JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Isolation and Identification of Lactic Acid Bacteria Isolated from Some Processed Meat Products in the Factories of Khartoum State, Sudan

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Abstract:
The objectives of this study were to identify and count Lactic Acid Bacteria (LAB) in different types of processed meat products and identify count. A total of 11 samples of these processed meat {4 frankfurter (F), 4 burger (B), 3 pastrami (P) were used in this study. Bacteria isolated on MRS medium were identified as lactic acid bacteria. Counts of these bacteria were generally high. The mean count was higher in frankfurter samples than the mean for the pastrami and burger samples. A total of 25 strains of LAB were isolated from the eleven samples mentioned above, in addition to 2 samples of sausage (S) and 2 of baby faeces (bf) which were chosen as potential bacteriocin producers and identified as *Lactobacillus plantarum* (*L. plantarum*) and *Lactobacillus curvatus* (*L. curvatus*). The cell shapes were rods and cocci and all isolates were Gram-Positive, catalase-negative, non-spore formers and capable of growth under anaerobic conditions.

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Keywords: Processed meat, lactic acid bacteria (LAB), *L. plantarum*, *L.curvatus*.

Introduction:

Meat production is growing globally and its estimated by FAO/WHO (2001), that it will reach nearly 320 million tons by the year 2016. The rising demand for meat in developing countries is mainly as a result of the fast progression of societies becoming more urbanized. This is also apparent in Khartoum State where for the past few years; there has been a significant increase in the number of meat processing factories, as well as the assortment of products they produce. In 2009, the number of meat processing factories in Khartoum State was nine as compared to year 2000, when there were only two factories. In addition, developing countries has also shown a continuous increase in meat consumption from an average of 10 kg per capita/year in the 1950s to 26 kg in the year 2030, FAO/WHO (2001). Processed meat is very popular, particularly among young population. Fast food products such as beef burgers or beef frankfurters are linked with urbanized (Heinz and Hautzinger, 2007).

Lactic acid bacteria (LAB) are Gram positive, non-spore former, usually non motile, non acid fast, non respiring rods or coccobacilli with frequently in chain, devoid of cytochrome, catalase negative. They grow well under anaerobic conditions but may grow in microaerophilic as well as aerobic conditions. They exhibit optimum growth as slightly lower acidic conditions (pH 5.5- 6.0), while growth is often restricted at neutral or somewhat alkaline conditions (pH above 7.0 to 7.5). They are strictly fermentative during sugar fermentation (Schleifer and Ludwig, 1995; Axelsson, 2004). Lactic acid bacteria include various major genera: *Lactobacillus*, *Lactococcus*, *Carnobacterium, Enterococcus, Lactosphaera*, *Leuconostoc*, *Melissococcus*, *Oenococcus*, *Pediococcus*, *Streptococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella*. Other genera are*: Aerococcus, Microbacterium, Propionibacterium* and *Bifidobacterium* (Carr *et al.*, 2002). Lactic acid bacteria have been used in the production of a variety of dairy, vegetables and meat fermented food in many countries. They are generally recognized as safe (GRASS), due to their typical association with food fermentations and their long tradition as food-grade bacteria. Lactobacilli have been used for many centuries in food fermentation processes and are considered as GRAS organisms that can safely be used and also for medical and veterinary

applications (Fuller, 1989; Tannock, 1997). The aim of the present study was isolation and identification of lactic acid bacteria isolated from processed meat products from some factories in Khartoum State, Sudan.

Materials and Methods

Collection of samples

Eleven samples of frozen beef meat products in plastic packaged {4frankfurter (F), 4burger (B) and 3pastrami (P)} from four different meat processing factories in Khartoum-State, Sudan. All these samples were collected from Elmohandesin markets, and 2 baby faeces samples from house-healthy new born infant age 17 days. All samples were transported in sterile insulated iced containers until arrival to the laboratory. Glassware and other materials (forceps, spoons, spatulas, mortar and pestle) used throughout this study were sterilized using a hot air oven at 160°C for 3 hours (Harrigan, 1998); knives were sterilized by direct flaming after dipping into spirit.

Ten grams each of the test samples were separately blended with 90ml sterile peptone water and homogenized for 30 seconds in Lab – Blender. The samples were further serially diluted (10^{-1}, 10^{-2}, 10^{-3}) and one hundred microliters (0.1ml) of suitable dilutions were spread directly onto the surface of duplicate plates of de Man, Rogosa, Sharpe (MRS) medium (de Man *et al.*1960). Samples were incubated under anaerobic conditions using an anaerobic jar system (GasPak; BBL) with a gas-generating kit (Oxoid BR0038B) at 37°C for 48-72 hours. After incubation plates with 30-300 colonies were enumerated and using colony counter (Quebec colony counter). Results were expressed as colony-forming units (CFU) per gram of the sample (Collin and Lyne,1984). Colonies of bacteria having different appearances were randomly sub-cultured from MRS agar plates and purified isolates were plated on MRS agar. All isolates were examined for gram reaction, production of catalase, oxidase activity and spore formation. Only gram-positive, catalase -negative, oxidase -negative and non-spore-forming isolates were selected and stored on MRS agar slopes in a refrigerator for further analyses.

Identification of lactic acid bacteria

Representative discrete colonies of LAB were picked from plates used for the isolation of LAB from different source mentioned above. These colonies were transferred to, and purified on MRS agar plates. The pure colonies were tested for Gram stain, spore staining, oxidase test and catalase reaction. The pure culture were streaked onto MRS agar slants and stored at 5 C for identification. Identification of the isolates was carried out using Bergey's Manual (Sneath *et al*., 1983: Barrow and Feltham, 1993: Harrigan, 1998 and Salminen *et al*., 2004).

Results and Discussion.

Counts of bacteria isolated on MRS medium (presumptive LAB)

Bacteria isolated on MRS medium were presumptively identified as lactic acid bacteria (De Man *et al.*, 1960). Counts of these bacteria were generally high. The mean count was higher in frankfurter samples ($1.3x10^7$cfu/g) than the mean for the pastrami and burger samples (mean 1.9×10^6cfu/g and 5.0×10^5cfu/g, respectively) as shown in Table 1. The highest mean count was detected in frankfurter samples ($1.3x10^7$) and ranged from $8.5x10^5$cfu/g to $4.5x\ 10^7$cfu/g. Abdel-Rahman and El-Basiony (1984) reported that aerobic plate count and lactobacilli count of 50 samples of Egyptian pastrami (dried meat product) obtained from Assiut city market ranged from $1x10^4$ to $9x10^6$ CFU, respectively. The high level of LAB on frankfurter may be attributed to the smoke which contains bactericidal chemicals, which are deposited on the surface of the product, and the heat causes development of a surface layer of coagulated protein. LAB (lactobacilli) is the main flora of commercial curing brines. They are mainly located below the surface of the product, while yeast and micrococci are mainly present on the surface (FAO, 2009). Also, slime formation is favored by a moist surface and is usually confined to the outer casing removal of this material with hot water leaves the product essentially unchanged. The usual source of these organisms to processed meats is milk solids. The souring results from the utilization of lactose and other sugars by the organisms and the production of acid (James *et al.*, 2005).

Isolation of bacteriocin-producing LAB from various sources

A total of 25 strains of lactic acid bacteria (LAB) were isolated from the eleven processed meat samples 4 frankfurter (F), 4 burger (B) and 3 pastrami (P) in addition to two samples of sausages (S_1,S_2) and two of baby faeces. Table 2 lists these isolates, the sources from where they were obtained, as well as their cell shapes, Gram reaction, catalase activity, spore formation and capability of growth under anaerobic conditions. Cell shapes included rods and cocci. All isolates were Gram-positive, catalase-negative, non-spore formers and capable of growth under anaerobic conditions. These are characteristics of lactic acid bacteria (Axelsson, 2004). Identification of lactic acid bacteria (LAB).

Table 3 shows results produced by two bacteriocin-producing isolates of lactic acid bacteria (obtained from baby faeces) which were subjected to morphological, cultural and biochemical characterization according to Barrow and Feltham (1993). The two isolates were catalase negative, oxidase negative and produced acid from glucose with no gas.

Morphologically, they were Gram-positive, rod shaped, non-motile and non-spore forming. Results revealed that the identified isolates were: *Lactobacillus plantarum* (bf1) and *Lactobacillus curvatus* (bf2). The human intestinal microflora consists of a wide variety of bacterial species, including *Lactobacillus plantarum* as one of the most predominant Lactobacillus species (Ahrné *et al.*, 1998)[17]. Also Chung and Yousef (2005) reported that the most active isolate from fermented food was identified as a *Lactobacillus curvatus.*

Table 1. Lactic acid bacteria (LAB) counts of processed meat samples obtained from different factories

Sample code	Source and factory	LAB (CFU/g)	Mean(CFU/g)
B1	Burger-factory 1	$4.0x10^5$	$5.0x10^5$
B2	Burger-factory 2	$4.8x10^5$	
B3	Burger-factory 3	$8.0x10^5$	
B4	Burger-factory 4	$3.2x10^5$	
F1	Frankfurter-factory 1	$1.2x10^6$	$1.3x10^7$
F2	Frankfurter-factory 2	$4.5x10^7$	
F3	Frankfurter- factory 3	$8.5x10^5$	
F4	Frankfurter-factory 4	$4.9x10^6$	
P1	Pastrami-factory 1	$4.0x10^6$	$1.9x10^6$
P2	Pastrami-factory 2	$1.1x10^6$	
P3	Pastrami-factory 3	$5.0x10^6$	

* n=3

Table 2: Sources of isolates of lactic acid bacteria and some of their characteristics

Serial	Isolate code	Source	Cell shape	Gram reaction	Catalase activity	Spore formation	anaerobic growth
1	F1	Factory 1	R	+	-	-	+
2	F2	Factory 2	R	+	-	-	+
3	F3	Factory 3	R	+	-	-	+
4	$B4_1$	Factory 4	C	+	-	-	+
5	$B2_1$	Factory 2	C	+	-	-	+
6	$B2_2$	Factory 2	R	+	-	-	+
7	$B3_1$	Factory 3	C	+	-	-	+
8	$B3_4$	Factory 3	R	+	-	-	+
9	$B4_3$	Factory 4	R	+	-	-	+
10	P1	Factory 1	R	+	-	-	+
11	P2	Factory 2	R	+	-	-	+
12	S1	Factory 1	R	+	-	-	+
13	S2	Factory 2	R	+	-	-	+
14	bf1	Baby faeces	R	+	-	-	+
15	bf2	Baby faeces	R	+	-	-	+

R = rod shaped; C = coccus.
F1, F2, F3 =Frankfurter
$B4_1$, $B2_1$, $B2_2$, $B3_1$, $B3_4$, $B4_3$ = Burger
P1, P2, P3 = Pastrami

S1, S2 = sausage
bf1, bf2 =Baby faeces

Table 3: Identification of two isolates of lactic acid bacteria isolate from baby faeces

Isolate code / Biochemical tests	bf1	bf2
Gram stain	+	+
Shape	Rod	Rod
Endospore staining	_	_
Motility test	_	_
Growth anaerobically	+	+
Catalase test	_	_
Oxidase test	_	_
Glucose (acid)	+	+
Glucose (gas)	_	_
O/F test	F	F
Nitrate reduction	_	_
Growth at 15 C°	+	+
Growth at 45 C°	_	_
Ammonia from arginine	_	_
V.P.	_	_
Amygdalin	+	_
Arabinose	+	_
Cellobiose	+	+
Fructose	+	+
Galactose	+	+
Gluconate	+	+
Lactose	+	+
Maltose	+	+
Mannitol	+	_
Mannose	+	+
Melezitose	+	_
Melibiose	+	_
Raffinose	+	_
Rhamnose	_	_
Ribose	+	+
Salicin	+	+
Sorbitol	+	_
Sucrose	+	_
Trehalose	+	_
Xylose	+	_
Esculin	-	+
Growth at pH 9.6	_	_
Growth at 6.5% Nacl	+	+
Genus	Lactobacillus	Lactobacillus
Species	Plantarum	Curvatus

O/F: Oxidation/fermentation test.
V.P.: Voges-proskauer test.

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