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Antibacterial activity of the bacteriocins producing- lactic acid bacteria isolated from some processed meat products against selected indicator bacterial strains

Huda M Selman [1], Ahmed A Mahdi [2], Nagat A El Rofaei [3] and Elnasri M Mutwali [4,*]

[1] *Department of Biology, Faculty of Education, Alzaiem Alazhari University, Khartoum, Sudan.*
[2] *Department of agricultural Biotechnology, Faculty of Agriculture, University of Khartoum, Sudan.*
[3] *Biotechnology Department, Faculty of Science and Technology, Omdurman Islamic University, Omdurman, Sudan.*
[4] *Department of Biology, Faculty of Education, Alzaiem Alazhari University, Khartoum, Sudan.*

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Abstract

In this study, a total of 25 Lactic Acid Bacteria (LAB) isolates from 15 samples [4 burgers, 4frankfurters, 3 pastramis, 2 sausages and 2baby faeces] were screened for their ability to produce inhibitory substances against three microorganisms which were isolated from meat products: (*Salmonella typhi, Staphylococcus aureus and Escherichia coli*). Other indicators (reference target organisms) were obtained from the Central Public Health Laboratory, Khartoum State, Sudan. These were: *Salmonella typhi* ATCC14023, *Staphylococcus aureus* ATCC29213 and *Escherichia coli* ATCC25922. The antagonistic activities of the isolates were screened by the direct spot-on-lawn method and well-diffusion method. A total of 25 isolates of LAB were obtained, 15 of which produced promising inhibition zones against all or some of the indicator bacterial strains.

Keywords: Antibacterial activity; Indicator bacterial strains; Inhibitory substances; Khartoum State; Sudan

1. Introduction

Meat and meat products are the main food sources in daily diets of people in developing countries that affected by several factors. Meat products diversity and their ease to use and do not require for cooking of them increased their using, especially in young people [1, 2]. Lactic Acid Bacteria (LAB) are characterized as Gram-positive cocci or rods, non-aerobic but aerotolerant, able to ferment carbohydrates for energy and lactic acid production. The metabolic pathway from glucose may be homofermentative or heterofermentative. Lactic acid bacteria are also able to produce small organic substances that contribute with aroma and give specific organoleptic attributes to the products [3]. 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* [4]. LAB can exert a bioprotective or inhibitory effect against other microorganisms as a result of the competition for nutrients and/or of the production of bacteriocins or other antagonistic compounds such as organic acids, hydrogen peroxide and enzymes. Food processors face a major challenge with consumers demanding safe foods with a long shelf life, but also expressing their preference for minimally processed products, less severely damaged by heat and freezing and not containing chemical preservatives. LAB antimicrobial activity is due to the production of organic acids (in particular, lactic acid and acetic acid), carbon dioxide, hydrogen peroxide and diacetyl [5]. Also some of these bacteria produce antagonistic substances, called bacteriocins, which in small amounts are very active against pathogens [6,7] .The incorporation of these compounds as biopreservative ingredients into model food has been shown to be effective in the control of pathogenic

* Corresponding author: Elnasri M Mutwali
Department of Biology, Faculty of Education, Alzaiem Alazhari University, Khartoum, Sudan.

and spoilage micro-organisms [8] .The aim of this study was to evaluate the antibacterial activity of the bacteriocins producing lactic acid bacteria isolated from some processed meat products against selected indicator bacterial strains.

2. Material and methods

14 samples of frozen meat products in plastic packaged (4 frankfurter, 4 burger, 3 pastrami and 2 sausages) from four different meat processing factories in Khartoum State, Sudan were collected from Elmohandsein markets. They were obtained in sterile insulated iced containers and immediately transported to the laboratory. And baby faces sample obtained from house-healthy new born infant age 17 days.

2.1. Isolation of bacteriocin producing LAB

In this study, a total of 25 LAB isolated from 14 samples mentioned previously, were screened for their ability to produce inhibitory substances against three microorganisms, which were isolated from meat products and identified according to Bergey's Manual [9, 10, 11, 12] as: *Salmonella typhi*, *Staphylococcus aureus* and *Escherichia coli*. Other indicators (reference target organisms) were obtained from the Central Public Health Laboratory, Khartoum State, Sudan. They were: Staphylococcus aureus ATCC29213, Salmonella typhi ATCC14023, Escherichia coli ATCC25922.

2.2. Susceptibility testing

The antagonistic activities of the isolates were initially screened by the direct spot-on-lawn method [13], which were afterwards confirmed by the well diffusion method [14].

2.3. Screening by the spot-on-lawn method

The inhibitory activities of 25 LAB isolates was confirmed with the spot-on-lawn assay as described by Schillinger and Lücke [15], Lewus et al. [13]), and Van Reenen et al. [16]. Overnight cultures of LAB isolates to be tested were spotted (5µl) onto the surface of MRS agar medium (4 spots in each plate) and incubated anaerobically at 37°C for 24 h to allow colonies to develop. Anaerobic conditions were used to minimize the formation of hydrogen peroxide and acetic acid. Each plate was overlaid with 7 ml of soft agar (0.75%) seeded with 0.5 ml of overnight cultures of the indicator organisms. The plates were incubated at 37°C for 24 h and clear zones around the spots were observed. The antagonism was detected by the formation of a growth inhibition halo of the indicator microorganisms around bacteriocins test isolates. Isolates producing clear inhibition zones were selected and used for further testing by the agar well diffusion method.

2.4. Testing by the well-diffusion method

Only 15 strains showing promise of antagonistic activity against the indicator bacteria were further used for confirmation of their activity by the well-diffusion method [14].In this method, cell-free supernatants of the test isolates were used to inhibit growth of the indicator bacteria.

2.5. Preparation of the cell-free supernatants

For the detection of antibacterial activity of each isolate, the method of Çadirci and Çitak [17] was followed, in which a 24-hour old culture of the isolate was used to inoculate MRS broth (2%) in test tubes. The inoculated tubes were incubated at 30°C for 72 hours without shaking. At the end of the incubation period, the cells were separated by centrifugation at 6000 rpm for 25 minutes in a centrifuge (Zentrifugen / Hettich D-78532 Tuttlingen, Germany). The pH of the culture was adjusted to pH 5.5 with sterile7.0M NaOH and cells were then desorbed at pH 2-2.5 in the presence of sterile 0.1N NaCl [18; 19]. The cell-free supernatants were carefully decanted, cellulose acetate filter-sterilized (pore size 0.45 µm, Sartorius Stedium Biotech GmbH37070 Geettingen, Germany) and were kept at 4ºC for use in the determination of their antagonistic activities against the 6 indicators bacteria, and for their characterization.

2.6. Conducting the well-diffusion test

Molten Nutrient Agar (N.A.) medium (48°C) was first seeded with washed cells of the indicator bacteria, and the inoculated medium was immediately poured into sterile Petri dishes. After solidification, the medium was allowed to dry for at least 30 minutes at room temperature. Four wells of uniform diameter (about 5 mm) were aseptically bored in the agar using a sterile Pasteur pipette. Fifty µl of each of the 15 cell-free supernatants of each test isolate (72 hours-old) were dispensed into three wells, while sterile MRS agar medium was poured into the fourth well to serve as a control treatment. Plates were left to stand for at least five hours at room temperature to allow diffusion of the cell-free extracts. The plates were then incubated inverted at 30°C for 24 hours. At the end of the incubation period, diameters of the resulting inhibition zones, if any, were measured and the results recorded in cm.

3. Results and discussion

3.1. Screening for antagonistic activity using spot-on-lawn method

A preliminary screening of the possible antagonistic activity of the isolates against 6 indicator bacteria, three of which were reference strains (*Staphylococcus aureus* ATCC 29213, *Salmonella typhi* ATCC 14023 and *E. coli* ATCC 25922), in addition to three indicator strains from the same genera isolated from the processed meat samples. Screening was conducted by the spot-on-lawn method [13]. Table 1 Shows that 15 of the isolates produced inhibition zones against all or some of the indicator strains, 12 of which showed antagonistic activity against all six indicator bacteria. Six of these were obtained from burger, three samples from frankfurter, two from pastrami, two from sausage and two from infant faeces.

Most of the burger isolates showed inhibitory activity against all indicators except isolate B41 which failed to inhibit *Staphylococcus aureus*, while the frankfurter isolate F3 failed to inhabit *E. coli* ATCC 25922. The pastrami isolate P2 was active only against *E. coli* ATCC 25922 and *Salmonella typhi* ATCC 14023 and failed to inhibit the remaining indicators. However, isolates obtained from sausage and infant faeces showed inhibition activity against all indicators.

This study was started by the isolation of a large number of promising bacteriocin-producing bacteria from a wide variety of samples (meat products samples and infant faeces). Conventry et al. [20] reported that the detection rate of bac+ strains from LAB isolates can be as low as 0.2% and therefore needs a large number of isolates from food sources. A total of about 25 colonies isolated from these samples were examined for detection of antibacterial activity against a set of 6 indicators. Activity against Gram-negative strains by Gram-positive bacteriocin producers has rarely been reported [21; 22; 23; 24].

3.2. Screening by the well-diffusion method

More stringent screening of the 15 isolates was carried out by the well-diffusion method [14], using cell–free supernatants prepared from the test isolates. Supernatants were prepared from each strain after incubation at 30°C for 72 hours in MRS broth.

Table 1 Bacteriocin activity (measured as diameter of inhibition zones in cm) against six target organisms by the spot on-lawn assay

Indicator organisms/ DIZ							
Isolate code	**Source**	***Salmonella typhi* ATCC14023**	***Salmonella typhi* local**	***Escherichia coli* ATCC25922**	***Escherichia coli* local**	***Staphylococcus aureus* ATCC29213**	***Staphylococcus aureus* local**
$B2_1$	Factory2	1.8	2.0	1.5	2.0	2.0	1.5
$B2_2$	Factory2	2.3	4.0	3.6	2.6	3.0	3.5
$B3_1$	Factory3	3.0	3.0	2.7	2.7	2.3	3.0
$B3_4$	Factory3	4.0	3.2	2.4	2.0	2.2	3.5
$B4_3$	Factory4	3.5	3.3	2.7	2.2	2.5	4.0
$B4_1$	Factory4	3.8	2.2	3.6	1.8	0.0	0.0
F1	Factory1	2.0	4.2	2.3	3.0	2.5	1.2
F2	Factory2	1.2	4.0	2.8	1.8	3.0	1.2
F3	Factory3	2.0	2.5	0.0	1.2	2.5	2.7
P1	Factory1	2.4	3.7	2.2	2.0	3.4	2.5
P2	Factory2	2.0	0.0	1.8	0.0	0.0	0.0
S1	Factory1	2.0	1.5	1.0	2.0	3.0	2.5
S2	Factory2	2.0	2.3	2.0	1.5	2.5	2.5

Bf1	Baby faeces	2.8	2.0	1.5	2.5	2.2	2.0
Bf2	Baby faeces	3.0	2.6	2.7	3.0	2.5	2.0

DIZ: Diameter of Inhibition Zones. 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 2 Bacteriocin activity measurement (Inhibition zone diameters, cm) produced by supernatants from 5 isolates against six indicator bacteria by the agar well diffusion method

Indicator organisms (IZD)							
Isolate code	**Source**	***Salmonella typhi* ATCC14023**	***Salmonella typhi* local**	***Staphylococcus aureus* ATCC29213**	***Staphylococcus aureus* local**	***Escherichia coli* ATCC25922**	***Escherichia coli* local**
$B2_2$	Burger	1.7	1.5	1.4	1.3	0.0	0.0
S1	Sausage	2.0	1.3	1.2	2.5	1.2	1.3
S2	Sausage	1.8	1.5	1.0	2.4	1.0	1.3
Bf1	Baby faeces	1.9	1.5	1.4	2.0	1.7	1.1
Bf2	Baby faeces	2.3	2.0	1.5	2.7	1.5	1.4

IDZ: Inhibition Zone of Diameter.

Table 2 shows diameters of inhibition zones produced by the test isolates against the indicator bacterium. Only 5 isolates produced inhibition zones, 4 isolates of which produced inhibition against all indicators, while the isolate from Burger ($B2_2$) failed to show any inhibition against *E. coli*. The most predominant inhibition zones were produced by the isolates (S1, S2, Bf1 and Bf2). It is also to be noted that isolate Bf2 which was obtained from baby faeces produced prominent inhibition zones against all indicators in comparison with the other isolates except *Escherichia coli* ATCC 25922.

Only 5 isolates produced antibacterial activity in the cell-free supernatant by using the well-diffusion assay, 4 isolates produced inhibition against all indicators except isolate ($B2_2$) from burger, which failed to show any inhibition against *E. coli*. These negative results could show that bacteriocin production is not highly conserved in these strains. Some of the bacteriocins are plasmid-mediated proteins [25], so one should consider the possibility that some cultures could have lost their plasmids after consecutive transfers during the purification. Lewus *et a*l. [13] found that only a few of the strains that tested positive using the spot on-lawn method gave positive results in the well diffusion assay. They considered that allowing some time for the bacteriocins to diffuse into the agar prior to incubation, or increasing the well size, so that more sample could be applied, might increase the sensitivity of the assay. Also Schillinger and Lücke [15] observed similar findings on checking Lactobacillus sake strains that were positive in the agar spot test and negative in the well diffusion assay.

The results indicate, the diameters of the inhibition zones varied, and ranged between 1.1 and 2.7 cm. This revealed that the LAB inhibited all the pathogenic bacteria tested according to [15]who mentioned that inhibition was scored positive if the width of the clear zone around the colonies of the producer strain was 0.5 mm or larger. Local *Staph. aureus*, as a target organism, demonstrated the highest detection rate among indicator bacteria that ranged from 1.3 to 2.7cm in diameter in isolate $B2_2$ to bf2, respectively. These findings are in agreement with those obtained by Bromberg et al. [26] who determined the production bacteriocin- like substances by some lactic acid bacteria isolated from meat and meat products active against *Staphylococcus aureus*. The most prominent inhibition zones were produced by the isolates (S1, S2, Bf1 and Bf2). It is also to be noted that isolate (Bf2), which was obtained from baby faeces produced prominent inhibition zones against all indicators in comparison with the other isolates, except in *E. coli* ATCC 25922.

4. Conclusion

- The produced bacteriocins showed a wide spectrum of antimicrobial activity against spoilage and pathogenic indicator organisms.
- Only a few of the strains tested positive using the spot on-lawn method, and gave positive results in the well diffusion assay. This indicates the need for some time for the bacteriocins to diffuse into the agar prior to incubation, or increasing the well size so that more sample could be applied, which might increase the sensitivity of the assay.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have declared that no competing interests exist.

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