Antagonistic Activities of Lactic Acid Bacteria from Raw Cow Milk against Selected Food- Borne Pathogens

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Abstract: The aim of this study was to assess the antagonistic activities of lactic acid bacteria (LAB) isolated from raw cow milk on selected food-borne pathogens. The antagonistic activities of five different strains of LAB isolates; *Lactobacillus plantarum*, *Leuconostoc mesenteriodes, Lactococcus lactis*, *Lactobacillus acidiophilus* and *Lactobacillus bulgaricus* from raw cow milk on six selected food borne pathogens; *Staphylococcus aureus*, *Escherichia coli, Salmonella* sp., *Shigella* sp., *Enterobacter* sp. and *Serratia* sp. were examined using agar well diffusion method. The production and quantification of lactic acid, hydrogen peroxide and diacetyl by these LAB were determined using standard method of analytical chemistry and the LAB were further assayed for antibacterial effect and activity of their crude bacteriocin. The antagonistic effect against food borne pathogens ranged between 3.2 to 11.3 mm, lactic acid (1.2 to 3.0 ml), diacetyl (0.4 to 1.5 ml) and hydrogen peroxide (0.6 to 2.1 ml), and the bacterocins produced had strong inhibition zones of 2 to 6 mm. The bacterocins activity ranged between 1200 to 5800 AU/mL. The highest bacterocins activity (5800 AU/mL) was with *Lactobacillus acidophilus* at pH 4.1 while the least (1200 AU/mL) was with *Lactobacillus mesenteriodes* at pH 1.21. The results of this study showed that metabolites produced by lactic acid bacteria had strong inhibition against food borne pathogens, and could be used as biological preservative in food industry as an alternative to chemical preservatives.
Keywords: Lactic acid bacteria, raw cow milk, pathogens, antagonistic activities

INTRODUCTION

Modern technologies in food processing and microbiological food safety standard have reduced but not eliminated the likelihood of food related illnesses and products spoilage in industrialized countries. Food spoilage could damage original nutritive value, texture, flavor of the food and eventually render food harmful to people (Nath *et al.,* 2013). Food-borne diseases and infections are very vital in food industry. According to the USDA (United State Department of Agriculture), 48 million people suffer food-borne illnesses and 3000 are reported as deadly cases (FDA and administration, 2013). New meals, manufacturing processes and the growing demand for minimally processed products (ready to eat) increases the possibility of microbial contamination. Furthermore, food safety is a global issue, and an increase in import and export of food products could lead to introduction and establishment of new diseases in geographical areas that have never experienced the food-borne pathogens (Murinda *et al.,* 2004; Oliver *et al.,* 2005).

The major pathogens in food include *Escherichia coli*, *Campylobacter jejuni, Listeria monocytogenes, Salmonella* sp., *Coxiella burnetii, Mycobacterium bovis, Mycobacterium paratuberculosis, Yersinia entercocolitica* and *Staphlococcus aureus* e.t.c (Oliver *et al.,* 2005). Consumers are concerned about the synthetic chemicals used as preservative in food industries as their persistence use could pose major threat to consumers. Despites improved manufacturing facilities and implementations of effective process control procedures such as HACCP (Hazards Analysis and Critical Control Point) in the food industries, these number of food-borne illnesses are on increased trend. There is a growing need in food industry for natural preservative and/or new method of conservation (Silver *et al*., 2018) and consumers are more favourable to preservatives of natural origin than that of chemical origin. Alternative food preservation technology such as bio-preservation is a reliable option to extend the shelf life and to enhance the hygienic quality, minimizing the impact on the food organoleptic and nutritional quality using the natural microflora and (or) their antibacterial products (Nath *et al.,* 2013).

Different strains of microorganisms with potentials use as bio-preservative agents have been reported (Ghanbari *et al.,* 2013). Dairy products are one group of food commonly use to obtain strains with antagonistic features. The most important microorganisms with antagonistic characteristics and potential use in food industry are Lactic acid bacteria (LAB). They have been traditionally associated with food and considered safe (Garcia *et al.,* 2010).

LAB are gram positive bacteria, non-sporulating, anaerobic facultative, catalase and coagulase negative, tolerant to acidic conditions and with low content of guanine and cytosine in their DNA. They belong to the group *firmicutes, Lactobacillales* order and the most representative genera are *Aerococcus, Alloiococcus, Enterococcus, Leuconostoc, Pediococcus, Steptococcus, Tetragenococcus, Vagococcus* and *Weissella* and in phylum *Actinobacterium* with genera *Atopobium* and *Bifidobacterium* (Giraffa *et al.,* 2012). The lactic acid bacteria not only have their effect on food flavor but they are also known to produce and excrete compounds with antimicrobial activity, such as bacterocins, lactic acid, diacetyl and diacetyl (Saranraj *et al.,* 2013). They have a long history of safe application in dairy and other industries. Since they possess no health risks. Lactic acid, bacterocins and other bioactive compounds excreted by LAB strains are a great substitute for chemical preservatives. Among the most protective and health promoting LAB cultures; are *Lb. helveticus* (*Lactobacillus helveticus*), *Lb. plantarum* (*Lactobacillus plantarum*), *Lb. reuteri* (*Lactobacillus reuteri*) *Lactococcus lactis*, *Streptococcus thermophilus* (Ranadheera *et al.,* 2017) and *Enterococcus faecium* (Cavallini *et al.*, 2011). These metabolites can inhibit pathogenic and spoilage microorganisms extending the shelf-life and enhancing the safety of food products. There are many potential applications of protective cultures in various food systems. These organisms have been isolated from grains, dairy and meat products, fermenting vegetables and the mucosal surface of animals. Some studies had shown isolation and characterization of LAB from cow milk without much attention on the antagonistic activities of LAB isolated from raw cow milk on food borne pathogens. Therefore, the aim of this study is to determine antagonistic activities of LAB isolated from raw milk against common food-borne pathogens and as well quantify lactic acid, diacetyl and hydrogen peroxide produced by these Lactic acid bacteria.

MATERIALS AND METHODS

Collection and identification of isolates

A total of 5 Lactic acid bacteria isolates and 6 food-borne pathogens previously isolated from raw cow milk samples were collected from the department of Microbiology, University of Ibadan, Oyo state Nigeria. The lactic acid bacteria were identified base on their morphological appearance on MRS (Mann Rogosa Sharpe), Gram stain reaction and biochemical reactions while the food-borne pathogens were identified base on morphological appearances on Eosin Methylene Blue agar, *Salmonella-Shigella* agar (for isolation of *Salmonella* sp. and *Shigella* sp.), Gram stain reaction and biochemical reactions.

Antagonistic activities of lactic acid bacteria isolate on food-borne pathogens

This was determined by the method of Takahiro *et al.,* (1991). The antagonistic activity of five selected LAB isolates; *Lactobacillus plantarum*, *Leuconostoc mesenteriodes*, *Lactococcus latis*, *Lactobacillus acidophilus* and *Lactobacillus bulgaricus* against six indicator organisms which are *Staphylococcus aureus*, *Escherichia coli, Salmonella* sp, *Shigella sp. Enterobacter* sp. *and Serratia* sp. was determined. The LAB isolates were grown in MRS broth (Ph 6.5), inoculated with 1% of an overnight culture and incubated at 37ºC for 18-24hours. After incubation, cells were removed from the growth medium by centrifugation (10,000g for 15min, 4ºC) to obtain aliquots of cell free supernatants. The antimicrobial spectrum of the inhibitory substances from LAB was determined using

the well diffusion method. The indicator organisms were cultured on nutrient agar for 24hours at 37ºC then used to prepared cell suspension in 9ml distilled water. 20ml of Muller Hinton agar cooled to 45ºC was mixed with 0.5ml of the indicator bacteria suspension in petri dish and incubated aerobically for 2hours at 37ºC to properly solidify. 9mm diameter wells were made and filled with aliquots of about 50µl of the cell free supernatants. Plates were incubated at 37ºC for 24hours. A caliper was used to subsequently measure the obtained Inhibition zones by measuring the diameter of the clear zones around the wells (Sumathi and Reetha, 2012).

Quantitative determination of antimicrobial compounds produced by lactic acid bacteria

For these measurements, the test organisms were grown on MRS (Mann Rogosa Sharpe) broth for 72 hrs. After, antimicrobial compounds were quantified using these methods.

The production of lactic acid was determined by transferring 25 ml of cell free supernatant of test organism into 100ml of flask. This was titrated with 0.1ml of phenolphthalein indicator (0.5% in 5% alcohol). The titratable acidity was calculated as lactic acid % w/v (Sanni *et al.,* 1999). Each milliliter of NaOH is equivalent to 90.08mg of lactic acid. The titratable acidity was then calculated as stated in Association of Official Analytical Chemist (2013).

Titratable acidity=

$$\frac{\text{M1 NaOH} \times \text{NaOH} \times \text{M.E} \times 100}{\text{Volume of Sample}}$$

Where M1 NaOH = Volume of NaOH used
N NaOH = Normality of NaOH
M.E = Equivalent factor

For the quantitative determination of hydrogen peroxide, 20 ml of diluted H_2S0_4 acid was added to 25ml of cell free supernatant of the test organism. Titration was carried out with 0.1 M potassium permanganate ($KMnO_4$). Each ml of 0.1M of potassium permanganate is equivalent to1.79mg of hydrogen peroxide solution. Decolonization of the sample was regarded as end point. The volume of hydrogen peroxide (H_2O_2) was then calculated.

$$H_2O_2 = \frac{\text{Volume of } KMnO_4 \text{ used} \times \text{molarity of } KMnO_4 \times \text{equivalent factor} \times 100 \text{ Volume of } H_2SO_4}{\text{volume of sample}}$$

For diacetyl, 25ml of 24 hours old broth cultures was transferred into conical flasks and 7.5 ml of hydroxylamine solution was used for the residual titration. The flasks were titrated with 0.1N HCl to a greenish-yellow end point using bromophenol blue as indicator. The equivalent factor of HCl to diacetyl is 21.5mg (Merih *et al.,* 2011).

Antagonistic activities of bacteriocin produced by Lactic acid bacteria on food-borne pathogens

To determine the bacteriocin activity, agar well diffusion method of Schillinger and Lucke (1989) and Takuhiro *et al.* (1991) were used. Lactic acid bacteria were propagated in 100 ml of De Man Rogosa Sharpe (MRS) broth (pH 5.8) at 30^{o}C for 48 hours. For extraction of bacterocins, cell free solution of bacterocins was obtained by centrifugation at 10,000 rpm for 20 minutes. The culture was adjusted to pH 7.0 using 1M NaOH to exclude the antimicrobial effects of organic acid, followed by filtration of the supernatant through 0.2µm pore size cellulose acetate filter (Schillinger and Lucke, 1989) to obtain crude bacteriocin for each sample. Inhibition activity from hydrogen peroxide (H_2O_2) was eliminated by the addition of 5mg/ml catalase (Daba *et al.,* 1991). Aliquots of 50 µl from each cell free supernatant (crude bacteriocin) was placed in agar wells in Petri dishes seeded with the bio assay strain (indicator microorganism): *Staphyloccus* sp., *Escherichia coli, Salmonella* sp., *Shigella* sp., *Enterobacter* sp., and *Serratia* sp.) and incubated overnight at 37^{o}C on Muller Hinton agar. A caliper was used to subsequently measure the obtained inhibition zone.

The LAB strains were classified as bacteriocin producers when the wells formed an inhibitory zone and the clear area around the test wells was used to indicate inhibitory activity. Therefore, the diameters (mm) of these zones were measured and recorded.

Bacteriocin activity of Lactic Acid Bacteria, growth rate and pH

Lactic acid bacteria were propagated in 100 ml of De Man Rogosa Sharpe (MRS) broth (pH 5.8) at 30 ^{o}C for 48 hours. For extraction of bacterocins, cell free solution of bacterocins was obtained by centrifugation at 10,000 rpm for 20 minutes. The culture was adjusted to pH 7.0 using 1M NaOH to exclude the antimicrobial effects of organic acid, followed by filtration of the supernatant through 0.2µm pore size cellulose acetate filter (Schillinger and Lucke, 1989) to obtain crude bacteriocin for each sample. Inhibition activity from hydrogen peroxide (H_2O_2) was eliminated by the addition of 5mg/ml catalase (Daba *et al.,* 1991). The cell free broth culture was tested (screened for pH growth of producer organisms and bacteriocin activity against the indicator (test) microorganisms (Graciela *et al.,* 1995). The growth of producer organism was determined when the broth culture of the bacteriocin produced were subjected to colometric analysis and the pH of the medium was determined ((Brinkten *et al.,* 1994). The bacteriocin activity was determined by the cell free supernatant serially diluted two folds in deionized water. The bacteriocin activity was defined as the reciprocal of the highest dilution showing inhibition of microorganisms multiplied by 100 and it is expressed as activity unit per milliliter (Au/ml) (Graciela *et al.,* 1995).

Statistical analysis

This was carried out using Duncan multiple range tests to separate the means and compare the antagonistic activities measured.

RESULTS

A total of 5 lactic acid bacteria isolates were selected and checked for antibacterial activity against 6 different food-borne pathogens. The inhibition zone ranged from 3.20±0.24mm – 11.3±0.03mm (Millimeters). The largest inhibition zone (11.3±0.03mm) was with *Lactobacillus acidophilus* against *Staphylococcus aureus* while the smallest inhibition (3.20±0.24mm) was with *Lactobacillus mesenteriodes* against *Enterobacter* sp. The LAB with the largest antagonistic activity against *Salmonella* sp., *Shigella* sp. and *Enterobacter* sp was *L. acidophilus*. However, only *L. plantarum* was antagonistic against *Serratia* sp. while the other Lactic acid bacteria yielded no zone of inhibition.

Comparatively, the antagonistic activities of the five studied LABS were significantly different ($p<0.05$) from one another. Notably, the antagonistic activities of *L. plantarum, L. lactis and L. acidophilus* were similar given the similar ranking of the means of the zone of inhibition (Table 1). Only *L. acidophilus* had significantly different ($p<0.05$) antagonistic activity against *Enterobacter* sp. *Staphylococcus aureus* is the most susceptible pathogens to all the LAB isolates.

Table 1: Antagonistic activities of Lactic acid bacteria against food-borne pathogens (mm).

LAB	*Staph aureus*	*E. coli*	*Salmonella* sp.	*Shigella* sp.	*Enterobacter* sp.	*Serratia* sp.
LP	10.30±0.20^{a}	8.60±2.00^{b}	8.70±0.21^{b}	8.60±2.03^{ab}	5.70±0.58^{b}	5.00±0.40^{a}
LM	4.60±0.90^{c}	4.20±1.02^{c}	4.10±0.06^{c}	3.40±0.46^{d}	3.20±0.24^{d}	0.00±0.00^{b}
LL	10.80±1.00^{a}	7.80±0.20^{ab}	7.30±0.40^{bc}	7.10±0.21^{b}	4.60±0.33^{cd}	0.00±0.00^{b}
LA	11.30±0.03^{a}	10.70±0.50^{a}	10.20±0.50^{a}	9.30±0.42^{a}	9.40±0.42^{a}	0.00±0.00^{b}
LB	8.50±0.00^{b}	7.30±1.00^{ab}	7.10±0.38^{bc}	5.80±0.58^{c}	4.40±0.12^{cd}	0.00±0.00^{b}

The results in the table above were presented in means± standard deviation. The means with different superscripts were significantly different from one another down the column while those with the same superscripts were similar. LAB = Lactic Acid Bacteria, LP = *L. planetarium,* LM = *L. mesenteriodes* LA = *L. acidiophilus,* LB = *L. bulgaricus,* LL = *L. latis*

Quantitative determination of antimicrobial compounds produced by lactic acid bacteria

In the quantification of lactic acid, diacetyl and hydrogen peroxide produced by Lactic acid bacteria at incubation period of 24 to 72 hours (Figure 1), Lactic acid production ranged from 1.2 to 3.0 ml. In all the LAB isolate, the metabolites production was highest after 48hours. The highest producer of Lactic acid was *Lactobacillus. plantarum* (3.0ml) while the least producer was *Lactobacillus acidophilus* (1.2ml). The production of diacetyle ranged from 0.4-1.5ml while for hydrogen perioxide it ranged from 0.6 to 2.1ml. The highest producer of hydrogen peroxide was *Lactobacillus plantarum* (2.1ml) while the least was *Lactobacillus bulgaricus* (0.6ml).

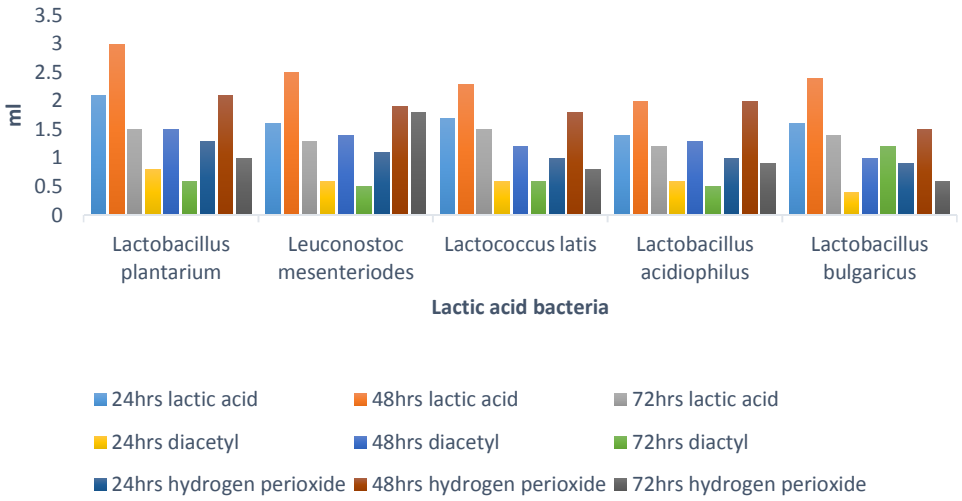

Figure 1: Quantities of lactic acid, diacetyl and hydrogen peroxide produced by LAB isolated from raw cow milk (ml).

Antagonistic activities of bacteriocin produced by Lactic acid bacteria on food-borne pathogen

There was significant difference ($p<0.05$) in the antagonistic activities of the compared bacteriocin producing LABS against the six test pathogens in this study (Table 2). *L. plantarum, L. latis and L acidiophilus* had the strongest antagonistic activities while the same trend was observed for the *Escherichia coli*, *Salmonella* sp. *Shigella* sp. and *Serratia* sp. *Enterobacter* sp. *L. mesenteriodes* had the least antagonistic activities against the test- food pathogens as observed from the outcome of this experiment.

Table 2: Antagonistic activities of bacterocins producing Lactic Acid Bacteria against food borne pathogens (mm)

LAB	*Staph.*sp	*E.coli*	*Salmonella* sp.	*Shigella* sp.	*Enterobacter* sp.	*Serratia* sp.
LP	6.00±0.02^{a}	6.00±1.00^{a}	6.00±0.30^{a}	6.00±0.44^{a}	5.00±0.61^{b}	4.00±0.95^{a}
LM	3.00±0.10^{b}	3.00±0.57^{b}	3.00±0.20^{b}	3.00±0.08^{b}	3.00±0.28^{c}	2.00±0.70^{b}
LL	6.00±0.70^{a}	6.00±0.33^{a}	6.00±0.10^{a}	6.00±0.19^{a}	5.00±0.50^{b}	4.00±0.17^{a}
LA	6.00±1.10^{a}	6.00±0.21^{a}	6.00±0.60^{a}	6.00±0.36^{a}	6.00±0.18^{a}	4.00±0.24^{a}
LB	5.00±0.40^{ab}	5.00±2.00^{ab}	5.00±0.23^{ab}	5.00±013^{ab}	4.00±0.22^{bc}	4.00±0.81^{a}

The results in the table above were presented in means± standard deviation. The superscripts indicate the ranking of the post hoc test using Duncan Multiple Range Test. The means with different superscripts were significantly different from one another down the column while those with the same superscripts were similar. LAB = Lactic Acid Bacteria, LP = *L. planetarium,* LM = *L. mesenteriodes*
LA = *L. acidiophilus,* LB = *L. bulgaricus,* LL = *L. latis*

Bacteriocin activity of lactic acid bacteria, growth rate and pH

Growth, pH and bacteriocin producing activity of lactic acid bacteria isolated from raw cow milk (Table 3). The five LAB studied had significantly different ($p<0.05$) bacteriocin activity at the compared growth and pH. condition in the experiment. The best condition that supports the highest Bacteriocin activity (5800.00±19.00 AU/mL) with *L. acidiophilus* at pH 4.10±0.12 while *L mesenteriodes* yielded the minimum Bacterocin activity of 1200.00±8.77AU/mL when the growth was 0.25±0.01 cfu/ml and the pH. of the medium was 1.21.

Table 3: Bacteriocin activity of lactic acid bacteria growth rate and pH

Lab isolates	Growth rate	pH of medium	Bacteriocin activity (Au/ml)
L.plantarium	0.81 ± 0.20^{ab}	4.50 ± 0.50^{a}	5600.00 ± 21.81^{ab}
L.mesenteriodies	0.25 ± 0.01^{c}	1.21 ± 0.03^{b}	1200.00 ± 8.77^{c}
L.latis	0.91 ± 0.01^{a}	3.90 ± 0.11^{a}	5600.00 ± 15.30^{ab}
L.acidiophilus	0.97 ± 0.02^{a}	4.10 ± 0.20^{b}	5800.00 ± 19.00^{a}
L.bulgaricus	0.71 ± 0.01^{b}	4.50 ± 0.12^{b}	5400.00 ± 29.05^{b}

The results in the table above were presented in means± standard deviation. The superscripts indicate the ranking of the post hoc test using Duncan Multiple Range Test. The means with different superscripts were significantly different from one another down the column while those with the same superscripts were similar.

DISCUSSION

In this study, the antagonistic activities of LAB isolated from raw cow milk on food-borne pathogens (mm) shows the bacterial pathogens; *Staphylococcus* sp., *E. coli*, *Salmonella* sp., *Shigella sp.*, *Enterobacter* sp. and *Serratia* sp. were inhibited by the LAB isolates at varying degrees. *Staph aureus* is the most susceptible pathogens to all the LAB isolates compare to *E. coil* and the other gram-negative pathogens. The result indicated that *Lactobacillus plantarum* is the only LAB isolate whose metabolites were able to have inhibitory effect on *Serratia* sp. This is in agreement with the findings of Sumathi and Reetha *et al.,* (2012) who reported the inhibition of *Staph* sp., *E. coli*, *Enterobacter* sp., *Shigella* sp. and *Salmonella* sp. by LAB with similar range of inhibition (0.5 to 13mm). Savadogo *et al.,* (2004) also reported that gram positive bacteria are much more susceptible to metabolite of lactic acid bacteria than gram negative bacteria indicator. The susceptibility of gram-positive bacteria is attributed to the particular nature of their cellular envelop. Despite their thick peptidoglycan layer, gram positive bacteria are more receptive to certain cell wall targeting antibiotics than gram negative bacteria due to the absence of the outer membrane containing lipopolysaccharides. This result indicates that all of the LAB isolate from the raw milk are capable of synthesizing inhibitive substance of pathogens. These inhibitive substances produced by these LAB act differently on the pathogens isolated from the raw cow milk. The inhibitory activity was maximal at the beginning of the stationary phase and remained stable long after growth had ceased, even in the presence of the producer cells. However, inhibition of pathogens by LAB is dependent on optimal growth of LAB and this in turn is also attributed to strain dependent, temperature, salinity and pH of the medium used. This has been previously published in Carbohydrate fermentation profile and physiological studies of Lactic acid bacteria from native raw cowmilk (Adediran and Aforijiku, 2020).

In the quantification of lactic acid, diacetyl and hydrogen peroxide produced by LAB isolated from raw cow milk (figure 1), all the LAB isolate production was highest at 48hours. Lactic acid production ranged from 1.2 to 3.0ml. The highest producer of Lactic acid was *Lactobacillus. plantarum* (3.0ml) while the least producer was *Lactobacillus*

acidophilus (1.2ml). The amount of hydrogen peroxide produced ranged from 0.6 to 2.1ml and the highest producer of diacetyl was *Lactobacillus plantarum* (1.5ml) while the least produced was *Lactobacillus acidophilus* (0.4ml). In all the metabolites produced, lactic acid has the highest production. This is because lactic acid bacteria produced lactic acid as the main product of fermentation. This work is in agreement with the work done by Adeniyi *et al.* (2009) who reported that all the LAB isolate from salad vegetables has peak production of lactic acid, diacetyle and hydrogen peroxide at 48hours. Adeniyi *et al.* (2009) also reported that *Lactobacillus. plantarum* produced the highest amount of lactic acid (1.80ml). Hydrogen peroxide is one of the primary metabolites that may be produced by lactic acid bacteria and which contribute to their antagonistic action. Hydrogen peroxide produced was considered high enough for antimicrobial activity. This is in consistency with the work done by Karshima *et al.* 2013 who reported that most Lactic acid bacteria isolated from raw milk are able to produce hydrogen peroxide which ranges from 1.0-3.5ml.

Zone of Inhibition of Indication (Mm) Caused by Bacteriocin Produced by Lactic Acid Bacteria Isolated from Raw Cow milk shows that *L. acidiophilus, L. latis* and *L. plantarium* have maximum antagonistic activity of 6mm. The bacteriocin produced by *L. mesenteriodes* has the least antagonistic/inhibitory activities and *Serratia* sp. was the least effected by all the lactic acid producing-bacteriocin. The resistance of *Serretia sp.*, a gram-negative bacterium is attributed to the particular nature of their cellular envelope (Savadogo *et al.,* 2004). This peculiarity is due to the presence of specific lipopolysaccharides on the membrane surface of cell wall which serve as a protective barrier. This result indicates that all of the LAB isolate from the raw milk are capable of Synthesizing inhibitory substances. These inhibitory substances produced by these LAB act differently on the pathogens isolated from the raw cow milk. The result also shows that *Lactobacillus plantarum* is the only isolate out of the five LAB isolates whose metabolites is able to have inhibitory effect on *Serratia sp*. This could be due to the large amount of lactic acid (3.0ml) produced by the LAB and the relative efficacy of lactic acid bacteria against gram negative bacteria. The small water-soluble molecule lactic acid is able to cause sublethal injury by disrupting the Lipopolysaccharides layer of the gram-negative bacteria (Savadogo *et al.,* 2004). All LAB strains were able to effectively inhibit *Staph* sp. follow by *Salmonella* sp., *Enterobacter* sp. and *Shigella* sp. The least are *Serratia* sp. and *Enterobacter* sp. In this study, after 48 hours of incubation, *L. acidiophilus*, *L. lactis* and *L. plantarium* had maximum antagonistic activity of 6mm. The bacteriocin produced by *L. mesenteriodes* had the least antagonistic/inhibitory effect on all the six test pathogens. Bacterocins have been reported to be inhibitory against a number of other bacteria. Ogunbanwo *et al.* (2003) shows that bacterocin from *L.plantarium* has inhibitory activities of 8mm on *staph. aureus*, 6mm and 7mm on *Shigella* sp.,7mm and 8mm on *Salmonella* sp. Raja *et al.* (2009) reported the production of bacteriocin of *Lactobacillus lactis cremoris* from kefir and controlled the food spoilage bacteria.

The activity of bacteriocin by the LAB isolates was pH and growth dependent. *Lactobacillus acidophilus*, *Lactococcus lactic* and *Lactobacillus plantarum* produced high bacteriocin activity (5800.00±19.00, 5600.00±15.30 and 5600.00±21.81 Au/ml) with high growth rate (0.97±0.02, 0.91±0.01 and 0.81±0.20 respectively) which made them potent bacteriocin producers while the least activity (1200 Au/ml) was with low growth rate of 0.25±0.01. This is in agreement with the findings of Ogunbanwo *et al.* (2003) who reported that *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1 isolated from Ogi exhibited bacteriocin activity between 3200 and 6400 Au/ml against *Escherichia coli*. *L. bulgaricus* exhibited bacteriocin activity of 5400 and *L. mesenteriodes* possess the least which is

1200. It was generally observed that *bacterocins* from the producer organisms had no inhibitory effects on the organisms producing it. The implication is that both the bacteriocin and the bacteriocin producing LAB could be used for bio-preservation of foods without adverse effects. it was also observed that different LAB has varying bacteriocin producing potentials as such those species of LAB identified as excellent potential bacteriocin producers are recommended to food processing industries to be employed in bio-preservation of foods to enhance extension of shelf life of food products and to reduce the risk of the use of chemical preservatives and additives, as they could pose health risk generally.

CONCLUSION

The Lactic acid bacteria; *Lactobacillus plantarum*, *Leuconostoc mesenteriodes, Lactococcus lactis*, *Lactobacillus acidiophilus* and *Lactobacillus bulgaricus* had antagonistic activities against the selected food-borne pathogens; *Staphylococcus aureus*, *Escherichia coli, Salmonella* sp., *Shigella* sp., *Enterobacter* sp. and *Serratia* sp. in varying degrees. These inhibitory activities of the LAB were further supported by the presence of Lactic acid, diacetyle and hydrogen peroxide with varying quantification. Further research on the isolation and characterization of the antimicrobial agents; Lactic acid, diacetyl, hydrogen peroxide and bacteriocins that are responsible for the antagonistic activity is of great importance. There is also need for assessing the antimicrobial potential of these metabolites using in vivo models for these biological metabolites to be recommended as bio-preservatives in food industries to enhance shelf life of food products, and as an alternative to synthetic antimicrobials, chemicals and commercial food preservatives.

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