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BIOPROTECTIVE POTENTIAL OF BACTERIOCINS FROM SOME *Lactobacillus* species ISOLATED FROM FOODS

Iyabo Christianah Oladipo[1], Deborah Oluyinka Anwooko[1], Dupe Ibukun Daramola[1], Funmilayo Bose Babalola[1], Ganiyat T. Abidoye[1], Olaitan Dorcas Olawale[1], Kehinde Mary Iyanda[1], Ouwakemi Ruth Ajiboye[1], Daniel Anuoluwapo Osunpidan[1], Aminat Theresa Ogunremi[1] and Afolake A. Olanbiwoninu[2]*

Address(es): Dr. Iyabo Christianah Oladipo,
[1]Ladoke Akintola University of Technology, Faculty of Pure and Applied Sciences, Department of Science Laboratory Technology, Ogbomoso 210214, Oyo State, Nigeria, phone number: +2348032183477.
[2]Ajayi Crowther University,, Faculty of Science, Department of Biological Sciences, Oyo, Oyo state, Nigeria.

*Corresponding author: xtiecoker@gmail.com

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ABSTRACT

Lactobacillus species isolated from *ogi*, *kunnu*, yoghurt and palm-wine were found to produce bacteriocins. The bacteriocins had broad spectra of antimicrobial activities against both Gram-positive and negative bacteria. The effects of the bacteriocins on *Escherichia coli* infections in rats were evaluated. *Sprague-Dawley* rats were infected with *E. coli* and treated with 1280 AU/ml of the bacteriocins from *L. plantarum* MO21, *L. plantarum* MP12, *L. casei* MK21, *L. casei* MO11, *L. brevis* MK11 and *L. buchneri* MY21. *Escherichia coli* infection caused upregulation of aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, total protein, globulin, cholesterol, bilirubin and glucose levels in sera of the infected rats which were down-regulated in the bacteriocin treated rats. Gastric and GIT damage caused by *E. coli* infection were reduced in the bacteriocin-treated groups. Therefore, it is concluded that these bacteriocins may have useful biomedical applications.

Keywords: *Lactobacillus* species; bacteriocin; gastric tissue; total protein and globulin

INTRODUCTION

The lactic acid bacteria (LAB) are rod- shaped bacilli or cocci characterized by an increased tolerance to a lower pH range. This aspect partially enables LAB to outcompete other bacteria in a natural fermentation, as they can withstand the increased acidity from organic acid production e.g. lactic acid. Lactic acid bacteria are generally recognized as safe (GRAS) and play an important role in food and feed fermentation and preservation either as the natural micro-flora or as starter culture added under controlled conditions. The preservative effect exerted by lactic acid bacteria is mainly due to the production of organic acids such as lactic acid which result in lowered pH (**Daeschel, 1989**). Lactic acid bacteria also produce antimicrobial compounds including hydrogen peroxide, $C0_2$, diacetyl and bacteriocin (**Cintas *et al.*, 2001**).

In probiotic and biotherapeutics, some strains of *Lactobacillus* and other lactic acid bacteria possess potential therapeutic properties including anti-inflammatory and anti-Cancer activities as well as other features of interest (**Arimah and Ogunlowo, 2014**). *Lactobacilli* are considered to have probiotic uses. Many people take *L. acidophilus* to help maintain the pH level of the intestine through the production of lactic acid, which allows for the proliferation of sensitive yet beneficial microbes that are important parts of the fecal floral. *L. acidophilus* is also used as a feed additive for livestock because it supposedly helps the digestibility of feeds through the production of certain enzymes. University of Nebraska research has shown, in the largest feeding study ever conducted that calves fed with feed supplemented with *L. acidophilus* had up to 80% less *E. coli* in their manure. This is the most promising method in inhibiting *E. coli* in livestock to date (**Altermann *et al.*, 2005**).

Gastrointestinal disorder is caused by various factors including antibiotic administration (**Van der Waaij *et al.*, 1982**) and as a result of infectious agents such as toxigenic *Escherichia coli*, *Salmonella enteritis* and viruses (**Silva *et al.*, 1999**). Innovative approaches have been tried as alternative to antibiotics in treating gastrointestinal diseases and these include using live biotherapeutic agents such as yeast and *Lactobacillus* species (**Fuller, 1992**). Also, some people use *Lactobacillus* for general digestion problem; irritable bowel syndrome (**Niedzielin *et al.*, 2001**), colic in babies (**Savino *et al.*, 2007**), crohn's disease (**Marteau *et al.*, 2006**), and a serious gut problem called necrotizing enterocolitis (NEC) in new born babies (**Luoto *et al.*, 2010**). It is also used for infection with *Helicobacter pylori* the type of bacteria that causes ulcers (**Sakamoto *et al.*, 2001**) and for other types of infection including urinary tract infections (UTIS) and to prevent respiratory infection in children attending daycare centers. It is also used for skin disorder such as fever blisters, canker, sores, eczema (**Woo *et al.*, 2010**), acne, high cholesterol, lactose intolerance and to boost the immune system (**Berggren *et al.*, 2011**).

Lactobacilli have been used for decades against infectious diseases (**Bernet *et al.*, 1994**) and their antimicrobial activities against pathogens have been extensively studied. These organisms have been widely used as probiotics (**Tannock, 1999**). Many of these lactic acid bacteria are known to produce antibacterial substance including bacterocin which can inhibit the growth of several pathogenic bacteria. Bacteriocin from lactic acid bacteria are natural antimicrobial peptides or small proteins with bactericidal or bacteriostatic activity against genetically related species (**Klaenhammer, 1988**). Bacteriocin can be classified broadly as those synthesized by Gram-positive and those by Gram-negative organisms, among those synthesized by Gram- positive, *Lactobacilli* bacteriocins are of commercial value (**Garneau *et al.*, 2002**).

Probiotics are live microorganisms that when consumed in an adequate amount as part of the food, confer a health benefit on the host (**FAO/WHO, 2001**). An experimental focus of probiotic LAB strains has indicated that this potential might play a considerable role during in vivo interactions occurring in the human gastro intestinal tract (**Avonts and De Vuyst, 2001; Kim *et al.*, 2003**). Therefore the aim of this research is to isolate and identify *Lactobacillus* species from some foods and to carry out *in vivo* assay of the bacteriocin produced by the isolated *Lactobacillus* species.

MATERIAL AND METHODS

Sample Collection

Four food samples which are palm wine, *ogi*, *kun-nu* and yoghurt were purchased from local producers in Ogbomosho, Oyo State, Nigeria.

Microbiological Analysis

One millilitre of each sample was measured, dispensed into 9 mL of sterile peptone water in McCartney bottles, and homogenized by thorough shaking. The samples were diluted into 10-fold dilutions and appropriate dilution was plated on deMann Rogosa Sharpe agar (Oxoid) to obtain LAB. Plates were incubated at $30^{O}C$ for 48 h under anaerobic conditions. Colonies were randomly picked from the agar plates and the strains were streaked out repeatedly to check for purity and sub-cultured on fresh agar plates of the isolation media, followed by microscopic examinations. The stock cultures were routinely maintained on MRS agar slants kept in refrigerator at 4^{O}C. The organisms were kept in freezing medium, by inoculating pure cultures into MRS broth with 20% glycerol and stored at -20^{O}C.

Phenotypic Characterization

The cell morphology of the presumptive LAB strains were viewed by using a phase contrast microscope (Olympus CH3-BH-PC, Japan) after Gram staining and testing for catalase activity. Strains were preliminarily identified based on phenotypic properties such as the ammonia (NH_3) production from arginine, growth at 15^{O}C and 45^{O}C, ability to grow at 6.5% of sodium chloride (NaCl), hydrogen peroxide production, pH reduction in MRS broth, and gas (CO_2) production from glucose, according to **Dykes (1995)**. All the strains were tested for the sugar fermentation patterns.

Identification of Strains

Strains identification was carried out according to physiological and biochemical characteristics, as described by **Schleifer and Kilpper-Balz (1984)**. To confirm the identity of the isolates, total genomic DNA was extracted using the method described by **Oladipo *et al*.** (**2013**). Identification was carried out by sequencing of the 16S rRNA genes using the primers designated as FD1 (5'-AGAGTT TGATCCTGGCTCAG - 3') forward and RD1 (5'-AAGGAGGTGATCCAGCC-3') for reverse (**Weisburg *et al*., 1991**).

Determination of bacteriocin production and antimicrobial spectra

This was carried out using the modified method of **Oladipo *et al*.** (**2014a, b; 2015a**). Briefly, *Lactobacillus species* were propagated in 100mL MRS broth for 72 hours at 30^{0}C anaerobically. For extraction of bacteriocin, a cell-free solution was obtained by centrifuging the culture (6708 g for 20 minutes at 4^{0}Cwith Beckman L5050B) and was adjusted to pH 7.0 by means of 1M NaOH to exclude the antimicrobial effect of organic acid. Inhibitory activity from hydrogen peroxide was eliminated by the addition of 5mg/mL catalase and filter sterilized through 0.22 mm filters. The antimicrobial activities of the supernatants were determined by well diffusion assay, 10μL aliquots of supernatants were placed in wells (3-mm diameter) cut in cooled soft LB agar plates previously seeded with indicator microorganisms. After 2 hours at 4^{O}C, the plates were incubated at 30 ^{O}C or 37^{O}C for growth of the target organism; after 24 hours, the plates were examined for growth inhibition zones (**Daba *et al*., 1991**).

Purification of Bacteriocins

The crude bacteriocin samples produced were treated with solid ammonium sulphate to 0, 30, 35, 40, 45, 50, 55, 60, 65 and 70% saturation. The mixtures were stirred for 2 hours at 4^{0}C and later centrifuged at 6708 g for 1 hour (4^{0}C). The precipitates were re-suspended in 25 ml of 0.05M potassium phosphate buffer (pH 7.0). Dialysis was followed in a tubular cellulose membrane against 2 liters of the same buffer for 18 hours in spectrapors No. 4 dialysis tubing. Assay of the bacteriocin activity was carried out (**Rammelsberg and Radler, 1990**). Agar well diffusion assay procedure was used, aliquots of 50μL from each bacteriocin dilution were placed in wells in plates seeded with the bioassay strain. The plates were incubated overnight at 30^{0}C and the diameters of the inhibition zones were taken.

***In vivo* Assay**

Infection and Treatment

The weights of all the animals were recorded prior to infection and all rats were confirmed to be healthy. The animals were randomly divided into 8 groups of 10 animals each. The rats in Group 1, the control were not infected but received normal saline (0.4μL) while the other seven groups were infected by oral administration with 0.1ml of *E. coli* (5.7×10^{7} CFU/mL). Group 2 was infected but received no bacteriocin, Groups 3 to 8 animals were given 1280 AU/mL of the bacteriocin from *L. plantarum* MO21, *L. plantarum* MP12, *L. casei* MK21, *L. casei* MO11, *L. brevis* MK11 and *L. buchneri* MY21 respectively. This treatment was administered twice in a week for 3 weeks (**Oladipo *et al*., 2014a**).

Clinical Examination

A modification of the method of **Oladipo *et al*.** (**2014a; 2015b**) was used for clinical examination of the experimental animals. The animals were weighed on a daily basis; blood samples were drawn from all the animals after three weeks of treatment and the blood was allowed to cloth at room temperature and then centrifuged at 310 g for 12 minutes. The serum was collected and used for analysis, which included total protein, blood glucose, albumin, globulin, and enzymes activities (AST and ALT).

Serum Biochemical parameters Analysis

Glucose Analysis: The quantitative determination of serum glucose was carried out using commercially available diagnostic experimental kits purchased from Diagnosticum Limited (Budapest, Hungary).

Albumin Assay: Quantitative colorimetric albumin determination was carried out by using Albumin Assay kit which was purchased from BioSino Biotechnology & Science Inc. (China). Serum total protein was determined according to Lowry's method. Total protein minus albumin equals globulin.

Transaminases Assay: The determination of AST and ALT was based on the fact that phenyl hydrazone which was produced after incubating the substrate with the enzyme was measured spectrophotometrically. The amount of phenyl hydrazone formed was directly proportional to the enzyme quantity. Stanadard kits for the determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were obtained from Span diagnostics, Surat (Gujurat). Colorimetric procedure in which the oxaloacetate and/or pyruvate formed in either the AST or ALT reaction is combined with 2, 4-dinitrophenylhydrazine to yield a brown-coloured hydrazone which is measured at 505 nm.

Histological assessment of the gastric tissues and GIT

The animals were anesthetized by ketamine (12 mg kg^{-1} body weight) followed by cervical dislocation for killing, stomachs and small intestines were removed and fixed in 10% formalin and embedded in paraffin. The sections (5μm) were cut with a microtome, stained with hematoxylin and eosin, and assessed under an Olympus microscope (Olympus Optical Co., GMBH, Hamburg, Germany). Images were captured using Camedia software (E20P 5.0 Megapixel; Hamburg, Germany) at 20X magnification (**Oladipo *et al*., 2014a; 2015b)**.

Statistical analysis

Statistical analysis of the data obtained was carried out using GraphPad Instat 3 software. Comparison between groups was made using one-way analysis of variance.

RESULTS AND DISCUSSION

Identification of bacterial strains

The isolates were found to be Gram's positive rod, catalase, oxidase, methyl red, coagulase, urease and indole negative. They were unable to hydrolyze casein and gelatin but they were all able to grow at pH of 3.9 and 9.2. In this present study, six organisms isolated from food samples (*ogi*, *kunnu*, yogurt and palm wine) were characterized and identified to be *Lactobacillus* species using polyphasic taxonomy approach as described by **Oladipo *et al*. (2013)**. The sequences were deposited in the GenBank database and accession numbers assigned to each strain as shown in Table 1.

Determination of bacteriocin production and antimicrobial spectra

Determination of the antimicrobial activity was carried out for all the *Lactobacillus* isolates and the bacteriocin produced showed antimicrobial inhibitory activity against *Serratia marcescens, Micrococcus luteus, Bacillus cereus, Bacillus subtilis, Escherichia coli, Lactococcus lactis, Staphylococcus aureus and Bacillus pumilus* but no activity was shown against *Proteus mirabilis, Proteus vulgaris, Klebsiella pneumonia, Enterobacter cloacae and Pseudomonas aeruginosa. Lactobacillus plantarum* MO21 and *Lactobacillus plantarum* MP12 had broad spectra of activities against the indicator organisms used (Table 2). The culture supernatant of *Lactobacillus* species was found to posses' antimicrobial activity against Gram positive and Gram negative bacteria. This supports the previous findings of **Nes *et al*. (1996)** who reported that bacteriocins are proteinaceous compounds with inhibitory activity against more or less related bacterial genera. **Juven *et al*. (1992)** also reported that the ability of the *Lactobacilli* to produce metabolites such as bacteriocins has been suggested as being responsible for their ability to inhibit other bacteria.

In-vivo evaluation of the effects of the bacteriocinogenic strains and their bacteriocins

Table 3 shows the result of weight gained by the experimental rats during *in-vivo* assay. No death was recorded and their weights were measured on a weekly basis for three weeks. The control had the highest weight gained after the first, second and third week while the infected but not treated group showed the lowest weight gained. No changes in rats' behavior, daily activity or physiology of the experimental rats was observed and the weight gained was regular. The weight gain result reveals that the body weight of the rats increased during the 3weeks of experiment. The low gained weight recorded in the untreated rats may be as a result of the infection induced by *E. coli* while the high gained weight recorded in the bacteriocin treated rats confirmed the findings of **Fuller and Gibson (1997)** who reported that bacteriocin has been used as growth promoters due to their ability to suppress the growth and activities of growth depressing micro flora and their ability in enhancing absorption of nutrients through the production of digestive enzymes.

Biochemical evaluation of sera

The result of the biochemical analysis showed an increase in the values of AST, ALT, albumin, total protein, globulin, cholesterol, bilirubin and glucose of the untreated rats due to the prolong infection caused by *E. coli.* But the bacteriocin treatment normalized the blood serum of the treated groups (Table 4). With regards to serum biochemical analysis, the high level of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) of the untreated group is an indication of liver damage or dysfunction caused by the administration of *E. coli.* Alanine aminotransferase (ALT) is principally found in the liver and is regarded as being more specific than AST for detecting liver cell damage (**Johnston, 1999**; **Cheesborough, 1991**). A rise in ALP activities has been linked with an increased osteoblastic activity (**Baron *et al*. 1994**) and lack of bile flow (cholestasis). **Devaraj (2012)** reported that when body tissue or an organ such as the heart or liver is diseased or damaged additional AST is released into the bloodstream. The lower AST and ALT values in rats treated with bacteriocin indicate liver function improvement brought about by bacteriocin treatment. As these parameters represent liver function, increase in their levels will indicate liver damage (**Gad, 2007**). Similar positive effect on the biochemical parameters of rats' serum were also observed by other authors with different probiotic treatment (**Fukushima and Nakano, 1995**).
Biochemical analysis showed high total protein, albumin, globulin, glucose, cholesterol and bilirubin in the rats from untreated group compared to the control and treated rats. **Amdekar *et al*. (2010)** reported that *E. coli* infection can cause the rise in total protein of blood serum and **Koneko (1989)**, reported that increase in total protein and globulin in serum have been associated with bacteria septicaemia and liver disease. The observed elevated bilirubin levels in the blood of infected rats may arise from free radical damage caused by the pathogen. This damage may be to the liver, red blood cells or the heart. **Baranano *et al*. (2002)** reported that elevated bilirubin levels in blood shows risk of cardiovascular diseases, increased breakdown of red blood cells and liver failure. **Koneko (1989)** reported that increase in total protein and globulin concentration in serum has been associated with bacterial septicemia and liver disease which was also observed in untreated rats. The lower Cholesterol level in the treated group when compared to untreated rats confirmed the ability of bacteriocin to function as anticholesterol substance. **Casas and Dobrogosz (2000)** said generally that *Lactobacilli* have anticholesterolaemic effect and **Bertazzoni *et al*. (2001)** found that *Lactobacilli* have direct effect on cholesterol levels by assimilation and removal from the growth medium.

Histological assessment of gastric tissues and GIT

The histopathology of the stomach showed that the *E. coli* infection caused disruption at the junction between the sub mucosal and muscular layer, infiltration by inflammatory cells but this was normalized by bacteriocin treatment as no visible lesions was observed in the stomach of the bacteriocin treated rats (Fig. 1). Also, the histopathology of the intestine of the untreated rats showed blunted and disintegrated villi tips and there were large parasitic sections along with little mucosal debris in the intestinal lumen which was cured by the bacteriocin treatment (Fig. 2). The result of the histopathology of the stomach of rats after three weeks of bacteriocin treatment showed no visible lesion as compared to the control. Also, the mucosa, submucosa and muscular layer were unaffected. The protection of the gastro intestinal tract was observed in the intestine of the bacteriocin treated rats because no visible lesions was observed and the serosa, villi and lumen was intact after three weeks of bacteriocin treatment. This indicated that bacteriocin treatment was able to cure the infection caused by the *E. coli* and this is also in accordance with **Oyetayo *et al*. (2003)** whose histopathological result also confirmed the protective effect of the *Lactobacillus*. There has been a number of studies that reveal the probiotic potential of *Lactobacilli* as health promoting bacteria in man and animals (**FAO/WHO, 2001**). They play a major role in protecting the immune system against pathogens residing in the human body.

Table 1 *Lactobacillus* species used in this study

Name of isolates	Source of isolation	Accession number
Lactobacillus plantarum MO21	*Ogi*	KJ739519
Lactobacillus plantarum MP12	*Palm wine*	KJ739520
Lactobacillus casei MK21	*Kunnu*	KJ739521
Lactobacillus casei MO11	*Ogi*	KJ739522
Lactobacillus brevis MK11	*Kunnu*	KJ739523
Lactobacillus buchneri MY21	*Yoghurt*	KJ739524

Accession number were assigned by GenBank of National Centre for Biotechnological Information (www.ncbi.nlm.nih.gov/genbank/)

Table 2 Antimicrobial activity of *Lactobacillus* species against indicator organisms

Indicator organisms	*L. plantarum* MO21	*L. plantarum* MP12	*L. casei* MK21	*L. casei* MO11	*L. brevis* MK11	*L. buchneri* MY21
Serratia marcescens	10.1± 0.21	11.0± 0.22	10.0± 0.11	9.0± 0.23	10.0± 0.12	9.5± 0.13
Micrococcus luteus	13.0± 0.23	12.0± 0.31	11.0± 0.31	10.0± 0.14	11.0± 0.14	8.0± 0.24
Proteus mirabilis	-	-	-	-	-	-
Proteus vulgaris	-	-	-	-	-	-
Bacillus cereus	10.0± 0.30	11.0± 0.21	9.0± 0.14	11.0± 0.12	9.0± 0.23	10.0± 0.31
Bacillus subtilis	11.5± 0.25	10.5± 0.11	8.0± 0.13	12.0± 0.23	10.0± 0.15	11.0± 0.23
Klebsiella pneumoniae	-	-	-	-	-	-
Escherichia coli	12.0± 0.10	11.5± 0.10	9.0± 0.11	9.0± 0.15	11.0± 0.16	8.5± 0.12
Shigella flexneri	12.5± 0.12	12.0± 0.14	-	-	-	-
Lactococcus lactis	11.0± 0.11	10.5± 0.13	10.0± 0.25	8.0± 0.31	8.5± 0.11	12.0± 0.24
Enterobacter cloacae	-	-	-	-	-	-
Staphylococcus aureus	13.0± 0.20	12.0± 0.12	11.0± 0.12	9.5± 0.16	9.5± 0.10	10.0± 0.20
Pseudomonas aeruginosa	-	-	-	-	-	-
Aeromonas hydrophilia	9.0± 0.21	9.2± 0.22	-	-	-	-
Salmonella typhimurium	10.0± 0.21	10.0± 0.23	9.0± 0.24	8.7± 0.22	-	-
Bacillus pumilus	11.0± 0.22	10.5± 0.25	8.0± 0.26	8.5± 0.24	10.0± 0.24	13.0± 0.11

Each value is a mean of 3 replicates ± standard deviation, - = Not Detected, diameter of zones of inhibition are in millimeter

Table 3 Weight gained by experimental rats during experimental period

Weeks of treatment	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8
Week 0	150± 0.47	150± 0.41	150± 9.81	150± 9.56	150± 0.47	150± 9.40	150± 0.47	150± 9.47
Week 1	158± 0.29	150± 0.22	152± 0.26	151± 0.22	151± 0.22	152± 0.26	152± 0.27	151± 0.26
Week 2	187± 1.41	153± 0.90	170± 1.47	171± 0.99	170± 0.79	173± 1.20	172± 1.00	173± 1.01
Week 3	200± 1.21	158± 0.89	190± 0.81	194± 0.92	196± 0.91	194± 0.99	192± 0.96	193± 1.09

Each value is a mean of 3 replicates ± standard deviation, weight of experimental animals are in gram

Table 4 Biochemical analysis of the blood serum of experimental rats

Biochemical parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Untreated
Total protein (g/dl)	1.9± 0.21	4.9± 0.41	3.9± 0.41	3.9± 0.21	3.9± 0.41	3.9± 0.51	3.9± 0.25	3.9± 0.11	4.9± 0.41
Glucose (g/dl)	119.6± 24.99	132.9± 20.02	130.0± 15.56	130.9± 20.02	130.9± 20.02	130.2± 15.86	130.4± 27.97	130.2± 15.56	132.9± 20.02
Globulin (g/dl)	0.9± 0.41	1.2± 0.49	1.04± 0.47	0.9± 0.21	1.0± 0.49	0.9± 0.21	1.0± 0.50	1.0± 0.17	1.2± 0.49
Cholesterol (g/dl)	69.7± 8.71	78.0± 9.61	70.3± 9.81	70.3± 9.81	70.3± 9.81	70.3± 9.99	71.0± 9.60	59.9± 5.49	78.0± 9.61
Bilirubin (g/dl)	0.45± 0.94	0.72± 0.10	0.49± 0.09	0.63± 0.10	0.69± 0.18	0.62± 0.21	0.67± 0.04	0.62± 0.10	0.72± 0.10
ALT (IU/l)	55.0± 8.30	71.0± 23.00	62.0± 29.00	67.0± 16.00	60.0± 27.00	63.0± 14.00	63.0± 23.00	49.0± 9.30	71.0± 23.00
AST (IU/l)	280.1± 20.49	304.6± 45.40	280.1± 40.31	292.8± 23.20	292.1± 23.20	292.8± 23.20	292.1± 22.31	282.2± 20.49	304.6± 45.40
Albumin (g/dl)	2.8± 0.29	4.0± 0.11	3.0± 0.11	3.0± 0.07	3.0± 0.11	3.0± 0.07	3.0± 0.03	3.0± 0.24	4.0± 0.11

Each value is a mean of 3 replicates ± standard deviation

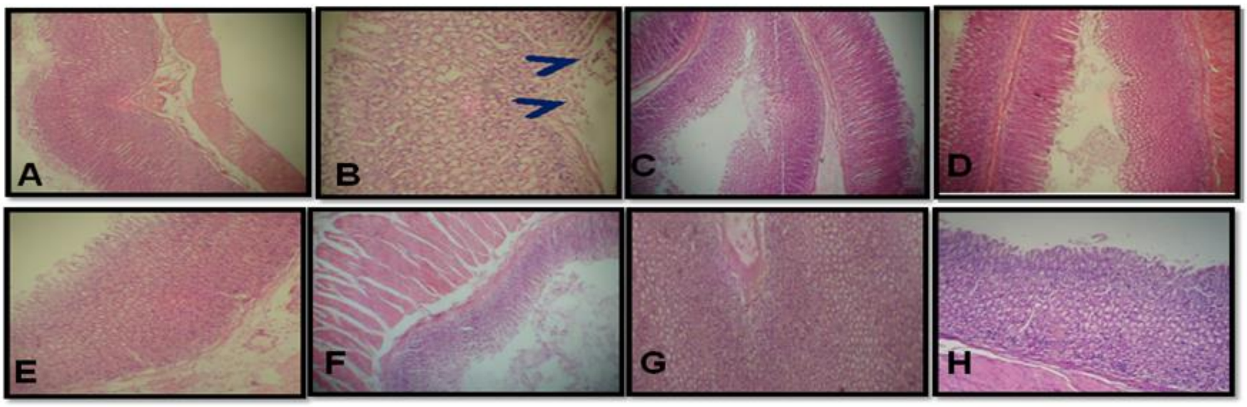

Figure 1 Histology of rat gastric tissues after infection with *E. coli* and treatment with bacteriocin.
A - H = Group1 – Group 8

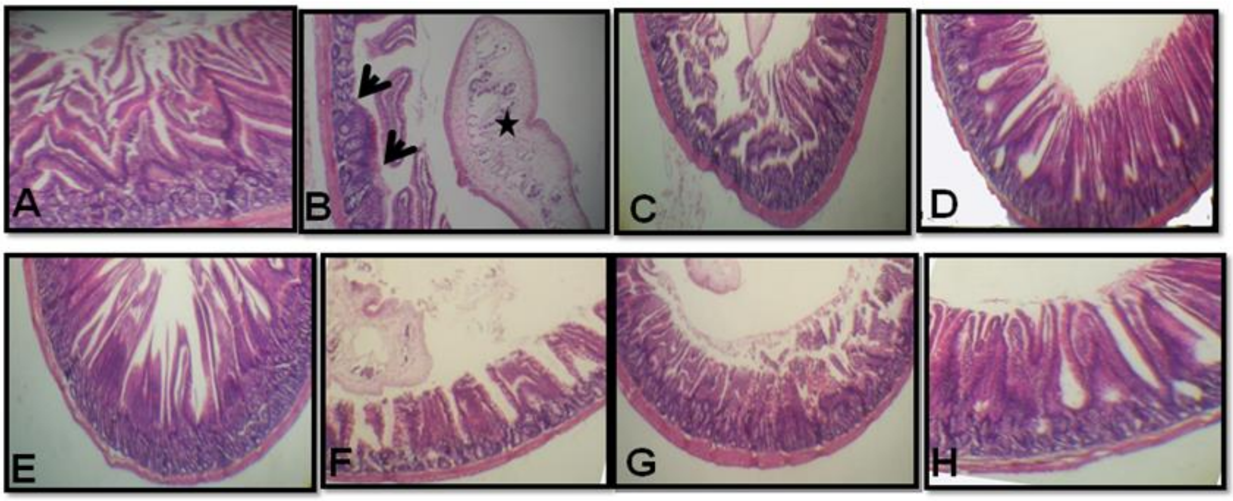

Figure 2 Histology of the small intestine of experimental rats after infection with *E. coli* and treatment with bacteriocin.
A - H = Group1 – Group 8

CONCLUSION

Conclusively, the antimicrobial characteristics of the *Lactobacillus* species has a positive impact on its use as starter culture for traditionally fermented foods with a view of improving the hygiene and safety of the food products so produced and the bacteriocin produced is responsible for an important effect in the disruption of *E. coli* plasma membrane and protection of the gastro intestinal tract as revealed in Sprague dawley rats. Hence , the improvements of liver function, protection of gastric tissues and GIT from infection are evidences supporting the probiotic nature of the strains . Therefore, the special characteristics of these *Lactobacillus* species can positively contribute to their use as probiotics. The use of these *Lactobacillus* species and their bacteriocins may be beneficial for biomedical purposes.

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