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Bacteriocin Activity of Lactic Acid Bacteria Isolated from Nunu, a Spontaneously Fermented Milk

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Abstract
According to literature, lactic acid bacteria have been used for ages as a natural preservative for dairy products as well as protective cultures.This is mainly due to their production of antimicrobial substances like organic acids and bacteriocins and their ability to compete against pathogenic and spoilage bacteria for nutrients. Nunu is a fermented dairy product enjoyed by the people of West Africa. This work was carried out for the purpose of identifying the antagonistic effects of bacteriocins of four lactic acid bacteria namely *Lb. fermentum, Lb, plantarum, Lb. mesenteroides* and *W. confusa* against some pathogenic/spoilage organisms which include *Strep. faecalis, Sal. typhimirum, E. coli* ATTC 24522*, B. cereus* and *P. aeruginosa*. Each of the LAB has different strains isolated at different times during the fermentation process.The activity shown by the bacteriocin against Gram negative bacteria is an unusual phenomenon and defies the observations of Klaenhammer, (1988) that bacteriocins are proteins which show inhibitory activity against closely related organisms. However, in this study, the bacteriocins of LAB strains were effective against Gram negative bacteria including *E. coli* ATCC 24522*, P. aeruginosa* and *Sal. typhimirum.* This was even to the extent that the highest antagonistic effect, 15.5 mm, was exhibited by *P. aeruginosa* against *Lb. mesenteroides* 6-7, though some the bacteriocins showed some antagonistic effects against the Gram positive bacteria with the highest being 14.5 mm shown by *Lb. mesenteroides* 0-23 against *Strep. faecalis*. *B. cereus* being another Gram positive bacterium was inhibited by only the bacteriocins of *W. confusa* 24-17.The present findings was supported by the observations made earlier by Mandal et al., (2008). According to the present study, starter cultures for nunu production can be made from *Lb. mesenteroides* 6-7 and *W. confusa* 24-17 and the fermentation allowed to take place within 48 hours.

1.0. Background

Fermentation of various foods by lactic acid bacteria (LAB) is one of the oldest forms of biopreservation practiced by mankind (Soomro *et al*., 2002). Fermentations provide a way to preserve food products, to enhance nutritive value, to destroy undesirable factors, to make a safe product, to improve the appearance and test of some foods, and to reduce the energy required for cooking (Lopez, 1992). These significant changes causing desirable biochemical effects result in the development of new aroma, flavor, taste and texture thereby increasing the sensory quality, palatability and acceptability of the product. Beside these, fermentation is relatively a low energy preservation that promotes the shelf life of products (Cooke *et al.*, 1987). Food fermentations have a great economic value and it has been accepted that these products contribute in improving human health (Savadogo *et al*., 2006).

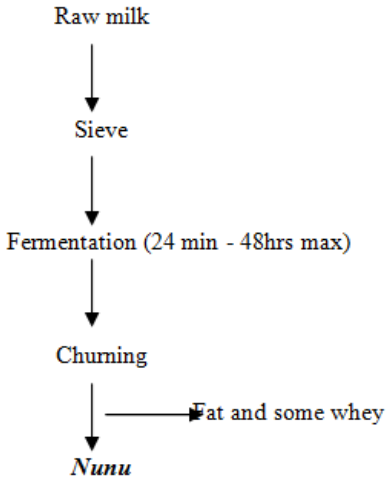
Indigenous fermented foods and beverages play a major role in the diet of Africans. Milk is the most abundant fermented animal product in Africa, although the extent to which milk is used in the dairy diet varies to a great extent (Jespensen, 2003). Africans in general enjoy soured milk products. Fermented milk products are of great significance for their therapeutic value for alleviating lactose intolerance, social value and as a means of generating income (Beukes *et al*., 2001). The art of making these products is handed down from one generation to the next (Caplice and Fitzgerald, 1999).

Nunu is a spontaneously fermented yoghurt-like milk product consumed as a staple food commodity in parts of the Saharan West Africa (Akabanda *et al*., 2010). Nunu is yoghurt-like in taste (a sharp acid taste) and can be consumed alone or with sugar and *fura*. The latter is made of millet dough mixed with spices, compressed into balls and cooked for about 30 minutes (Owusu-Kwarteng *et al*., 2010). The cooked *fura* is crumbled in a bowl of *nunu* into what is called *fura de nunu.*

Raw milk has low keeping quality and at room temperature, spontaneous microbial spoilage occurs turning the product sour. This is brought about by the activity of lactic acid bacteria (Wouters *et al*., 2002). Nunu is processed by collecting fresh cow milk and allowing it to ferment for a day or two. The Fulanis in Ghana ferment the milk in calabashes, or rubber buckets. High temperatures promote the acidification and temperatures around 35 ^{o}C in the season from March to June results in a finished product after around 24 hours. On the other hand, temperatures around 15-17 ^{o}C from October to February can prolong the fermentation up to 48 hours. Figure 1 illustrates the production process of Nunu. Nunu production and consumption are much more practiced in the northern parts of Ghana than in the south (Akabanda *et al*., 2010). It, however, does not appeal to majority

of the people because of the apparent unhygienic conditions under which it is prepared, and also its short shelf-life (Yahuza, 2001).

Figure 1.1: A Flow diagram of *nunu* processing (Akabanda *et al*., 2010)



A potential growth area for fermented milk includes added value products such as low calorie, reduced-fat varieties and those fortified with physiologically active ingredients including fibers, phytosterols, omega-3-fatty acids, whey based ingredients, antioxidant vitamins, isoflavones that provide specific health benefits beyond basic nutrition (Khurana and Kanawjia, 2007). Milk's nutritional composition makes it not only suitable for human nutrition but also ideal for microbial life. The growth of microorganisms in food could make the food grossly unwholesome and harmful to consumers. Outbreaks of milk-borne diseases have occurred despite pasteurization, as a result of either improper pasteurization or product recontamination (Hartman, 1997).

It is noticed that raw milk often contains microorganisms which may likely cause food-borne diseases (Adeyisun *et al*., 1995; Headrick *et al*., 1998). Even when the milk is fermented, the fermentation process with attendant drop in pH may not rid the product of these organisms and may be carried to consumers (Ogbonna, 2011).

Many lactic acid bacteria have important roles in the production of fermented foods, and some of these bacteria have been shown to be capable of inhibiting the growth of a wide variety of food spoilage organisms. Protection of food from spoilage and pathogenic microorganisms by LAB is through the production of organic acids, hydrogen peroxide, diacetyl (Messens and De Vugst, 2002), antifungial compounds such as fatty acids (Corsetti *et al*., 1998) or phenullactic acid (Lavermicocca *et al*., 2000) and/or bacteriocins (De Vugst and Vandamme, 1994). The common occurrence of LAB in foods and feeds coupled with their long-lived use contributes to their natural acceptance as GRAS (Generally Recognized As Safe) for human consumption (Aguirre and Collins, 1993).

Bacteriocins are proteinaceous antibacterial compounds, which constitute a heterologous subgroup of ribosomally synthesized antimicrobial peptides (De Vugst and Vandamme, 1994). Although bacteriocins may be found in many Gram-positive and Gram-negative bacteria (Riley and Wertz, 2002), those produced by LAB have received particular attention in recent years due to their potential application in the food industry as natural preservatives (Ennahar *et al*., 1999). Bacteriocins produced by LAB are small, ribosomally synthesized, antimicrobial peptides or proteins that possess activity towards closely related Gram-positive bacteria, whereas producer cells are immune to their own bacteriocin(s) (De Vugst and Vandamme, 1994). Even though antimicrobial peptides occupy an inhibition spectrum narrower than that of antibiotics (McAuliffe *et al*., 2001), bacteriocins produced by LAB have been reported to permeate the outer membrane of Gram-negative bacteria and to induce the inactivation of Gram-negative bacteria in conjunction with other enhancing antimicrobial environmental factors, such as low temperature, organic acid and detergents (Alakomi *et al*., 2000; Elliason and Tatini, 1999).

Depending on the producer organism and classification criteria, bacteriocins can be classified into several groups (Ennahar *et al*., 2000; McAuliffe *et al*., 2001) in which classes I and II are the most thoroughly studied. Class I, termed lantibiotics, constitutes a group of small peptides that are characterized by their content of several unusual amino acids (Gruder *et al*., 2000). The class II bacteriocins are small, non-modified, heat stable peptides (Nes and Holo, 2000). The important ones are nisin, diplococcin, acidophilin, bulgarican, helveticins, lactacins and plantaricins (Nettles and Barefoot, 1993). The lantibiotic nisin which is produced by different *Lactococcus lactis* spp. is the most thoroughly studied bacteriocin to date and the only bacteriocin that is applied as an additive in food worldwide (Delves-Broughton *et al*., 1996).

1.1. Problem Statement/Justification

Milk is highly valued among natural foods since it provides essential nutrients in higher amounts than other staple foods (Oyawoye *et al*., 1997). Milk is utilized in the production of at least 400 different fermented products all over the world (Willey *et al*., 2008). *Nunu* is produced by spontaneous fermentation of milk, and numerous attempts have been made to study microorganisms that are associated with the process (Akinyanju, 1989; Akabanda et al., 2010).

Nowadays consumers are concerned about the synthetic chemicals used as preservatives in food, leading to a trend towards less processed food (Soomro *et al*., 2002). These untreated foods harbour pathogens which can multiply under refrigeration and without oxygen. A potential solution to this problem is in the use of protective cultures and/or their metabolites in food preservation. Protective cultures are selected based on their ability to grow in a product and inhibit food poisoning or spoilage organisms (Modzelewska-Kapitola *et al*., 2005).

Bacteriocins produced by LAB may be considered natural preservatives or biopreservatives that can possibly fulfill these requirements. Biopreservation refers to the use of antagonistic microorganisms or their metabolic products to inhibit or destroy undesired microorganisms in foods to enhance food safety and extend shelf life. The present study investigates the use of bacteriocins from lactic acid bacteria as protective cultures in nunu.

1.2. Objectives

1. To determine the antibacterial activities of some LAB bacteriocins against some selected strains of pathogenic microorganisms.
2. To determine a novel LAB strain(s) that can be used as a starter culture for nunu fermentation.

2.0. METHODOLOGY

2.1. LAB used for the experimental work

A total of 27 LAB were used for the experiment out of the total 189 strains of LAB isolated from Nunu of which ten (10) were *Lb. fermentum*, seven (7) *Lb. plantarum*, seven (7) *Lb. mesenteroides* and three(3) *Weisella confusa*. They were coded as follows:

Lb. fermentum

Ferm 1	Ferm 2
4-16	0-16
10-1; 10-7	0-17;18-3
10-3; 20-18	0-19; 18-4

Lb. plantarum

Ferm 1	Ferm 2
2-5	2-1
6-8	6-20
6-24	6-30; 8-26

Lb. mesenteroides

Ferm1	Ferm 2
0-12	6-7
0-21	6-14
0-23	
0-25	
0-35	

Weisella confusa

Ferm 2
6-8
12-19
22-7
24-17

The first number of the codes refers to the hour the strains were isolated and the second number also refers to the isolate's number at that hour. The strains were kept at -80 °C in 20% w/w glycerol and prior to use, the strains were streaked on to MRS agar plates (de Man Rogosa and Sharpe agar, Merck KGaA, 64271 Darmstadt, Germany) and incubated aerobically at 35°C±2 for 24 hours after which they were sub-cultured into MRS broth (de Man Rogosa and Sharpe broth, Merck KGaA, 64271 Darmstadt, Germany) and incubated at 35°C±2 for 24 hours. They were later kept at 4^0C until use.

2.2. Test strains

Five different strains of pathogenic organisms were used. These are *Salmonella typhimirum, Streptococcus faecalis, Bacillus cereus, Pseudomonas aeruginosa and Escherichia coli* ATCC 25422. E. coli ATCC 25422 was obtained from Centre for Scientific Research into Plant Medicine, Mampong-Akuapem, in the Eastern Region of Ghana. The remaining four were obtained from the University of Copenhagen and were lent out by

Akabanda Fortune (PhD). These strains were streaked on Nutrient Agar (Oxoid Ltd, Basingstoke, Hampshire, England) and aerobically incubated (J.P. Selecta, SA. Ctra, N11 Km: 585. 1 Abrera Barcelona, Spain) at 35^0C±2 for 24 hours. Afterwards they were kept at 4^0C.

2.3. Preparation of Media

The media prepared which included Nutrient agar (Oxoid Ltd, Basingstoke, Hampshire, England), Peptone water (Merck KGaA, 64271 Darmstadt, Germany), MRS agar (de Man Rogosa and Sharpe agar, Merck KGaA, 64271 Darmstadt, Germany), MRS broth (de Man Rogosa and Sharpe broth, Merck KGaA, 64271 Darmstadt, Germany)

and Mueller-Hinton agar (Oxoid Ltd, Basingstoke, Hampshire, England) were prepared under aseptic conditions per the manufacturers' instructions and autoclaved at 121^{0}C for 15 minutes and dispensed aseptically into Petri dishes and test tubes. They were then incubated (J.P. Selecta, SA. Ctra, N11 Km: 585. 1 Abrera Barcelona, Spain) overnight for sterility and kept at 4^{0}C until use.

2.4. Agar Well Diffusion Assay

2.4.1. Preparation of Cell Free Supernatant (CFS) of the LAB grown in the MRS broth

Cell free filtrates were prepared according to Kalalou et al., 2004. The LAB cultured on the MRS agar (de Man Rogosa and Sharpe agar, Merck KGaA, 64271 Darmstadt, Germany) were inoculated each into 10 ml of MRS broth and incubated (J.P. Selecta, SA. Ctra, N11 Km: 585. 1 Abrera Barcelona, Spain) for 24±2 hours at 37^{0}C. After the incubation, the pH of each of the LAB was checked and made neutral by adding 1M NaOH (Harris reagent, Philip Harris plc, Shenstone, England) when the inocula are acidic and 1M HCL (Harris reagent, Philip Harris plc, Shenstone, England) when basic. This was done to keep the pH of the inocula neutral by using the pH meter (Crison instruments, SA. Reira Principal, 34-36. 08328 Alella, Spain).

10 ml of each of the inocula was transferred into the centrifuge tubes and centrifuged (J.P. Selecta, SA. Ctra, N11 Km: 585. 1 Abrera Barcelona, Spain) at 6000 rpm for 15 minutes. The solvent were then filtered using the syringe filter (Sartorius AG, 37070 Goettingen, Germany) with a pore size of 0.02 µm and a disposable syringe (Opso Saline Ltd with technical assistance of Segae Precision Co. Ltd, Korea).

2.4.2. Preparation of test strains

The nutrient agar plates (Oxoid Ltd, Basingstoke, Hampshire, England) were checked for purity by colony appearance and one colony on each plate was inoculated into 5 ml peptone water (Merck KGaA, 64271 Darmstadt, Germany)16-hour incubation at 37^{0}C in an incubator (J.P. Selecta, SA Ctra, N11 Km: 585. 1 Abrera Barcelona, Spain). They were later sub-cultured into another 5 ml of peptone water (Merck KGaA, 64271 Darmstadt, Germany) and incubated (J.P. Selecta, SA Ctra, N11 Km: 585. 1 Abrera Barcelona, Spain) for additional 2 hours at 37^{0}C to achieve a turbidity compared to 0.5 McFarland's standards.

2.4.3. Bacteriocin Activity

The plates were made by dispensing 20 ml of Mueller-Hinton agar (Oxoid Ltd, Basingstoke, Hampshire, England) into sterile Petri dishes and allowed to solidify. The plates were incubated (J.P. Selecta, SA Ctra, N11 Km: 585. 1 Abrera Barcelona, Spain) at 37^{0}C overnight for sterility.

Mueller-Hinton agar plates (Oxoid Ltd, Basingstoke, Hampshire, England) were flooded with the test strains in suspension from the peptone water (Merck KGaA, 64271 Darmstadt, Germany) and allowed to dry at room temperature on a level surface. A sterilized cork borer of an external diameter of 5 mm was used to create wells on the Mueller-Hinton agar plates (Oxoid Ltd, Basingstoke, Hampshire, England). The bacteriocins extracted from the various LAB were carefully dispensed into the wells created and filled to the brim (about 0.2 ml). A duplicate of each plate was made. The plates were kept in a refrigerator for 2 hours for complete diffusion of the bacteriocins. The plates were then incubated (J.P. Selecta, SA Ctra, N11 Km: 585. 1 Abrera Barcelona, Spain) at 37^{0}C for 24 hours after which the inhibition zones were measured in millimeter (mm) using a rule.

2.5. Statistical Analysis of Results

The experiments were done in duplicates and the mean of the results calculated. The mean results were then analyzed using Microsoft Office Excel 2007 version.

3.0 Results

The antimicrobial activities of the bacteriocins isolated from the various LAB strains were tested against some pathogenic bacteria using the agar well diffusion assay. Out of the 27 strains of LAB used, 17 of them had antagonistic effect against at least one pathogen representing 62.96% whereas the remaining 10 representing 37.04% had no antagonistic effect against any of the pathogens.

The largest zone of inhibition was shown by *Lb. mesenteroides* 6-7 against *Pseudomonas aeruginosa* with a mean inhibition of 15.5 mm followed by *Lb. mesenteroides* 0-23 against *Streptococcus faecalis* with a mean inhibition of 14.5 mm. Most of the bacteriocins isolated showed no antagonism against the pathogens whilst others were able to inhibit at least one of the pathogens. *Lb. mesenteroides* 6-7 showed the highest zone on all the pathogens except *Bacillus cereus* (fig.4.3).

The bacteriocin of *Weisella confusa* 24-17 was exceptional as it was the only isolate that was able to exhibit antagonistic activity against *B. cereus* with a mean inhibition zone of 11.0 mm (fig.4.4).

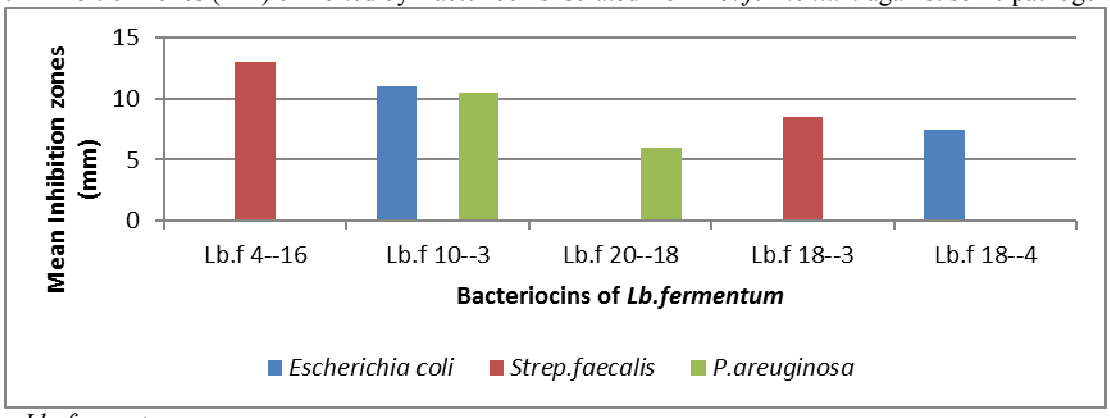
It must also be noted that ten (10) strains of *Lb. fermentum* were used and out of these five strains representing 50% were able to exhibit antagonistic effect against at least one pathogenic organism used. Only one strain, *Lb. fermentum* 10-3, was able to inhibit two (2) pathogens namely *E. coli* and *P. aeruginosa* with mean inhibition zones of 11 mm and 10.5 mm respectively. Among those that exhibited antagonistic effect, a highest mean inhibition zone of 13 mm was recorded and this was shown by *Lb. fermentum* 4-16 against *Strep.*

faecalis whereas the least mean inhibition zone of 6 mm was recorded and exhibited by *Lb. fermentum* 20-18 against *P. aeruginosa*. This can be seen in the table and figure below.

Tab. 4.1 Results of bacteriocin activities of *Lb. fermentum* against some pathogens

LAB	*Escherichia coli*			*Streptococcus faecalis*			*Pseudomonas aeruginosa*			*Salmonella typhimirum*			*Bacillus cereus*		
Ferm 1	1	2	Mean	1	2	Mean	1	2	Mean	1	2	Mean	1	2	Mean
4-16	0.0	0.0	0.0	14.0	12.0	13.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10-1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10-3	11.0	11.0	11.0	0.0	0.0	0.0	11.0	10.0	10.5	0.0	0.0	0.0	0.0	0.0	0.0
10-7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20-18	0.0	0.0	0.0	0.0	0.0	0.0	6.0	6.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0
Ferm 2															
0-16	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0-17	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0-19	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18-3	0.0	0.0	0.0	9.0	8.0	8.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18-4	8.0	7.0	7.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Fig 4.1 Inhibition zones (mm) exhibited by Bacteriocins isolated from *Lb. fermentum* against some pathogens



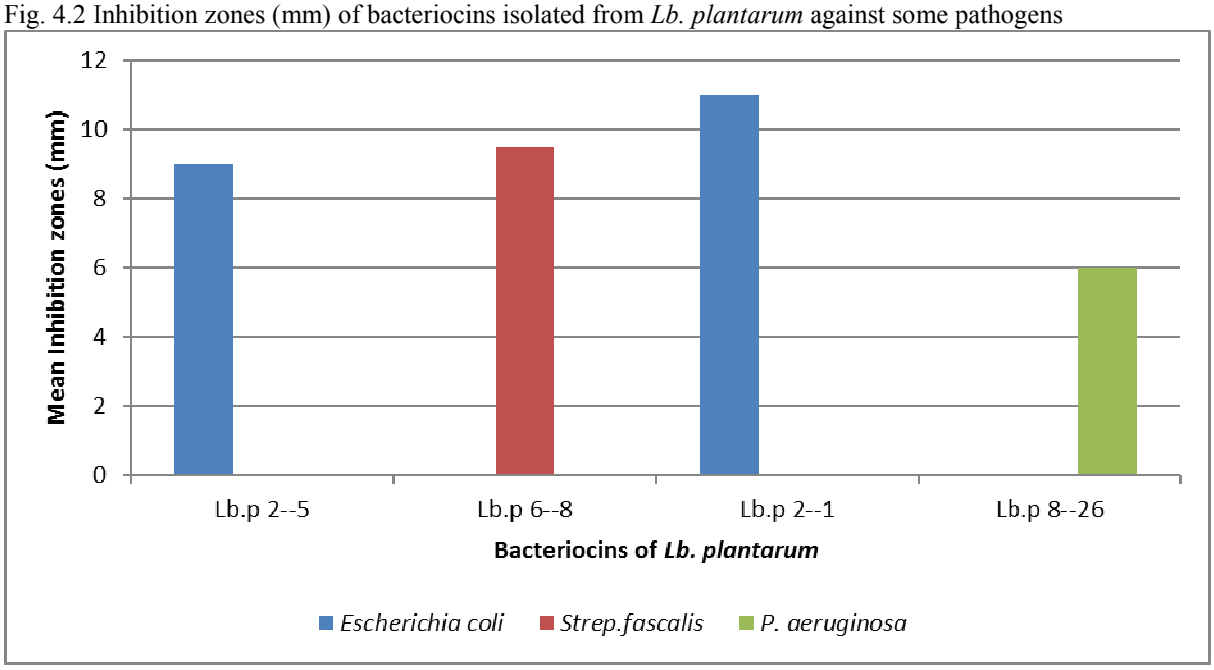
Lb.f = *Lb. fermentum*

As shown in table 4.2, seven (7) strains of *Lb. plantarum* were used and out of which four (4) strains representing 57.14% were able to inhibit at least one pathogen used. The highest mean inhibition of 11 mm was recorded and this was shown by *Lb. plantarum* 2-1 against *E. coli* and the least mean inhibition was 6 mm by *Lb. plantarum* 8-26 against *P. aeruginosa*.

Tab 4.2 Results of bacteriocin activities of *Lb. plantarum* against some pathogens

LAB	*Escherichia coli*			*Streptococcus faecalis*			*Pseudomonas aeruginosa*			*Salmonella typhimirum*			*Bacillus cereus*		
Ferm 1	1	2	Mean	1	2	Mean	1	2	Mean	1	2	Mean	1	2	Mean
2-5	0.0	0.0	0.0	7.0	11.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6-8	0.0	0.0	0.0	11.0	8.0	9.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6-24	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ferm 2															
2-1	11.0	11.0	11.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6-20	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6-30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
8-26	0.0	0.0	0.0	0.0	0.0	0.0	6.0	6.0	6.0	0.0	0.0	0.0	0.0	0.0	0.0

Fig. 4.2 Inhibition zones (mm) of bacteriocins isolated from *Lb. plantarum* against some pathogens



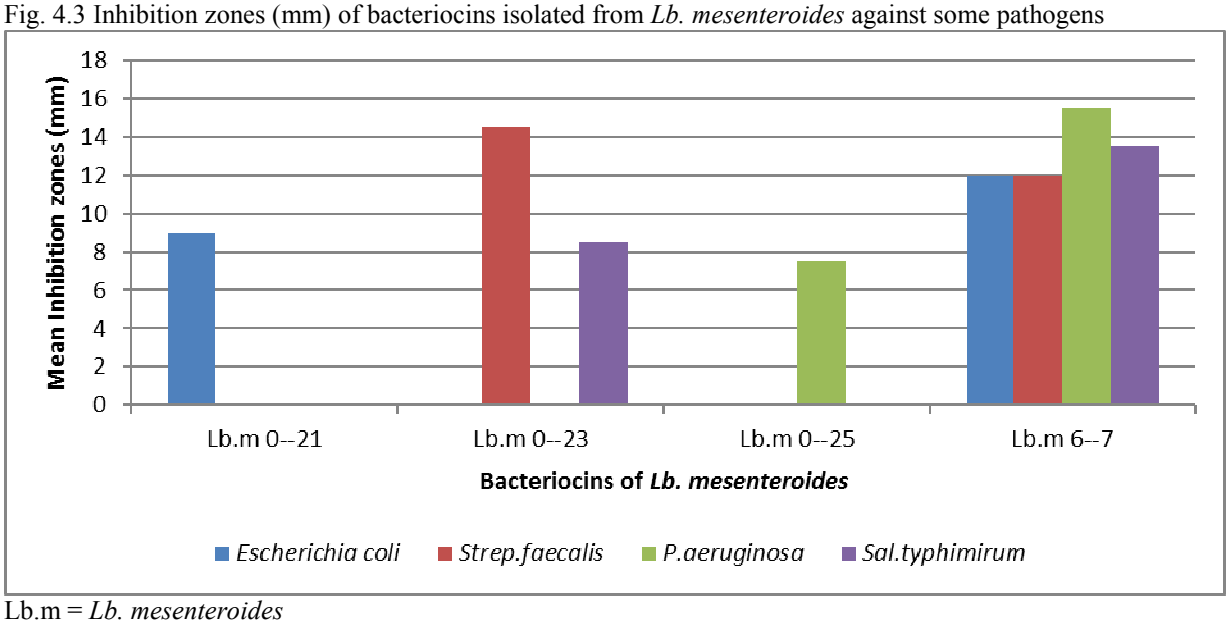
Lb.p = *Lb. plantarum*

Also seven (7) strains of *Lb. mesenteroides* were used and out of these four (4) strains representing 57.14% were able to exhibit antagonistic effect against at least one pathogenic organism. The strain *Lb. mesenteroides* 6-7 showed inhibition against four (4) of the pathogens namely *E. coli, Strep. faecalis, P. aeruginosa* and *Sal. typhimirum*. Also the strain *Lb. mesenteroides* 0-23 showed inhibition against two (2) pathogens namely *Strep .faecalis* and *Sal. typhimirum* with mean inhibition zones of 14.5 mm and 8.5 mm respectively. The least mean inhibition exhibited by *Lb. mesenteroides* was 7.5 mm shown by the strain *Lb. mesenteroides* 0-25 against *P. aeruginosa* whereas the highest mean inhibition was 15.5 mm by strain *Lb. mesenteroides* 6-7 against *P. aeruginosa*. It must also be noted that among the 27 LAB strains used only *Lb. mesenteroides* had antagonistic effect against *Sal. typhimirum.*

Tab 4.3 Results showing bacteriocin activities of *Lb. mesenteroides* against some pathogens

LAB	*Escherichia coli*			*Streptococcus faecalis*			*Pseudomonas aeruginosa*			*Salmonella typhimirum*			*Bacillus cereus*		
Ferm 1	1	2	Mean	1	2	Mean	1	2	Mean	1	2	Mean	1	2	Mean
0-12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0-21	10.0	8.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0-23	0.0	0.0	0.0	14.0	15.0	14.5	0.0	0.0	0.0	9.0	8.0	8.5	0.0	0.0	0.0
0-25	0.0	0.0	0.0	0.0	0.0	0.0	7.0	8.0	7.5	0.0	0.0	0.0	0.0	0.0	0.0
0-35	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ferm 2															
6-7	12.0	12.0	12.0	11.0	13.0	12.0	16.0	15.0	15.5	15.0	12.0	13.5	0.0	0.0	0.0
6-14	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Fig. 4.3 Inhibition zones (mm) of bacteriocins isolated from *Lb. mesenteroides* against some pathogens



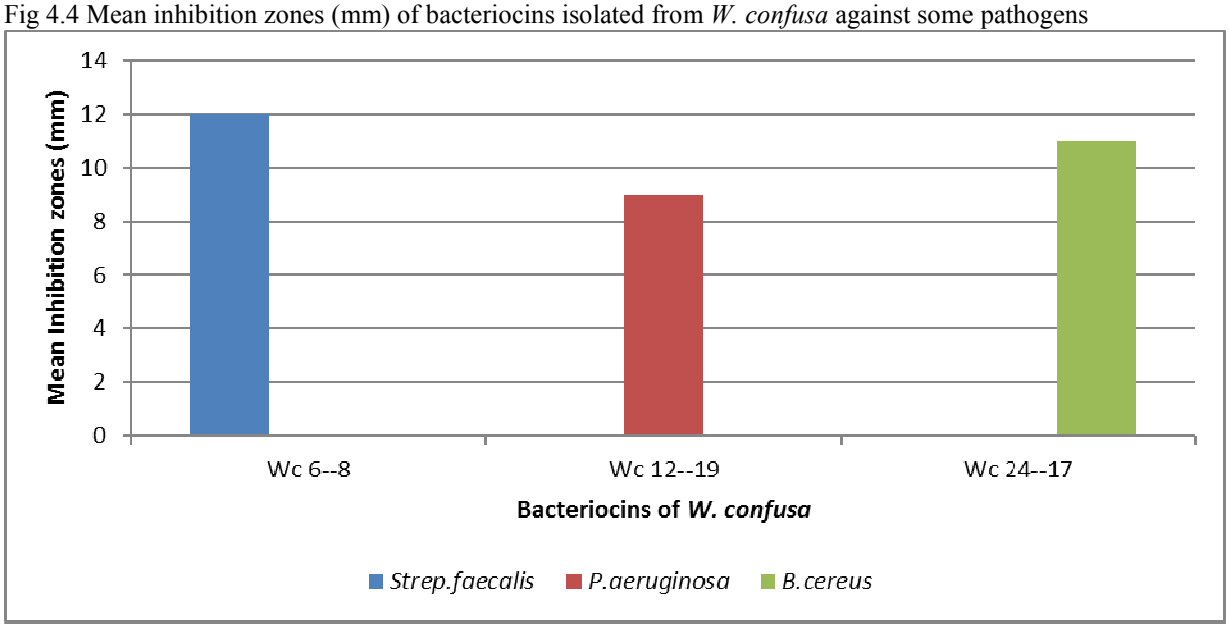
Lb.m = *Lb. mesenteroides*

Lastly, *W. confusa* showed 100% antagonistic effect against at least one of the pathogens used. This was the only strain that inhibited *B. cereus* with a mean inhibition of 11 mm by 24-17. The least mean inhibition of 9 mm was shown by *W. confusa* 12-19 against *P. aeruginosa.* This can be seen in table 4.4.

Tab 4.4 Results of bacteriocin activities of *W. confusa* against some pathogens

LAB	*Escherichia coli*			*Streptococcus faecalis*			*Pseudomonas aeruginosa*			*Salmonella typhimirum*			*Bacillus cereus*		
FERM 2	1	2	Mean	1	2	Mean	1	2	Mean	1	2	Mean	1	2	Mean
6-8	0.0	0.0	0.0	13.0	11.0	12.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12-19	0.0	0.0	0.0	0.0	0.0	0.0	10.0	8.0	9.0	0.0	0.0	0.0	0.0	0.0	0.0
24-17	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	12.0	10.0	11.0

Fig 4.4 Mean inhibition zones (mm) of bacteriocins isolated from *W. confusa* against some pathogens



Wc = *Weisella confuse*

4.0 Discussion

The use of bacteriocins for bio-preservation is a relatively recent technology for eliminating or controlling pathogenic or spoilage bacteria in food. Bacteriocins such as Nisins are active against a wide range of bacteria (Klaenhammer, 1993; Tagg *et al*., 1976), but the cost of producing and purification of this antimicrobial peptide

and the possibility of the development of resistant strains are concerns that limit the use of this peptide (Gravesen *et al*., 2002).

The rationale behind this project is the production of this vital antimicrobial peptide known as bacteriocins from some LAB and their antagonistic effects against some food spoilage or pathogenic organisms. Although several LAB strains have been reported as bacteriocin producers, their effectivity ranges from narrow to broad spectrum types (Hata *et al*., 2009). Bacteriocin of *Lb. fermentum* was found to have antimicrobial potential against methicillin resistant *Staphylococcus aureus* (Nawaz *et al*., 2009). In the same way, some other researchers observed the inhibition of pathogenic *Salmonella* and *Escherichia* by the bacteriocin of *Lb. acidophilus* (Sattari *et al*., 1999; Cocconnier *et al*., 1997).

The sensitivity of Gram negative bacteria to bacteriocins produced by lactic acid bacteria is not common. However, in this study, the bacteriocins of LAB strains were effective against Gram negative bacteria including *E. coli* ATCC 24522*, P. aeruginosa* and *Sal. typhimirum.* This was even to the extent that the highest antagonistic effect was exhibited by *P. aeruginosa* against *Lb. mesenteroides* 6-7, though some of the bacteriocins showed some antagonistic effects against the Gram positive bacteria with the highest being 14.5 mm shown by *Lb. mesenteroides* 0-23 against *Strep.faecalis*. *B.cereus* being another Gram positive bacterium was inhibited by only the bacteriocins of *W.confusa* 24-17. These results indicate that some of our lactic acid bacteria strains found in nunu are capable of synthesizing inhibitive substances against pathogenic bacteria. Also these inhibitive substances (bacteriocins) produced by our lactic acid bacteria strains act differently on the pathogenic reference indicator strains. Although Klaenhammer, (1988) stated that bacteriocins are proteins which show inhibitory activity against closely related organisms, the present findings is rather supported by the observations made by Mandal *et al*., (2008) where bacteriocins of Gram positive bacteria are able to inhibit growth of both Gram positive and Gram negative bacteria.

It is worth noting that the diameters of inhibitory zones produced by *Lb. mesenteroides* were larger as compared to that of *Lb. fermentum, Lb. plantarum and W. confusa.* Different reactions of the test strains to different bacteriocins indicate the different mode of actions of bacteriocins. This difference points out the presence of weak neutralization mechanism in the test strains that failed to show some antagonistic effect or clear zones of inhibitions for bacteriocin of the various LAB used. For bacteriocin of *Lb. mesenteroides*, such neutralization mechanism cannot be predicted due to presence of clear zone of inhibition. Bacteriocins of *Lb. fermentum* are known to have effect on *E. coli* (Riaz *et al.,* 2010) but in this particular study they did not show much significant result since only two of the isolates, 10-3 and 18-4, had effect on *E. coli* out of the ten isolates.

Bacteriocins of LAB are known to have effect on Gram positive bacteria (Klaenhammer, 1988). The inability of the LAB strains to show antagonistic effect against the two Gram positive bacteria used especially *B. cereus* may be as a result of the concentrations of the bacteriocins used. It is hereby assumed that if the concentrations were increased from 20µl to 50µl, better results would have been reached with the bacteriocins showing better antagonistic effect against the Gram positive bacteria used.

According to the present study, starter cultures for nunu production can be made from *Lb. mesenteroides* 6-7 and *W. confusa* 24-17 and the fermentation allowed to take place within 48 hours. This is so because the LAB isolated during the 48-hour fermentation had better chances of inhibiting the pathogens tested against. With the combination of the two LAB in question, there seems a better chance of the organisms having better antagonistic effect against the five pathogens worked on.

5.0 Conclusions

Of all the isolated bacteriocins from 27 LAB strains used against the five spoilage microorganisms, only 17 of them had antagonistic effects against at least one of the pathogens used. The bacteriocin of *Lb. mesenteroides* 6-7 was the only isolate that inhibited the proliferation of four pathogens namely *E. coli, P. aeruginosa, Sal. typhimirum* and *Strep. faecalis* with only *Strep. faecalis* being Gram positive bacterium. Also the bacteriocin *W. confusa* 24-17 was the only isolate the inhibited *B. cereus.*

These isolated strains which showed better activity can positively boost their use as starter cultures for the fermentation of nunu. This showed that LAB are capable of inhibiting pathogenic microorganisms in the food environment and display crucial antimicrobial properties with respect to food preservation and safety with a view to improving the quality and microbiological safety of nunu. They can also be used more specifically to selectively inhibit certain high-risk bacteria like *E. coli, Sal. typhimirum, Strep. faecalis, P. aeruginosa* and *B. cereus* in food.

Furthermore, due to their synergistic properties, *Lb. mesenteroides* and *W. confusa* can also help to reduce the addition of chemical preservative in food and can alternatively satisfy the consumer's demands for safe, fresh-tasting, ready-to-eat and minimally processed foods.

Hence, it could be concluded that the bacteriocins isolated from *Lb. mesenteroides* and *W. confusa* may offer novel, safe alternative for the protection and preservation of high value nunu.

6.0 Recommendations

Based on the results reached, further purification of the bacteriocins isolated from *Lb. mesenteroides* and *W. confusa* are required to enable the commercial application of this natural antimicrobial compound.

It is also recommended that further research be carried out on characterization of the bacteriocins.

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