.

NO.	Type	Plate count agar
1	Cow	$6/8 \times 10^8$
2	Sheep	$3/3 \times 10^8$
3	Water buffalo	$3/4 \times 10^8$
4	Camel	$3/5 \times 10^8$
5	Goat	3×10^8
6	Industrial	$2/2 \times 10^8$

Table 3. Average counts of lactic acid bacteria in dough samples in MRS Agar in Gorgan city.

NO.	Type	Plate count agar
1	Cow	$1/8 \times 10^8$
2	Sheep	$3/3 \times 10^8$
3	Water buffalo	$6/7 \times 10^8$
4	Camel	$3/7 \times 10^8$
5	Goat	$4/1 \times 10^8$
6	Industrial	$3/9 \times 10^8$

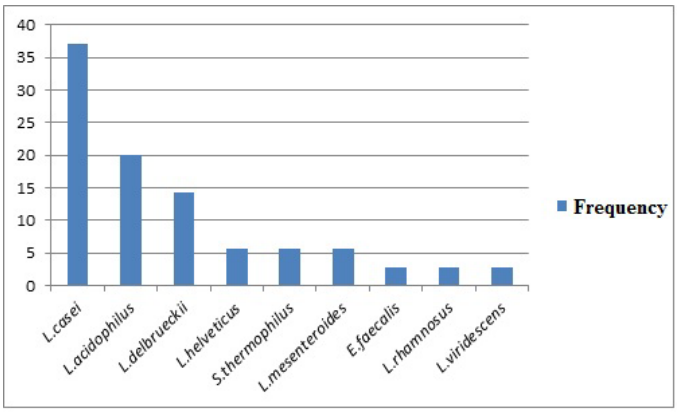

Figure 1. Frequency of lactic acid bacteria isolated from the dough samples in Gorgan city.

Isolation and identification of lactic acid bacteria

35 species of lactic acid bacteria were isolated and identified from 15 samples of dough that the types and numbers of these bacteria shown in Tables 4 and 5, according to type of dough.

The highest frequency of lactic acid bacteria isolated from the dough samples, is belongs to the species include *Lactobacillus casei*, *Lactobacillus acidophilus* and *Lactobacillus Delbrueckii*. It is shown the number and percentage of different species of lactic acid bacteria isolated from dough samples, in Figure 1.

According to Table 5, the maximum number of lactic acid bacteria is in sheep and the minimum number is in camel dough. Any kind of lactic acid bacteria have been isolated from camels dough due to high yeast.

Antagonistic effect of LAB

Lactic acid bacteria isolated from dough samples showed antagonistic activity against pathogenic bacteria with the production of the antimicrobial compound. The results are shown in Table 6. *Lactobacillus casei*, *Lactobacillus delbrueckii* and *Lactobacillus rhamnosus*, isolated from sheep dough, inhibit the growth of all pathogenic bacteria. *Lactobacillus casei* has the highest antibacterial activity against pathogenic bacteria.

Lactobacillus acidophilus isolated from sheep and goat dough, have no antimicrobial effect on pathogenic bacteria. So in total, the lactic acid bacteria isolated from sheep dough, produce greater antimicrobial compounds against pathogenic bacteria.

Table 4. Identification of lactic acid bacteria based on morphological and biochemical tests.

Biochemical tests / Lactic Acid Bacteria	Sucros	Lactose	Maltose	Raffinose	Rhamnose	Mannose	Mannitol	Fructose	Sorbitol	Galactose	Xylose	Arabinose	Growth at		
													Nacl %6/5	10^0c	45^0c
L. casei	+	+	+	-	-	+	+	+	+	-	-	+	-	+	-
L. acidophilus	+	+	+	+	+	-	+	-	+	-	+	-	+	-	-
L .fermentum	+	+	-	+	-	+	+	-	+	+	+	_	-	+	-
L. delbrueckii	-	-	-	-	+	+	-	+	-	+	-	+	-	-	-
L.helveticus	+	+	+	-	-	-	-	-	+	-	-	+	-	+	-
L. viridescens	-	-	+	-	-	+	-	-	-	-	-	+	+	+	+
L. rhamnosus	+	+	+	+	+	+	+	-	+	-	+	-	+	+	+
L. mesenteroides	+	+	+	-	-	+	-	-	-	+	-	+	-	+	+
S. termophilus	+	+	+	+	+	+	+	+	+	-	+	_	+	-	-
E. fecalis	-	+	+	-	-	+	-	-	-	+	-	-	+	+	+

Table 5. Types and number of lactic acid bacteria isolated from dough in Gorgan city on the type of livestock.

NO.	Type of livestock	Number of samples	Lactic acid bacteria	
			Number	Type
1	Cow	3	4	*L. casei*
			3	*L. acidophilus*
			1	*L. fermentum*
2	Sheep	3	4	*L. delbrueckii*
			2	*S. thermophilus*
			1	*L. mesenteroides*
			1	*E. faecalis*
			1	*L. casei*
			1	*L. acidophilus*
			1	*L. rhamnosus*
			1	*L. helveticus*
3	Water buffalo	2	8	*L. casei*
			1	*L. delbrueckii*
			1	*L. acidophilus*
			1	*L. helveticus*
4	Camel	4	0	-
5	Goat	1	1	*L. acidophilus*
6	Industrial	2	1	*L. acidophilus*
			1	*L. viridescens*
			1	*L. mesenteroides*

Kazemi *et al,* 2010, in the same study found the opposite result and *Lactobacillus acidophilus* showed the highest inhibitory effect with an inhibition zone diameter of 14 mm [14].

Conclusion

The fermented dairy products can be possibly a good source of potential probiotic organisms. In Iran, a number of researchers have reported the isolation of LAB from dairy products like butter, kashk and cheese [15]. However, there are some fermented dairy products in Iran, which have yet not been evaluated for their health benefit, mainly their probiotic properties. Lactic acid bacteria display numerous antimicrobial activities in fermented foods. This is mainly due to the production of organic acids, but also of other compounds, such as ethanol, H_2O_2, diacetyl, reuterin and bacteriocins. Several bacteriocins with industrial potential have been purified and characterized. Application of bacteriocin-producing starter cultures in fermented

Table 6. Results in inhibition of lactic acid bacteria isolated from traditional dough against pathogenic bacteria.

Pathogenic bacteria / LAB	*Escherichia coli*	*Bacillus cereus*	*Citrobacter freundii*	*Staphylococcus aureus*
L. casei from sheep sample	16.33 ± 0.57^{ab}	16.33 ± 0.57^{ab}	18.33 ± 0.57^{a}	13 ± 3.6^{b}
L. casei from water buffalo sample	13 ± 0^{a}	12.66 ± 0.57^{a}	9.33 ± 0.57^{b}	13 ± 0^{a}
L. casei from cow sample	-	11.33 ± 0.57^{a}	-	-
L. acidophilus from industrial sample	9.66 ± 1.15^{a}	12 ± 2^{a}	11 ± 0^{a}	9 ± 0^{a}
L. acidophilus from sheep sample	10.33 ± 3.21^{a}	12 ± 0^{a}	13 ± 0^{a}	9 ± 0^{a}
L. acidophilus from water buffalo sample	9 ± 0^{a}	-	9 ± 0^{a}	-
L. acidophilus from cow sample	-	-	-	-
L. acidophilus from goat sample	-	-	-	-
L. delbrueckii from water buffalo sample	-	15 ± 0^{a}	9 ± 0^{b}	-
L. delbrueckii from sheep sample	11 ± 0^{a}	13 ± 0^{a}	12.66 ± 3.51^{a}	11 ± 2^{a}
L. helveticus from water buffalo sample	-	15 ± 0^{a}	-	10 ± 0^{b}
L. helveticus from sheep sample	-	-	12 ± 0^{a}	-
L. rhamnosus from sheep sample	16 ± 0^{a}	13.66 ± 3.05^{a}	15.66 ± 4.04^{a}	14.33 ± 1.15^{a}
L. fermentum from cow sample	15 ± 0^{b}	16 ± 0^{a}	9.66 ± 0.57^{c}	9 ± 0^{c}
L. viridescens from industrial sample	16 ± 0^{a}	13 ± 0^{b}	10 ± 0^{c}	-
L. mesenteroides from sheep sample	-	11 ± 0^{a}	-	-
L. mesenteroides from industrial sample	-	10.5 ± 0.7^{a}	-	9 ± 0^{a}

foods has been studied during in vitro laboratory fermentations as well as on pilot-scale level. The promising results of these studies underline the important role that lactic acid bacteria may play in food industry as starter cultures to improve food quality and safety.

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