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The Prevalence of Lactic Acid Bacteria in Sausages at Khartoum State

Nagwa B. Elhag[1], El Rakha B. Babiker[2], Ahmed A. Mahdi[3]

[1]Department of Food Hygiene and Safety, College of Public & Environmental Health, University of Bahri, Khartoum North, Sudan

[2]Food Research Centre, Microbiology and Biotechnology Department, Agricultural Research Corporation, Shambat, Sudan

[3]Department of Botany and Agricultural Biotechnology, Faculty of Agriculture, Khartoum University, Shambat, Sudan

Abstract: ***Lactic acid bacteria (LAB) are useful microorganisms. They have been used in food preparation thousands of years ago. A total of 40 fresh sausage samples were collected randomly from 8 sources in Khartoum State, Sudan. These sources include (a) butcheries in Khartoum, Khartoum north, and Omdurman, (b) factory- processed sausages at retail outlets and (c) home-made sausages. The sausage samples were studied to count and identify LAB species associated with sausages samples. LAB counts were carried out on de Man and Rogosa (MRS) agar medium. The pure isolates of LAB isolates were characterized by using morphological properties, biochemical tests and their ability to ferment different sugars. The mean counts of LAB in butcheries samples ranged from 9.30x10^{6}cfu/g to 3.64x 10^{7}cfu/g, which was higher than the mean of factory-processed sausages (mean 2.12 x 10^{5}cfu/g - 4.41 x 10^{6}cfu/g), which was, in turn, higher than the load shown by home-made sausages (mean 5.04 x 10^{5}cfu/g). LAB isolates were identified as Streptococcus cremoris (40% of isolates), Enterococcus faecalis (20%), Lactobacillus acidophilus (15%), Lactobacillus delbruckii subsp. lactis (10%), Lactobacillus delbruckii subsp. Bulgaricus (7.5%), Lactobacillus jenseny, Lactobacillus vitulinus, and Streptococcus avium, each representing 2.5% of the isolates. The high counts of LAB and the prevalence of beneficial LAB in sausage samples make this product a good source for industrial LAB which can be applied in the production of a wide range of fermented foods and pharmaceuticals.***

Keywords: Beneficial LAB bacteria, Characterization of LAB, *Enterococcus*, *Streptococcus cremoris*, Fresh Sudanese sausages

1. Introduction

Lactic acid bacteria (LAB) are used for a long a time by man. LAB play an important role in food industry and food preservation. They are gram-positive, catalase negative, non spore-forming and anaerobic cocci or rod bacteria. These bacteria divided into homo-fermentative and hetero-fermentative according to the final product produced during fermentation [1]. LAB live in different environments rich in nutrients such as milk and milk products, meat, fermented products, beverages and vegetables. Also they will exist in soil, water, manure, sewage [2] and human [3]-[4]-[5]. Many researches were carried out on LAB and their benefits in different fields such as their role in production of fermented products as starter cultures to inhibit the spoilage bacteria and enhance the organoleptic characters of the final product [6], their ability to be use as propiotics [7], reduce cholesterol level [8], control intestine disorder [9], produce small organic compounds responsible for organoleptic properties [10], improve the immune system [11] and their role in processing of animal feeds like silage [12]-[13]. Beneficial LAB bacteria, especially *Lactobacillus* species can produce antimicrobial substances inhibit the growth of some pathogenic microorganisms [14]. As reported by [15] th these beneficial microorganisms are found to be most effective during periods of disease or stress and following antibiotic treatment. Also it has been recorded that they have ability to spoil different products such as meat, fish and beverage [16]-[17]. The aim of this research was the isolation and identification of LAB. To our best knowledge this the first study for isolation and identification of LAB from sausage samples.

2. Materials and methods

2.1. Sampling

A total of 40 sausage samples were collected from different eight sources: (a) butcheries in Khartoum, Khartoum North and Omdurman, (b) factory-processed sausages (F1, F2. F3, F4 factories) collected at retail outlets, and (C) homemade from household in Khartoum. Samples were collected in sterile ice cooled container and immediately transferred to the laboratory for microbiological analysis.

2.2 Preparation of serial dilution, enumeration and isolation of LAB

Thirty grams of each sample of fresh sausages were vigorously homogenized in sterile bottles containing 270ml of peptone water and then blended for 30 sec in sterile electric blender. Serial ten-folds dilutions were prepared according to the method described by [18] using the same diluents. Enumeration of LAB was detected using MRS agar medium (Hi-media Laboratories Pvt. Ltd. India). Readily prepared solidified MRS agar plates were inoculated with 0.1 ml of suitable dilutions using spread method, and then incubated anaerobically by using anaerobic jars with gas generating kits (Oxoid BR 0038b) at 37 ^{O}C for 2-3 days [18]-[19]. After incubation colonies were counted using colony counter (Quebec colony counter) and recorded as colony forming unit (cfu) per gram fresh weight of each sample. Predominant isolates from different morphologically differences were selected and purified by repeated streaking on MRS agar. The pure cultures were streaked onto MRS slant agar, stored at 5^{0}C for further studies and sub-cultured at two-month interval. LAB isolates were activated in MRS broth at 30oC for 24 h prior to use.

2.3. Characterization of LAB isolates

Characterization of the purified isolates was carried out using Bergeys Manual [20]- [21]-[18]-[22]. All purified isolates were subjected to gram staining, catalase test, growth at 15^{O}C and 45^{o}C in MRS broth [23]-[1], growth in 6.5 % and 18% NaCl in MRS broth, growth in 4.4 pH and 9.6 pH in MRS broth, production of gas from glucose and NH3 from arginine broth, production of acetoin in phosphate broth, action in litmus milk broth and fermentation of 1% sugars (amygdalin, arabinose, fructose, lactose, raffinose, salicin, sucrose, xylose, maltose, and mannitol).

3. Results and Discussion

Generally, the count of LAB in investigated samples was high (Table 1). The mean counts of LAB in butcheries samples ranged from 9.30x10^{6}cfu/g to 3.64x 10^{7}cfu/g, which was higher than the mean of factory-processed sausages (mean 2.12 x 10^{5}cfu/g - 4.41 x 10^{6}cfu/g), which was, in turn, higher than the load shown by home-made sausages (mean 5.04 x 10^{5}cfu/g). LAB counts of the investigated sausage samples were within the range of 2.12 x 10^{5} cfu/g to 3.64 x 10^{7}cfu/g. The counts of LAB in Mhom (a traditional meat sausage in Thailand), were 6.0x10^{6}-1.0x10^{7}cfu/g [24], The high counts of these bacteria in this study may be due to the suitable conditions that favor LAB growth, or they may be introduced from raw meat, spices, equipments and air during handling, processing, marketing and storage.

A total of forty isolates were presumptively identified as LAB according to the morphological and biochemical tests (Table 2). The identified isolates were gram-positive, rods or cocci, non-motile, non-spore forming, catalase-negative, oxidase-negative, and producing acid from glucose with no gas. Isolates were identified as:

Streptococcus cremoris represented 40% of the total isolates. It is used in dairy products to create cheese. It is known as *L. lactis* but it is more commonly known as *Streptococcus cremoris*. It gives the cheese its characteristics flavour and odour. It is selected for manufacturing cheese such as cheddar, Colby, cottage cheese, cream cheese and camembert cheese as well as other dairy products like cultured butter, sour cream and kefir. It can be used as single culture or in mixed strain cultures with other LAB. Some strains of *S. cremoris* produce the bacteriocin diplococcin, its activity spectrum was restricted to *S. cremoris* and *S. lactis* strians and non of the gram-positive or negative strains were inhibited [25].

This research makes a critical step towards understanding and manipulating *L. cremoris* for improving the flavour, texture and preservation of cheese produced manually.

Enterococcus faecalis represented 20% of the isolates. *Enterococcus faecium* and *Enterococcus faecalis* strains are used as probiotics. Enterococci belong to LAB and they are of importance in foods due to their involvement in food spoilage and fermentation as well as their utilization as probiotics as in human. They are used as starter cultures in the food industry as well as health supplements and probiotics by the pharmaceutical industry. This status requires a careful evaluation on the bases of pathogencity of the strains used to produce food and pharmaceuticals.

The pathogencity status may produce clinical symptoms similar to staphylococcal intoxication. The infectious dose is probably high (more than 10^{7} organisms). Food sources include sausages, cheeses, meat pie, pudding and raw milk. Entrance into the food chain is due to under processing and/ or poor and unsanitary food production [26].

Enterococci are poor acidifiers, and in traditional sausages of high pH they find good conditions for survival and growth [27]. However, they are still considered as GRAS (Generally Recognized as Safe) microorganisms [28]. Studies pointed out those meat enterococci, especially *Enterococcus faecium*, have a much lower pathogenicity potential than clinical strains, and some strains of *E. faecium* are already used as starter cultures or probiotics [27]-[29]. However the safety of the genus *Enterococcus* is difficult to assess, because certain strains are also associated with human disease. Enterococci are commensals of the mammalian tract, but at the same time can also occur in and dominate the microflora of foods [30]. The presence of enterococci in the sampled sausages indicates the poor hygienic quality of raw materials used in sausage production [28] also can be used as an indicator of faecal contamination [30].

Lactobacillus acidophilus represents 15% of the isolates. It is a benevolent type of microbe that can help improve the balance of bacteria in our bodies. We get acidophilus from plant sources as whole wheat foods, onion, tomato, banana and garlic. Honey always contains varying concentration of acidophilus [31]. Eating foods containing acidophilus can help to treat and prevent diarrhea caused by bacteria. It also fights the vaginal bacteria that cause yeast infections to women. It also helps in lowering cholesterol and helps to digest lactose in lactose sensitive people. The therapeutic potential of these bacteria in fermented dairy products is dependant on their survival during manufacture and storage. *L. acidophilus* has been reported to be beneficial organisms that provide excellent therapeutic benefits. It is present in the form of the tablets and suppositories and as freeze dried granules, powder and capsules.

Lactobacillus delbruckii subsp. *lactis* (10%) is none pathogenic. In fact it is widely used in the food industry and can be found in yoghurt, milk, vegetables and cheese [32].

Lactobacillus delbruckii subsp. *Bulgaricus* (7.5%) is important in the dairy industry as starter cultures for the production fermented milk, yoghurt and cheese [33]-[34].

g- *Lactobacillus jenseny*, *Lactobacillus vitulinus*, and *Streptococcus avium*, each representing 2.5% of the isolates.

The microbiological quality of Thai fermented meat sausages was studied by [24]. They identified the predominant LAB as *Lactobacillus curvatus*, *L. delbrueckii*, *L. acidophilus*, *L. paracasei*, *L. brevis*, *L. mesentroides*, *L.*

plantarum, *L. farciminis*, *Carnobacterium divergens*, *Pediococcus pentosaceus* and *Enterococcus canakci*. The predominant LAB strains associated with Turkish dry fermented sausage were isolated and identified as *Lactobacillus lactis* subsp. *lactis*, *L. curvatus* subsp. *curvatus*, *L. brevis*, *L. fermentum*, *Wieisiella viridescens*, *L. delbrueckii* subsp. *delbruceckii*, *Wieisiella confusa*, *Lactobacillus collinoides*, and *Leuconostoc mesentroides* subsp. *mesentriodes/ dextranicum* [35]. They claimed that the dominant microflora in sausage is *Lactobacillus plantarum*. *Lactococcus lactis* subsp. *lactis*, *L. casei*, and *Enterococcus casseliflavus*, and *Leuconostoc mesenteroides* were isolated from fresh sausages stored at40^{o}Cfor 10 days [36].

The whole Results explain that there is a diversity of LAB species associated with fresh Sudanese sausages sold in Khartoum State. Some of these LAB species can be used as a probiotics in the food industry as well as pharmaceuticals.

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Author Profile

Dr. Nagwa Babiker Elhag Khalifa received the B. Sc degree in Food Science and Technology from Alexandria University- Egypt in 1993, Faculty of Agriculture, M.Sc. degree in Food Microbiology from University of Khartoum Faculty of Agriculture - Sudan in 2003 and PhD degree in Food Microbiology and Biotechnology from University of Khartoum-Sudan Faculty of Agriculture in 2013. During this period I stay teaching in different Universities. Now I join University of Bahri - Food Hygiene and Safety teach and doing different researches.

Professor El Rakha Bashir. Babiker received the B. Sc degree in Food Science and Technology from University of Khartoum Faculty of Agriculture, M. Sc and PhD degree in Food Microbiology from Britain. Now she joined Food Research Center, Shambat. Sudan.

Professor Ahmed Ali Mahdi received the B. Sc degree in Food Science and Technology from University of Khartoum Faculty of Agriculture, M. Sc and PhD degree in Food Microbiology from Britain. Now he joined Sert University- Libya.

Table 1: Sources and LAB counts isolated on MRS medium

Sample No	*Sample source*	**Mean LAB counts (cfu/g)*
1	Khartoum butcheries	$9.30x10^{6}$
2	Khartoum North butcheries	$9.79x10^{6}$
3	Omdurman butcheries	$3.64x10^{7}$
4	**Factory 1	$2.93x10^{5}$
5	**Factory 2	$2.23x10^{5}$
6	**Factory 3	$2.12x10^{5}$
7	**Factory 4	$4.41x10^{6}$
8	Homemade	$5.04x10^{5}$

* Mean of 5 replicates.
** Factory samples at retail outlet.
LAB: lactic acid bacteria

Table 2: Presumptive Identification of LAB isolated from sausage samples collected from different sources

Isolates No	Isolates Code	Shape	Gas from glucose	NH3 from Arginine	Growth at		Growth in 6.5%Nacl	Growth in 18%Nacl	Growth in 4.4 pH	Growth in 9.6 pH	Amygdalin	Arabinose	Fructose	Lactose	Raffinose	Salicin	Sucrose	Xylose	Maltose	Mannitol	Action in Litms milk	Species
					15°C	45°C																
1	KhB1	Cocci	-	-	+	-	-	-	-	-	+	VW	+	-	+	-	-	-	+	-	+	*S. cremoris*
2	KhB2	Cocci	-	-	+	-	-	-	-	-	+	VW	+	-	+	-	-	-	+	-	+	*S. cremoris*
3	KhB3	Cocci	-	-	+	-	-	-	-	-	+	VW	+	-	+	-	-	-	+	-	+	*S. cremoris*
4	KhB4	Rod	-	-	-	+	-	-	-	+	+	-	+	+	-	+	+	-	+	-	+	*L. deblruckii sub lactis*
5	KhB5	Cocci	-	-	+	-	-	-	-	-	+	VW	+	-	+	-	-	-	+	-	+	*S . cremoris*
6	KNB1	Cocci	-	+	+	+	+	-	+	+	+	VW	+	+	+	+	+	-	+	-	+	*E. faecalis*
7	KNB2	Rod	-	+	-	+	+	-	+	+	+	-	+	+	+	+	+	-	+	-	+	*L .acidophilus*
8	KNB3	Cocci	-	+	+	+	+	-	+	+	+	VW	+	+	+	+	+	-	+	-	+	*E . faecalis*
9	KNB4	Rod	-	+	+	-	-	-	-	-	-	-	+	+	-	+	-	-	+	-	+	*L .deblruckii sub lactis*
10	KNB5	Cocci	-	+	+	+	+	-	+	+	+	VW	+	+	+	+	+	-	+	-	+	*E . faecalis*
11	OB1	Cocci	-	-	+	-	-	-	-	-	+	VW	+	-	+	-	-	-	+	-	+	*S . cremoris*
12	OB2	Cocci	-	-	+	-	-	-	-	-	+	VW	+	-	+	-	-	-	+	-	+	*S . cremoris*
13	OB3	Cocci	-	-	+	-	-	-	-	-	+	VW	+	-	+	-	-	-	+	-	+	*S . cremoris*
14	OB4	Cocci	-	-	-	+	+	-	W	W	+	-	+	+	-	+	+	-	+	-		*S . avium*
15	OB5	Cocci	-	-	+	-	-	-	-	-	+	VW	+	-	+	-	-	-	+	-	+	*S . cremoris*
16	LB1	Cocci	-	+	+	+	+	-	+	+	+	VW	+	+	+	+	+	-	+	-	+	*E. faecalis*
17	LB2	Cocci	-	-	+	-	-	-	-	-	+	VW	+	-	+	-	-	-	+	-	+	*S . cremoris*
18	LB3	Cocci	-	-	+	-	-	-	-	-	+	VW	+	-	+	-	-	-	+	-	+	*S . cremoris*
19	LB4	Cocci	-	+	+	+	+	-	+	+	+	VW	+	+	+	+	+	-	+	-	+	*E . faecalis*
20	LB5	Cocci	-	-	+	-	-	-	-	-	+	VW	+	-	+	-	-	-	+	-	+	*S . cremoris*
21	MB1	Cocci	-	+	+	+	+	-	+	+	+	VW	+	+	+	+	+	-	+	-	+	*E. faecalis*
22	MB2	Cocci	-	-	+	-	-	-	-	-	+	VW	+	-	+	-	-	-	+	-	+	*S. cremoris*
23	MB3	Rod	-	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	+	*L.. delbruki sub bulgaricus*
24	MB4	Rod	-	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	+	*L. delbruki sub bulgaricus*
25	MB5	Cocci	-	+	+	+	+	-	+	+	+	VW	+	+	+	+	+	-	+	-	+	*E. faecalis*
26	GB1	Rod	-	+	-	+	+	-	+	+	+	-	+	+	+	+	+	-	+	-	+	*L. acidophilus*
27	GB2	Rod	-	+	-	+	+	-	+	+	+	-	+	+	+	+	+	-	+	-	+	*L. acidophilus*
28	GB3	Rod	-	+	-	+	+	-	+	+	+	-	+	+	+	+	+	-	+	-	+	*L. acidophilus*
29	GB4	Rod	-	+	-	+	+	-	+	+	+	-	+	+	+	+	+	-	+	-	+	*L. acidophilus*
30	GB5	Rod	-	+	-	+	+	-	+	+	+	-	+	+	+	+	+	-	+	-	+	*L. acidophilus*
31	WB1	Rod	-	+	-	+	+	-	-	-	+	-	+	-	-	+	+	-	d	-	-	*L. jenseny*
32	WB2	Rod	-	-	+	-	-	-	-	W	+	-	+	+	+	+	+	-	+	-	-	*L. vitulinus*
33	WB3	Rod	-	+	+	-	-	-	-	-	-	-	+	+	-	+	-	-	+	-	+	*L deblruckii sub lactis*
34	WB4	Cocci	-	-	+	-	-	-		-	+	vw	+	-	+	-	-	-	+	-	+	*S. cremoris*
35	WB5	Cocci	-	-	+	-	-	-	-	-	+	VW	+	-	+	-	-	-	+	-	+	*S. cremoris*
36	HB1	Cocci	-	+	+	+	+	-	+	+	+	VW	+	+	+	+	+	-	+	-	+	*E. faecalis*
37	HB2	Rod	-	+	+	-	-	-	-	-	-	-	+	+	-	+	-	-	+	-	+	*L. deblruckii sub lactis*
38	HB3	Cocci	-	-	+	-	-	-	-	-	+	VW	+	-	+	-	-	-	+	-	+	*S. cremoris*
39	HB4	Cocci	-	-	+	-	-	-	-	-	+	VW	+	-	+	-	-	-	+	-	+	*S. cremoris*
40	HB5	Rod	-	+	+	-	-	-	-	-	-	-	+	+	-	-	-	-	+	-	+	*L . delbruki sub bulgaricus*

Legend:
(-)Negative Reaction
(+) Positive reaction
(w)Weak reaction
(vw) Very weak reaction
(d) Delayed reaction