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# Effect of Bacteriocins-producing Lactic Acid Bacteria on Target Microorganisms

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**ABSTRACT**

The problem of increased resistance of bacteria to antibiotics and the increasing demand for safe foods has enhanced the interest in replacing antibiotics by natural products (biopreservatives). In this study the antagonistic effect of lactic acid bacteria (LAB) producing-bacteriocins on nine target organisms was studied using the agar-well diffusion assay (AWDA). LAB was isolated from Sudanese fresh sausages, intestines of different animals, saliva, cheese and cucumber in MRS broth and on MRS agar media. The target organisms studied were *Salmonella* sp, *S. typhi*, *Staphylococcus aureus*, *Bacillus subtilis*, *B. cereus*, B. *stearothermophilus*, *B. pantotheticus*, *Escherichia coli*, and *Pediococcus* strain BFE 2306. Only 16 LAB isolates of 30 produced clear inhibition zones. These LAB isolates were identified as *Enterococcus faecalis* (3 isolates), *Enterococcus avium*, *Pediococcus pentosaceus* (3 isolates), *P. domanosus*, *Lactobacillus murinus* (2 isolates), *L. gasseri* (2 isolates), *L. acidophilus*, *L. plantarum*, *L. alimentarius*, and *L. casi* subsp. *Rhamanosus*. Crude bacteriocins and pellets of *Enterococcus faecalis*, *Pediococcos pentosaceus* and *Lactobacillus murinus* exhibited the strongest antimicrobial activity ranging between 40 and 1280 AU/ml, while most supernatants of bacteriocin-producers did not show antimicrobial activity against all indicator organisms (0.00-640 AU/ml). Sudanese products of animal sources can be a rich source for bacteriocins-producing LAB and can be used as biopreservatives.

***Keywords:*** *LAB, Bacteriocin, Pathogenic microorganisms, Antagonistic effect, Sudanese products*.


## INTRODUCTION

Lactic acid bacteria (LAB) is a biological tool for biopreservation and extending the shelf life of different food products (Deegan, 2005). The use of LAB in food processing can reduce the use of chemical preservatives, improves food palatability, and nutritional quality by increasing protein and vitamins availability. Also they produce detoxifying agents and inhibitory compounds e.g. $CO_2$, organic acids, ethanol, hydrogen peroxides and bacteriocins (Oyewole, 1997; Holzapfel, 2002; Hansen, 2002; Chelule, 2010). Bacteriocins are produced by prokaryotic and eukaryotic organisms (Papagianni, 2003). They are proteinaceous compounds which have antagonistic effect against not only closely related species but also spoilage and food-borne pathogens (Carolissen-Mackay, 1997; Aymerich, 2000; Leroy, 2004). These peptides are very important in food and feed, since sufficient amount can inhibit or kill pathogenic microorganisms that compete for the same nutritive demand (Deegan,

2005). They can be produced by gram-positive and gram-negative bacteria (De Vuyst and Vandamme, 1994; Jack, 1995). Jack, (1995) reported that *Bacillus* sp. *B. subtilis*, *B. thringiensis*, *B. stearothermophilus*, *B. licheniformis*, *B. magaterium*, *B. thermoleovorans*, *B. cereus* and *B. coagulans* may also produce bacteriocins. A colicin, warnerin and coagulin are bacteriocins produced by *Escherichia coli*, *Staphylococcus warneri* (FM10, FM20, and FM30) and *Bacillus coagulance* respectively. Bacteriocincs of LAB have a considerable attention nowadays because they are classified as "generally recognized as safe" (GRAS) and the possibility to be used as biopreservatives in food processing is growing (Carr, 2002; Patil, 2007). Different types of baceriocins are widely used in food industry e.g. nicin from *Lactococcus lactis* subsp. *lactis* (Rodriguez, 1996; Moreno, 2000) and pediocin from *Pediococcus pentosaceus* (Moreno, 2006). Different strains of enterococci are capable of producing enterocins which are active against *Listeria monocytogenes*, *Staphylococcus aureus*, and *Clostridium* spp. (Floriano, 1998; Franz, 1999; Gelsomino, 2001). Bacteriocins-producing LAB can be isolated from different types of food products such as dairy products (Foulquiè Moreno, 2006; Leroy, 2003), sausages (Cintas, 1997; Herranz, 2001), fish (Ben Embarek, 1994) and vegetables (Floriano, 1998; Bennik, 1998). Many Sudanese products are highly rich in hurdle lactic acid bacteria (LAB) that can be used as biopreservatives. Different strains of lactic acid bacteria (LAB) were recorded as part of traditional fermented milk products, but the knowledge of their health benefits and species properties need more explaining (Salih, 2011). Abdulla and Osman (2010) suggested that Sudanese dairy products may be a rich source of LAB. They found that the dominant species of LAB were *Lactobacillus xylosus*, *Lactococtcus lactis* sub *cremoris*, *Lactobacillus delbruccki*, and *Pediococcus cerviacae*. Several studies were carried out on different Sudanese products (e.g. meat, fish, dairy, vegetables, and cereal products) focusing on their microbial load, however the technology and microbiology of these products require more studies. This research was conducted to study the antimicrobial activity of bacteriocin-producing LAB against spoilage and pathogenic microorganisms.

## MATERIALS AND METHODS

### *Collection of samples*

Nine different sources [sausages, intestines of chicken, pigeons, sheep and cattle, saliva, cheese, cleaned sheep intestines (locally called musarn mejrure) and cucumber] (Table 1) were used as sources for the isolation of bactriocin-producing LAB. All samples were purchased from retail markets in Khartoum state except saliva. The samples were collected aseptically and immediately transported ice-cooled in an insulated ice container to the laboratory for microbial analysis.

### *Indicator microorganisms*

The selected microorganisms used for the determination of antagonistic activity of LAB were *Salmonella*, *S. typhi*, *Staphylococcus aureus*, *Bacillus subtilis*, *B. cereus*, B. *stearothermophilus*, *B. pantotheticus*, *Escherichia coli*, and *Pediococcus* strain BFE 2306. These organisms were obtained from the department of Veterinary Microbiology, Faculty of Veterinary Sciences, University of Khartoum. LAB BFE 2306 was kindely provided by Dr. Nuha Elkhatim, Food and Biochemistry Department, Faculty of Agriculture University of Khartoum.

### *Screening for bacteriocin producing LAB.*

Ten grams of each source (Table 1) were aseptically weighed and hand mixed well in 100 ml de Man Regosa and Sharp (MRS-Hi-media Laboratories Pvt. Ltd., India) broth for the enrichment of any resident LAB (de Man, 1960). The mixture was incubated at 37ºC for 24 hrs. The broth was then serially diluted and enumerated by the pour plate method. The plates were anaerobically incubated at 37 $^{O}$C for 48 hours using anaerobic jars with gas generating kits (Oxoid BR 0038b) till colonies were visible. Then the plates were overlaid with MRS soft agar (0.75% agar) seeded with 0.5 ml active broth of indicator organisms and was overlaid with the MRS soft agar medium and allowed to set. The plates were then incubated aerobically at 37$^{O}$C for 24 hours to allow the colonies to develop. Colonies showing zones of inhibition were considered as potential bacteriocin producers.

The inhibitory activity of 30 LAB isolates was confirmed by spot-on-lawn assay as described by Schillinger and Lücke (1989), Lewus, (1991), and Van Reenen, (1998). Overnight cultures of LAB isolates to be tested were spotted (5µl) onto the surface of MRS agar medium (4 spots in each) and incubated anaerobically at 37$^{O}$C for 24 h to allow colonies to develop. Each plate was overlaid with 7 ml soft agar (0.75%) seeded with 0.5 ml of overnight cultures of the indicator organisms. The plates were incubated at 37 $^{O}$C for 24 h and clear zones around the spots were observed. The inhibitory reaction was scored as positive if the width of the clear zone around the colonies of LAB isolates was 5 mm or larger (Ogunbanwo 2003; Lade 2005).

***Isolation, Purification and presumptive identification of bacteriocins producers:***

The 16 LAB isolates which produced clear inhibition zone with diameter 5mm or larger were isolated, purified and identified depending on their morphological, cultural and biochemical characterization (Sneath, 1986, Harrigan, 1998; Holt 1998; Barrow and Fealthman, 1993; Axelesson, 2004 and Salminen, 2004).

***Extraction of crude bacteriocins samples***

LAB isolates which produced inhibition zone (16 isolates) were seeded in 100 ml MRS broth individually and incubated at 30$^{o}$C for 72 h anaerobiacally (Oxoid gas generating kits). Then the broth was heated at 80$^{o}$C for 20 minutes in a water bath to kill living cells, and were then desorbed at pH 2-2.5 using sterile 0.1 N NaCl (Yang, 1992, Vera Pingitore, 2007). The cultures were then centrifuged at 10000 rpm for 20 min to obtain supernatants of crude bacteriocins. Crude bacteriocins obtained were adjusted to pH 6.5 using sterilized 1M NaOH or HCl (Daba, 1991; Todorov and Dicks, 2005). This was followed by filtration of the supernatants through a 0.2 μm pore-size cellulose acetate filter.

***Partial purification of crude bacteriocins extracts***

Crude bacteriocins were treated with 40 % ammonium sulphate $(NH4)_2 SO_4$), and the mixtures were stirred for 1 hour at 4$^{O}$C, and were then centrifuged at 10,000 rpm for 20 minutes (Vera Pringitore, 2007) and supernatants were decanted. The pellets and surface pellicle were dissolved in 10 ml sterile ultra pure water. Bacteriocins activity was carried out and the titer was determined for both pellets and the supernatants.

***Antagonistic activity of bacteriocins***

The agar well diffusion assay (AWDA) was used to determine the antagonistic effect of bacteriocin-producing LAB against indicator organisms (Schillinger and Lücke, 1989, and Takahiro, 1991). MRS and nutrient agar media were used for culturing LAB 2306 and the other indicator organisms respectively. Fifteen ml of molten agar (MRS for LAB 2306 as an indicator and NA for other indicator organisms) were first seeded with 1 ml ($10^{-5}$cfu/g) of overnight cultures of indicator organisms grown separately in sterile Petri dishes, and after solidification were dried for 15 minutes under a sterilized hood. Wells of uniform diameter (8 mm) were bored in the agar using sterile micropipette tips. Two-fold serial dilutions were conducted to dilute supernatants of each isolate (Graciela, 1995). Aliquots of 50 μl from each bacteriocin dilution were placed in the wells. The plates were incubated anaerobically (using Oxiod gas generating kits ) overnight at 30$^{O}$C for lactic acid bacteria and aerobically at 37$^{O}$C for non-lactic acid bacteria. Diameters of inhibition zones around the wells were recorded (Rammelsberg and Radler, 1990) and the antimicrobial activity of bacteriocin producers were determined. as the reciprocal of the highest dilution showing inhibition of the indicator organisms multiplied by 100 to express it in arbitrary units of activity per ml (Au $ml^{-1}$) (Graciela, 1995).

## RESULTS AND DISCUSSION

Sources, type of sources and codes of LAB isolates are shown in Table 1. Thirty presumptive bacteriocin producers were obtained from the investigated sausage samples and other sources. Sixteen isolates of LAB out of 30 isolates produced clear zones of inhibition (5mm) against the indicator organisms; and were therefore selected as potential bacteriocin producers. Table 2 revealed that the identified isolates (16 isolates) were Enterococcus faecalis (N1, N5, and N6), E. avium (N2), Pediococcus pentosaceus (N10, N14, and N16), P. domanosus (N9), Lactobacillus murinus (N13 and N15), Lactobacillus gasseri (N4 and N7), Lactobacillus acidophilus (N3), Lactobacillus plantarum (N8), Lactobacillus alimentarius (N12), and Lactobacillus casi subsp. rhamanosus (N11). Pinto, (2007) and Swetwiwathana, 2008 reported that Lactobacillus, Pediococcus, and Enterococcus spp. have the ability to produce bacteriocins that inhibit or kill gram-positive and gram-negative bacteria. Enterococcus faecium and Pediococcus acidilactici were found to play an important role as probiotics (Salminen, 1998). Lactobacillus gasseri has been known to produce the bacteriocin gassericin. Tahara, (1997) isolated L. gasseri JCM 2124 which produces at least two bacteriocins, named gassericin B2 and B3. Callewaert, (2008) and Sparo, (2008) found that bacteriocins from Enterococcus

Table 1. Presumptive bacteriocin producers isolated from sausages and other Sources

| Isolates serial No | Isolates code | Source | Type of sources |
|---|---|---|---|
| 1 | N1 | Sausages (Butcher- Khartoum market-Kh.S.B ) | *AS |
| 2 | N2 | Sausages (Butcher –Khartoum North market-KN.S.B) | *AS |
| 3 | N3 | Sausages (Butcher –O market-O.B) | *AS |
| 4 | N4 | Sheep Intestine (ShI) | *AS |
| 5 | N5 | Sheep Intestine (ShI) | *AS |
| 6 | N6 | Cattle Intestine (CI) | *AS |
| 7 | N7 | Cattle Intestine (CI) | *AS |
| 8 | N8 | Saliva (Sa) | *HS |
| 9 | N9 | Pigeon Intestine (PI) | *AS |
| 10 | N10 | Pigeon Intestine (PI) | *AS |
| 11 | N11 | Cheese (Cheese) | *AS |
| 12 | N12 | Intestine used for sausages filling (Int. S.F) | *AS |
| 13 | N13 | Intestine used for sausages filling (Int. S.F) | *AS |
| 14 | N14 | Chicken Intestine (ChI) | *AS |
| 15 | N15 | Chicken Intestine (ChI) | *AS |
| 16 | N16 | Cucumber (Cu) | ***NAS |

* AS. Animal Source.
** HS. Human Source.
*** NAS. None Animal Source.

Table 2. Presumptive identification of LAB isolated from sausages and other sources as bacteriocin producers

| Isolates No | Isolates code | Shape | Gas From glucose | NH3 from Arginine | Growth at 15ºC | Growth at 45ºC | Growth in 6.5%NaCl | Growth in 18% NaCl | Growth in 4.5% | Growth in 6.5% | Amygdalin | Arabinose | Fructose | Lactose | Raffinose | Salicin | Sucrose | Xylose | Maltose | Mannitol | Action in litmus milk | Species |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | N1 | Cocci | - | + | + | + | + | - | + | + | + | VW | + | + | + | + | + | - | + | - | + | *E. faecalis* |
| 2 | N2 | Cocci | - | - | + | + | - | - | W | W | + | - | + | + | - | + | + | - | + | - | - | *E. avium* |
| 3 | N3 | Rod | - | + | - | + | + | - | + | + | + | - | + | + | + | + | + | - | + | - | - | *L. acidophilus* |
| 4 | N4 | Rod | - | - | - | + | + | - | + | + | + | - | + | + | + | + | + | - | + | - | + | *L. gasseri* |
| 5 | N5 | Cocci | - | + | + | + | + | - | + | + | + | VW | + | + | + | + | + | - | + | - | + | *E. faecalis* |
| 6 | N6 | Cocci | - | + | + | + | + | - | + | + | + | VW | + | + | + | + | + | - | + | - | + | *E. feacalis* |
| 7 | N7 | Rod | - | - | - | + | + | - | + | + | + | - | + | + | + | + | + | - | + | - | + | *L. gasseri* |
| 8 | N8 | Rod | - | - | + | + | + | - | + | + | - | + | + | + | + | + | + | - | + | - | + | *L. plantarum* |
| 9 | N9 | tetrade | - | - | + | - | - | - | + | - | - | - | + | - | + | + | + | - | | - | + | *P. domanosus* |
| 10 | N10 | tetrade | - | - | + | + | + | - | + | + | - | - | + | + | - | - | - | - | + | - | - | *P. pentosaceus* |
| 11 | N11 | Rod | - | - | + | + | + | + | + | + | + | + | + | + | + | + | + | | + | | + | *L. casi sub rhamnosus* |
| 12 | N12 | Rod | - | - | + | - | + | - | + | + | + | + | + | - | - | + | + | - | + | - | + | *L. alimentarius* |
| 13 | N13 | Rod | - | - | + | + | + | - | + | + | + | + | + | + | + | + | + | - | + | + | + | *L. murinus* |
| 14 | N14 | tetrade | - | - | + | + | + | - | + | + | - | - | + | + | - | - | - | - | + | - | - | *P. pentosaceus* |
| 15 | N15 | Rod | - | - | + | + | + | - | + | + | + | + | + | + | + | + | + | - | + | + | + | *L. murinus* |
| 16 | N16 | tetrade | - | - | + | + | + | - | + | + | - | - | + | + | - | - | - | - | + | - | - | *P. pentosaceus* |

Legend:

(-)Negative Reaction. (w)Weak reaction.
(+) Positive reaction. (vw) Very weak reaction

*faecalis* and other enterococci species can be used as biopreservatives of food or as probiotics. Crude bacteriocin activity of LAB against target microorganisms is presented in Table 3. Crude bacteriocins of *Enterococcus faecalis* (N1), *Pediococcos pentosaceus* (N14) and *Lactobacillus murinus* (N13) exhibited a wide range (40-1280 AU/ ml) and the strongest antimicrobial activity on both gram-positive and garma-negative bacteria. The effect of bacteriocin producers on gram-negative bacteria

*Salmonella* sp., *Salmonella typhi* and *E coli* was either week or not detected. The same effect was observed on bacteriocins activity of supernatants of LAB against gram-negative bacteria (Table 4). Gram-negative bacteria are resistant to bacteriocins of lactic acid bacteria due to the effective barrier function of the outer membrane that is not found in gram-positive bacteria (Stevens, 1991).However, Audisio, (2001) and Pantev, (2003) claimed that enterocin or enterococcin obtained from enterococci have an antimicrobial activity against gram-negative bacteria such as *E. coli* and *Salmonella pullorum.*

All supernatants remaining after precipitation by $(NH)_2SO_4$ did not show antimicrobial effect against all indicator organisms (Table 4). The antimicrobial activity of most supernatants obtained from bacteriocin producers after precipitation by (NH4)2SO4, was either weak or not detected on the indicator organisms. This may be due to the complete precipitation of bacteriocin by (NH4)2SO4, or due to the low concentration of bacteriocins remaining after precipitation (Stevens, 1991). Activity of Pellets precipitated by $(NH)_2SO_4$ for all bacteriocin producers revealed a wide range against gram-positive and

Table 3. Crude Bacteriocin activity (AU/ml) against nine target organisms

| 1solates serial No | 1solates code | Bacteriocin-producers | Source of sample | Activity against target organisms | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *Salmonella sp.* | *B. cereus* | *E . coli* | *B.sterothermophilus* | *St. aureus* | | | | |
| 1 | N1 | *E. faecalis* | Kh,S. B | 160 | 1280 | 160 | 640 | 640 | 640 | 1280 | 40 | 1280 |
| 2 | N5 | *E. faecalis* | Sh.I | 40 | 80 | 0.00 | 1280 | 160 | 320 | 640 | 160 | 1280 |
| 3 | N6 | *E. faecalis* | C.I | 80 | 640 | 80 | 640 | 320 | 160 | 640 | 40 | 320 |
| 4 | N2 | *E. avium* | KN.S.B | 80 | 320 | 160 | 320 | 1280 | 160 | 1280 | 40 | 640 |
| 5 | N3 | *L. acidophilus* | O.B | 160 | 320 | 160 | 640 | 320 | 1280 | 1280 | 40 | 640 |
| 6 | N4 | *L. gasseri* | Sh.I | 80 | 80 | 0.00 | 40 | 0.00 | 0.00 | 0.00 | 0.00 | 160 |
| 7 | N7 | *L. gasseri* | C.I | 40 | 640 | 80 | 0.00 | 640 | 640 | 0.00 | 0.00 | 320 |
| 8 | N8 | *L. plantarum* | SP | 80 | 160 | 0.00 | 320 | 160 | 0.00 | 320 | 0.00 | 1280 |
| 9 | N9 | *P. domanosus* | P.I | 0.00 | 1280 | 160 | 1280 | 160 | 160 | 640 | 0.00 | 640 |
| 10 | N10 | *P. pentosaceus* | P.I | 160 | 1280 | 160 | 1280 | 1280 | 640 | 320 | 160 | 1280 |
| 11 | N14 | *P. pentosaceus* | Ch.I | 160 | 1280 | 160 | 320 | 1280 | 640 | 320 | 160 | 1280 |
| 12 | N16 | *P. pentosaceus* | Cu | 0.00 | 160 | 0.00 | 320 | 0.00 | 160 | 160 | 0.00 | 160 |
| 13 | N11 | *L. casi sub rhamnosus* | Cheese | 0.00 | 0.00 | 40 | 40 | 0.00 | 0.00 | 0.00 | 0.00 | 40 |
| 14 | N12 | *L. alimentarius* | In.S.F. | 160 | 320 | 40 | 160 | 640 | 160 | 160 | 160 | 160 |
| 15 | N13 | *L. murinus* | In.S.F | 40 | 1280 | 40 | 640 | 320 | 640 | 1280 | 40 | 1280 |
| 16 | N15 | *L. murinus* | Ch.I. | 40 | 1280 | 40 | 640 | 320 | 640 | 160 | 40 | 160 |

Legend:
AU: Arbitrary Unit

Table 4. Bacteriocin activity (AU/ml) of supernatants after bacteriocin precipitation by ammonium sulphate [(NH4)2SO4] against target organisms

| 1solates serial No | Isolates sources | 1solates code | Bacteriocin-producers | Source of isolates | Activity against target organisms | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *Salmonella sp.* | *B. cereus* | *E . coli* | *B.sterothermophilus* | *St. aureus* | | | *B.subtilis* | |
| 1 | AS | N1 | *E. faecalis* | Kh,S. B | 0.00 | 80 | 0.00 | 0.00 | 0.00 | 1280 | 0.00 | 0.00 | 0.00 |
| 2 | AS | N5 | *E. faecalis* | Sh.I | 0.00 | 80 | 0.00 | 0.00 | 0.00 | 1280 | 0.00 | 0.00 | 0.00 |
| 3 | AS | N6 | *E. faecalis* | C.I | 0.00 | 80 | 0.00 | 0.00 | 80 | 0.00 | 0.00 | 0.00 | 160 |
| 4 | AS | N2 | *E. avium* | KN.S.B | 0.00 | 0.00 | 0.00 | 160 | 0.00 | 0.00 | 80 | 0.00 | 40 |
| 5 | AS | N3 | *L. acidophilus* | O.B | 0.00 | 80 | 0.00 | 320 | 80 | 640 | 80 | 80 | 320 |
| 6 | AS | N4 | *L. gasseri* | Sh.I | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 40 | 0.00 | 0.00 | 40 |
| 7 | AS | N7 | *L. gasseri* | C.I | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 80 | 320 |
| 8 | HS | N8 | *L. plantarum* | SP | 0.00 | 160 | 80 | 0.00 | 80 | 0.00 | 0.00 | 0.00 | 160 |
| 9 | AS | N9 | *P. domanosus* | P.I | 0.00 | 0.00 | 80 | 40 | 0.00 | 320 | 0.00 | 80 | 0.00 |
| 10 | AS | N10 | *P. pentosaceus* | P.I | 0.00 | 40 | 80 | 40 | 0.00 | 160 | 0.00 | 0.00 | 0.00 |
| 11 | AS | N14 | *P. pentosaceus* | Ch.I | 0.00 | 1280 | 80 | 640 | 160 | 640 | 1280 | 160 | 320 |
| 12 | NAS | N16 | *P. pentosaceus* | Cu | 0.00 | 0.00 | 80 | 160 | 0.00 | 640 | 0.00 | 0.00 | 0.00 |
| 13 | AS | N11 | *L. casi sub rhamnosus* | Cheese | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 14 | AS | N12 | *L. alimentarius* | In.S.F. | 0.00 | 320 | 320 | 160 | 40 | 80 | 40 | 0.00 | 640 |
| 15 | AS | N13 | *L. murinus* | In.S.F | 0.00 | 40 | 0.00 | 0.00 | 0.00 | 320 | 320 | 0.00 | 80 |
| 16 | AS | N15 | *L. murinus* | Ch.I. | 0.00 | 40 | 0.00 | 0.00 | 0.00 | 320 | 320 | 0.00 | 80 |

Legend:
AU: Arbitrary Unit

Table 5. Bacteriocin activity (AU/ml) of pellets precipitated by ammonium sulphate [(NH4)2SO4] against target organisms

| 1solates No | 1solates Code | Bacteriocin-producers | Source of isolates | Activity against target organisms | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | *Salmonella* | *B. cereus* | *E. coli* | *B. stearothermophilus* | *St. aureus* | *B.subtilis* | | | |
| 1 | N1 | *E. faecalis* | Kh,S. B | 160 | 640 | 640 | 1280 | 1280 | 1280 | 1280 | 640 | 1280 |
| 2 | N5 | *E. faecalis* | Sh.I | 320 | 320 | 160 | 320 | 320 | 640 | 1280 | 160 | 1280 |
| 3 | N6 | *E. faecalis* | C.I | 320 | 640 | 160 | 1280 | 1280 | 1280 | 640 | 160 | 1280 |
| 4 | N2 | *E. avium* | KN.S.B | 160 | 1280 | 160 | 640 | 1280 | 320 | 640 | 160 | 320 |
| 5 | N3 | *L. acidophilus* | O.B | 640 | 1280 | 1280 | 1280 | 1280 | 640 | 640 | 640 | 640 |
| 6 | N4 | *L. gasseri* | Sh.I | 0.00 | 160 | 0.00 | 160 | 320 | 640 | 160 | 0.00 | 320 |
| 7 | N7 | *L. gasseri* | C.I | 80 | 160 | 0.00 | 160 | 640 | 640 | 40 | 0.00 | 1280 |
| 8 | N8 | *L. plantarum* | SP | 0.00 | 40 | 320 | 640 | 80 | 40 | 0.00 | 0.00 | 160 |
| 9 | N9 | *P. domanosus* | P.I | 160 | 160 | 0.00 | 640 | 320 | 160 | 0.00 | 0.00 | 1280 |
| 10 | N10 | *P. pentosaceus* | P.I | 160 | 160 | 40 | 640 | 320 | 160 | 640 | 0.00 | 640 |
| 11 | N14 | *P. pentosaceus* | Ch.I | 80 | 1280 | 1280 | 1280 | 1280 | 1280 | 640 | 160 | 1280 |
| 12 | N16 | *P. pentosaceus* | Cu | 0.00 | 40 | 40 | 160 | 0.00 | 160 | 0.00 | 0.00 | 640 |
| 13 | N11 | *L. casi sub rhamnosus* | Cheese | 0.00 | 320 | 640 | 160 | 0.00 | 160 | 80 | 40 | 40 |
| 14 | N12 | *L. alimentarius* | In.S.F. | 0.00 | 1280 | 0.00 | 1280 | 640 | 80 | 1280 | 40 | 1280 |
| 15 | N13 | *L. murinus* | In.S.F | 40 | 1280 | 160 | 640 | 1280 | 1280 | 1280 | 80 | 1280 |
| 16 | N15 | *L. murinus* | Ch.I. | 40 | 1280 | 160 | 320 | 1280 | 1280 | 1280 | 80 | 1280 |

Legend:
AU: Arbitrary Unit

gram-negative organisms (Table 5). Pellets obtained from bacteriocins of *Enterococcus faecalis* (N1), *Pediococcus pentosaceus* (N14); *Lactobacillus murinus* (N13) were the most active isolates against all indicator organisms. Their antimicrobial activity ranged between 40 and 1280AU/ml (Table 5). It was observed that *Salmonella* sp. affected by most LAB bacteriocin producers (Table 5). Partial purification or precipitation by (NH4)2SO4, increases the concentration of bacteriocins, thus increasing the activity. Ogunbanwo, (2003) reported that during the purification procedures each step resulted in a considerable loss of protein concentration while the specific activity increased and the optimal bacteriocin recovery was achieved by inducing ammonium sulfate and trichloroacetic acid precipitation. These results revealed that these bacteriocins could be used as food preservatives. Results presented in Table 3, 4, and 5 showed that bacteriocin-producing LAB isolated from non-animal sources recorded an antimicrobial activity ranging only between 0.00-320 AU/ ml against indicator organisms. These results all together revealed that isolates from animal sources showed a better performance than non-animal sources. Similar results were reported by Joshi, (2006).

## REFERENCES


Abdullah SA, Osman MM. 2010. Isolation and Identification of Lactic acid Bacteria from Raw Cow Milk, White Cheese and Rob in Sudan. *Pakistan* Journal of Nutrition 9(12): 1203-1206.

Audisio MC, Oliver G and Apella MC. 2001. Effect of different complex carbon sources on growth and bacteriocin synthesis of *Enterococcus faecium*. International Journal of Food Microbiology 63.235-241.

Axelsson L. 2004. Lactic acid bacteria: Classification and physiology. In: *Lactic Acid Bacteria.* Microbiological and Functional Aspects. (S. Salminen, and A. Von Wright, eds), Third edition. Marcel Dekker. New York, Basle, pp 1-72.

Aymerich MT, Garriga M, Monfort JM, Nes I and Hugas M. 2000. Bacteriocins-producing lactobacilli in Spanish-style fermented sausages: characterization of bacteriocins. Food Microbiology 17 (1): 33-45.

Barrow GISJ and Feltham RKA. 1993. *Manual for Identification of Medical Bacteria*, third edition. Cambridge University Press, England.

Ben Embarek PK, Jeppensen V and Hus HH. 1994. Antilisterial potential of *Enterococcus faecium* strains isolated from sous-vide cooked fish fillets. Food Microbiology 11: 525-536.

Bennik MHJ, Vanloo B, Brasseur R, Gorris LGM and Smid EJ. 1998. A novel bacteriocin with YGNGV motif from vegetable associated *Enterococci mundtii*: full chrcterization and interaction with target organism. Biochem. Bioph. Acta 1373, 47–58.

Callewaert R, Hugas M and De Vuyst L. 2000. Competitiveness and bacteriocin production of Enterococci in production of Spanish-style dry fermented sausages. *International Journal of Food Microbiol*ogy 57:33-42.

Carr FJ, Chill D and Maida N. 2002. The lactic acid bacteria. A literature survey. Crit. Rev. Microbiol. 28: 281-370.

Carolissen-Mackay V, Arendse G and Hastings JW. 1997. Purification of bacteriocins of lactic acid bacteria: problems and pointers. *International Journal of Food Microbiology* 34(1):1-16.

Chelule PK, Mokeona MP and Gqaleni N. 2010. Advantages of traditional lactic acid bacteria fermentation of food in Africa Current Research Technology and Education Topics in Applied Microbiology and Microbial Biotechnology. Mèndez- Vilas, A. (Ed.): 1160-1167. FORMATEX.

Cintas LM, Casaus P, Harvarstein LS, Hernandez PE and Nes IF. 1997. Biochemical and genetics chraterization of enterocin P, a novel sec-dependant bacteriocin from Enterococcus faeciumP13 with

a broad spectrum. Appl. Environ.Microbiol 63: 4321-4330
Daba H, Pandian S, Gosselin JF, Simard RE, Huang J and Lacroix C. 1991. Detection and activity of bacteriocin produced by *Leuconstoc mesenteroides*. Applied of Environmental Microbiology 57: 3450-3455.
de Man JC, Rogosa M and Sharpe ME. 1960. A medium for the cultivation of lactobacilli. *J*ournal of *Applied*. *Bact*eriology. 23:130.
De Vuyst L and Vandamme EJ. 1994. Antimicrobial potential of lactic acid bacteria, p. 91- 142. In: *Bacteriocins of Lactic Acid Bacteria*. (de Vuyst, L. and Vandamme E.J., ed.), Blackie Academic and Professional, Glasgow.
Deegan LH, Cotter PD, Hill C and Ross P. 2005. Bacteriocins; biological tools for biopreservation and shelf-life extension. International Dairy Journal. 160:1058-1071.
Floriano B, Ruiz-Barba JL and Jimenez-Diaz R. 1998. Purification and gentic characterization of enterocin 1 from Enterococcus faecium 6T1a, a novel antilisterial plasmid encoded bacteriocinwhich does not belong to the pediocin family of bacteriocins. Applied and Environmental Microbiology. 64: 4883-4890.
Foulquie´ Moreno MR, Sarantinopoulos P, Tsakalidou E and De Vuyst L. 2006. The role and application of enterococci in food and health. Int. J. Food Microbiol. 106: 1-24.
Franz CM, Haolzapfel WH and Stiles ME. 1999. Enerococci at the crossroad of food safety?. *International Journal of Food Microbiology*. 64: 1-24.
Fricourt BV, Barefoot SF, Testin RF and Hayasaka SS. 1994. Detection and activity of plantaricin F, an antibacterial substance from *Lactobacillus plantarum* BF001 isolated from processed channel catfish. *J. Food prot.* 57: 698-702.
Gelsomino R, Vancanneyt M, Condon S, Swings J and Cogan TM. 2001. Enerococci diversity in the cheese making environment of an Irish Cheddar-type cheese makonf factory. *International Journal of Food Microbiology*. 71: 177-188.
Graciela M, Vignolo M, de Kairuz Aida AP, de. Ruiz H and Oliver G. 1995. Influence of growth condition on the production of lactocin 705, a bcteriocin produced by *Lactobacillus casei* CRL.705. Journal of Applied. Bacteriology 78; 5-10.
Hansen EB. 2002. Commercial bacterial starter Cultures for fermented foods of the future. International. Journal of Food Microbiology 78: 119-131.
Harrigan WE. 1998. *Laboratory Methods in Food and Dairy Microbiology.* Academic press. USA.
Herranz C, Casaus P, Mukhopadhyay S, Martinez JM, Rodriguez JM, Nes F, Hernandez PE and Cintas LM. 2001. *Enterococcus faecium* P21: a strain occurring naturally in dry-fermented sausages producing the class II bacteriocins enterocin A and enterocin B. Food Microbiology 18: 115-131.
Holt JG, Krieg NR, Sneath PHA, Staley JT and Williams ST. 1994. Bergey's manual of determinative bacteriology, 9th ed. P. 529, p 566. Williams and Wilkins. M D.
Holzapfel EB. 2002. Appropriate starter culture technologies for small scale fermentation in developing countries. *Int. J. Food Microbiol.* 75:197-212.
Jack RW, Tagg JR and Ray B. 1995. Bacteriocins of gram-positive bacteria. Microbiol .Mol. Biol. Rev. 59: 171-200.
Joshi VK, Sharmal S and Neerja SR. 2006. Production, purification , stability and efficacy of bacteriocin from isolates of natural lactic acid fermentation of vegetables. Food Technol Biotechnol. 44(3): 435-439.
Lade, HS, Chitanand, MP, Gyananath, G and Kadam, TA. 2006. Studies on some properties of bacteriocins produced by *Lactobacillus* species isolated from agro-based waste. The Internet Journal of Microbiology ™ ISSN: 1937-8289. Vol. 2 (1-5).
Leroy, F and De Vuyst, L. 2004. Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends in Food Science and Technology*. 15: 67-78.
Leroy F, Foulquie´ Moreno MR and De Vuyst L. 2003. *Enterococcus faecium* RZS C5, an intresting bacteriocin producer to be used as a-culture in food fermentation. Int. J. Food Microbiol 88: 235-240.
Lewus CB, Kaiser A and Montville TJ. 1991. Inhibition of food borne bacterial pathogens by bacteriocin from lactic acid bacteria isolated from meat. Applied Environ. Microbiol 57: 1683-1688.
Moreno I, Lerayer AIS, Baldini VLS and Leitão MF, de F. 2000. Chraterization of bacteriocins produced by *Lactococcus lactis* strains. Braz .J. Microbiol 31: 184-192.
Moreno MR, Sarantinopoulos P, Tsakalidon E and De Vuyst L. 2006. The role and application of enterococcin in food and health. Int. J. Food. Microbiol 106: 1-24.
Ogunbanwo ST, Sanni AI and Onilude AA. 2003. Characterization of bacteriocin produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. *African Journal of Biotechnology*. 2 (8): 219-227.
Oyewole OB. 1997. Lactic fermented foods in Africa and their benefits. *Food Control.* 1997; 8(5-6):289-297.
Pantev A, Valcheva R, Danova S, Ivanova I, Minkov I, Vainkov I, Haertle T and Chobert JM. 2003. Effect of enterocin A 2000 on biological and synthetic phospolipid membranes. Int. J. Food Microbiol 80: 145- 152.
Papagianni M. 2003. Ribosomally synthesized peptides with antimicrobial properties: biosynthesis, structure, function and applications. Biotechnol. Adv 21: 465-499.
Patil M, Pal M, Pal V and Yaddula RK. 2007. Isolation of bacteriocinogenic lactic acid bacteria from rat intestine. J. Culture Collection 5: pp 58-63.
Pinto AL, Fernandes M, Pinto C, Albano H, Teixeira P, Castilho F and Gibbs PA. 2007. Partial characterization of bacteriocin produced by *Pediococcus pentosaceus* and *Enterococcus faecium* isolated from ready-to-eat seafood. Abstracts/Journal of Biotechnology. 131S: S211-5241.
Rammelsberg M and Radler F. 1990. Antibacterial polypeptides of *Lactobacillus* species. J. Appl. Bacterio 69: 177-184.
Rodriguez JM. 1996. Review: Antimicrobial spectrum, properties and mode of action of nisin, a bacteriocin produced by *Lactococcus lactis.* Food Sci. Technol. *Int* 2(2): 61-68.

Salih AM, El Sanosi MSM and El Zubeir IEM. 2010. A review on the Sdanese Traditional Dairy Products and Technology. International Journal Dairy Science Vol (6): 227. Issue 4.

Salminen S, Laine M, von Wright A, Vuopio-Varkila I, Korhonen T and Mattila-Sandhom. 1998. Development of selection criteria for proboitic strains to assess their potential in functional foods: a nordic and European approach. Biosci. Microflora 15: 61-70.

Salminen S, Wright AV and Ouwehand A. 2004. *Lactic Acid Bacteria. Microbiology and Functional Aspects.* Tird Edition. Marcel Dekker, Inc. New York. Basel.

Schillinger V and Lücke FK. 1989. Antimicrobial activity of *Lactobacillus sake* isolated from meat. Appl. Environ. Microbiol 55(8):1901-1906.

Sneath PHA, Mair NS, Sharp ME and Holt JG. 1986. Berg's Mannual of Systematic Bacteriology, Vol. 2, (Baltimre: Williams and Wilkins).

Sparo M, Muñez GG, Castro M, Calcagno ML, Garcia M, Allende MA, Ceci M, Najle R and Manghi M. 2008. Characteristics of an environmental strain, *Enterococcus faecalis* CECT7121, and its effects as additive on craft dry-fermented sausages. Food Microbiology. 25(4): 607-615.

Stevens KA, Sheldon BW, Klapes NA and Klaenhammer TR. 1991. Nisin treatment for inactivation of *Salmonella* species and other gram-negative bacteria. Appl. Environ.Microbiol. 57(12): 3613-3615.

Swetwiwathana A, Surapantanpisit Y, Zendo T, Nakayama J and Sonomoto K. 2008. Identification and partial characterization of pediocin PA-1 produced by *Pediococcus pentosaceus* associated in traditional Thai fermented beef (mum). Proceeding of 53rd International Cogress of Meat Science and Technology. Edited by Zhou and Zhang. China Agricultural University Press. pp. 61-62.

Tahara T, Yoshioka S, Utsumi R and Kanatani K. 1997. Isolation and partial characterization of bacteriocins produced by *Lactobacillus gasseri* JCM 2124. FEMS Microbiology Letters 148(1): 97-100.

Takahiro T, Emiko Y and Takatoshi I. 1991. Lacticin, a bacteriocin produced by *Lactobacillus delbrueckii* subsp. lactis. Lett. Appl. Microbiol 12: 43-45.

Todorov SD and Dicks LMT. 2005. *Lactobacillus plantarum* isolated from molasses produces bacteriocins active against Gram-negative bacteria. Enzyme and Microbiol, Technol 36: 318-326.

Van Reenen CA, Dicks LMT and Chikindas ML. 1998. Isolation, purification and partial characterization of plantiricin 423, a bacteriocin produced by *Lactobacillus plantarum*. J. Applied Microbiol 84: 1131-1137.

Vera Pringiton E, Salvucci E, Sesma F and Nader –Macias ME. 2007. Different for purification of antimicrobial peptide from Lactic Acid Bacteria (LAB). Communicating Current Research and Educational Tropics and Trends in Applied Microbiology. Méndez-Vilas (Ed). Pp 557-568.

Yang R, Johnson MC and Ray B. 1992. Novel Methods to extract large amount of bacteriocin from lactic acid bacteria. Applied and Environmental Microbiology 58: 3355-3359.