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Scientific Research

Partial Characterization of Bacteriocins from Two *Pediococcus acidilactici* Strains Isolated during Traditional Sorghum Beer Processing in Côte d'Ivoire

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Abstract

Two lactic acid bacteria strains (At1BEAE22 and At344E21) isolated during *tchapalo* production were identified on the basis of phenotypic analyses. Bacteriocins produced by these strains were tested for their antimicrobial activities using well diffusion agar method. Heat resistance, pH sensitivity and enzyme treatments were also analyzed. Results showed that both lactic acid bacteria strains were identified as *Pediococcus acidilactici*. Their bacteriocins inhibited growth of *Lactobacillus delbrueckii* F/31, *Listeria innocua* ATCC 33090, *Enterococcus faecalis*, *Enterococcus faecalis* ATCC 29212, *Streptococcus* sp, *Enterococcus faecalis* CIP 105042 and *Enterococcus faecium* ATCC 51558. These bacteriocins were heat stable at 60˚C for 30 min for all indicator bacteria. However, they remained active only against *Lactobacillus delbrueckii* and *Listeria innocua* at 121˚C for 60 min. Moreover, they were active in a wide range of pH (3 to 9) with a maximum activity observed at pH 5 and 6 on all indicator bacteria. But, bacteriocin from *Pediococcus acidilactici* At34E21 was more stable at acidic pH than basic one. The fact that the bacteriocin was inactivated by proteinase K and *α*-chymotrypsin indicated its proteinaceous nature, a general characteristics of bacteriocins.

Keywords

Bacteriocin, *Pediococcus acidilactici*, Lactic Acid Bacteria, *Tchapalo* Production

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1. Introduction

Lactic acid bacteria (LAB) are very important for human beings. Indeed, they are not only part of the commensal flora of human, but also play an important role in the conservation and improvement of organoleptic and nutritional qualities of foods [1]-[3]. They have interesting inhibitory properties related to different mechanisms that allow them to preserve food against spoilage and food-borne pathogens and thereby increase their shelf-life. These properties relate to the production of organic acid, hydrogen peroxide, diacetyl and bacteriocins [4]-[8].

Bacteriocins are a heterogeneous family of small, heat-stable peptides with potent antimicrobial activity that are produced by many bacterial species, including many probiotic strains. Those produced by Gram-positive bacteria have a bactericidal or bacteriostatic effect on other species and genus, but the activity is usually limited to other Gram-positive bacteria [8]. Lactic acid bacteria bacteriocins are considered as safe natural preservatives or bio-preservatives, because they are degraded by the proteases in gastrointestinal tract unlike traditional antibiotics and they also reduce the use of chemical preservatives in foods [9]. In addition, they are active against Gram-positive foodborne pathogens, such as *Bacillus cereus*, *Listeria monocytogenes*, *Staphylococcus aureus*, and *Clostridium botulinum* and against certain Gram-negative bacteria [5] [10]. It is also stated that bacteriocin-producing LAB originally isolated from foods are the best candidates for improving the microbiological safety of these foods because they are well adapted to food conditions and should therefore be more competitive than LAB isolated from other sources.

Research on bacteriocins from LAB has expanded over the last decades to include the use of bacteriocins or producer organisms as natural food preservatives and their potential utility in human health applications [4] [5] [11]. Thus, several types of bacteriocins from food-associated LAB are identified and characterized, of which the important ones are nisin, pediocin, diplococcin, acidophilin, bulgarican, helveticin, lactacin and plantaricin. They are produced by several species including *Lactbacillus pentosus*, *Lactobacillus plantarum*, *Lactococcus lactis*, *Enterococcus faecium*, *Leuconostoc pseudomesenteroides* and *Pediococcus acidilactici*. They are detected in foods such as dairy products, meats, barley, sourdough, red wine, fermented vegetables, traditional fermented products, etc. [12]-[14].

Like most traditional fermented foods, *tchapalo*, a traditional sorghum beer from Côte d'Ivoire, contains LAB. The main species present belong to the genus *Lactobacillus*, *Enterococcus*, *Pediococcus* and *Leuconostoc* [15]. But up to date, it is not clear whether any bacteriocin is produced in *tchapalo* processing by LAB. The bacteriocin produced by the strains isolated from this beverage has neither been characterized nor checked for its efficacy in various food products. In this paper, we report on bacteriocins from strains of *Ped. acidilactici* isolated during *tchapalo* processing. Effects of pH, temperature and proteolytic enzymes on bacteriocin activities from these strains were determined.

2. Materials and Methods

2.1. Isolation of Bacteriocins-Producing Bacteria

During *tchapalo* processing, samples of sorghum grain, sorghum malt, sorghum malt flour, mash, cooked sediment, wort, sour wort, sweet wort, traditional starter and *tchapalo* were collected from three areas (Abobo, Attecoubé and Yopougon) randomly selected in the district of Abidjan, Southern Côte d'Ivoire according to Aka *et al.* [16]. Samples were collected in sterile screw cap tubes and serially diluted (10^{-1} - 10^{-7}) in sterile distilled water. The diluted samples were plated onto MRS agar plates and incubated anaerobically at 30˚C, 37˚C and 45˚C for 48 h. Screening of bacteriocins produced by isolates was done on a total of 117 lactic acid bacteria strains by well-diffusion method [17] against indicator bacteria *i.e.*, *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecalis*, *Enterocuccus faecium*, *Streptococcus* sp., *Lactobacillus delbrueckii*, *Listeria innocua*, *Salmonella typhimurium*, *Escherichia coli* and *Pseudomonas aeruginosa* (**Table 1**). They were obtained from Laboratoire National de la Santé Publique (LNSP) and CSRS laboratory of microbiology culture collection. Among strains showing antibacterial activity against indicators bacteria, two appeared as the best ones and were selected and subjected to morphological, physiological, and biochemical tests, including Gram staining, motility, catalase, oxidase tests, as well as growth at different pH and at different temperatures. The strains were further identified using API 50 CH (Biomérieux, Marcy-l'Etiole, France), sub-cultured onto MRS agar slants which were incubated at 37˚C for 48 h and preserved in 20% glycerol at −20˚C.

Table 1. Media and culture conditions of indicator bacteria strains and antibacterial activity of the studied lactic acid bacteria.

Indicator bacteria strains	Sources	Media and culture conditions	Antibacterial activity of crude bacteriocins of *Pediococcus acidilactici*			
			At1BE22		At34E21	
			CCBA (mm)	MIC (AU/mL)	CCBA (mm)	MIC (AU/mL)
Bacillus cereus	DSM 31^T	Nutrient broth, 18 - 24 h, 37˚C	-	Nd	-	Nd
Staphylococcus aureus	ATCC 25923	Nutrient broth, 18 - 24 h, 37˚C	-	Nd	-	Nd
Enterococcus faecalis	Clinical LNSP	Brain Heart Infusion, 18 - 24 h, 37˚C	11	800	09	800
Enterococcus faecalis	ATCC 29212	Brain Heart Infusion, 18 - 24 h, 37˚C	13	1600	11	1600
Enterococcus faecalis	CIP 105042	Brain Heart Infusion, 18 - 24 h, 37˚C	10	800	09	400
Streptococcus sp.	Clinical LNSP	Brain Heart Infusion, 18 - 24 h, 37˚C	12	3200	11	1600
Enterococcus faecium	ATCC 51558	Brain Heart Infusion, 18 - 24 h, 37˚C	14	6400	13.5	1600
Lactobacillus delbrueckii	F/31	MRS, 18 - 24 h, 44˚C, anaerobiosis	16	3200	17	6400
Listeria innocua	ATCC 33090	Nutrient broth, 18 - 24 h, 37˚C	11.5	3200	14	6400
Salmonella typhimurium	ATCC 5066	Nutrient broth, 18 - 24 h, 37˚C	-	Nd	-	Nd
Escherichia coli	ATCC 28170	Nutrient broth, 18 - 24 h, 37˚C	-	Nd	-	Nd
Pseudomonas aeruginosa	ATCC 27853	Nutrient broth, 18 - 24 h, 37˚C	-	Nd	-	Nd

CCBA: crude concentrated bacteriocin activity; MIC: minimum inhibitory concentration, AU: arbitrary units (AU), -: negative result, Nd: not determined.

2.2. Extraction of Bacteriocins

Bacteriocin extractions were performed according to the modified method described by Savadogo *et al.* [4] below. Lactic acid bacteria strains At1BE22 and At34E21 were propagated each in 1000 mL MRS broth (pH 7.0). For extraction of bacteriocins, cell-free solutions were obtained by centrifuging (10,000 xg TGL-16M) the culture for 20 min at 4˚C; then precipitated with ammonium sulphate (60% saturation). The mixture was stirred for 2 h at 4˚C and later centrifuged at 12,000 xg for 1 h at 4˚C. The precipitates were resuspended in 100 mL of 0.1 M potassium phosphate buffer (pH 7.0) and was adjusted to pH 7.0 by means of 5 N NaOH to exclude antimicrobial effect of organic acid. Furthermore, the precipitates were sterilized using 0.2 µM pore size filter (Corning syringe filters, Sigma-Aldrich, Germany). These precipitates were kept at 4˚C until use.

2.3. Antibacterial Activity of Precipitated Bacteriocins

Antibacterial activities were assayed against indicator bacteria strains using well diffusion agar method described by Arici *et al.* [17]. The indicator test bacteria were incubated in medium broth for 18 - 24 h at 37˚C or 44˚C (**Table 1**). About 10^6 ufc/mL of the indicator bacteria to be tested for sensitivity were inoculated (1% v/v) into 20 mL of media solf agar (0.9% agar) and poured in the Petri dishes. After solidification, Petri dishes were dried for 30 min under a laminar flow hood. Wells of 5 mm diameter were cork bored in the agar. Aliquots (100 µL) of bacteriocin solutions were dispensed in the wells and plates were pre-incubated at 4˚C for 2 h and then incubated for 18 h. Antagonistic activity was expressed as the area of inhibition surrounded each agar well. The antagonistic activities of samples were determined for each isolate by the persistence of the inhibition zone measured in diameter (mm). Antibacterial tests were done in triplicate and the mean values recorded.

2.4. Minimum Inhibitory Concentration (MIC)

The precipitated bacteriocins were determined by two fold serial dilution in sterile 0.1 M potassium phosphate buffer (pH7.0) and the antimicrobial activity was assayed as described above. Antimicrobial activity was ex-

pressed as arbitrary units (AU) per milliliter. One AU was defined as the reciprocal of the highest dilution showing a clear zone of growth inhibition.

2.5. Characterization of Bacteriocins

Bacteriocin samples were characterized with respect to thermal and pH stability and susceptibility to denaturation by enzymes according to Diop *et al.* [2] and Ogunbanwo *et al.* [18].

2.5.1. Heat Resistance

The effect of temperature on bacteriocins was tested by heating the precipitated bacteriocins at 60˚C, 80˚C, 100˚C and 121˚C during 0, 15, 30 and 60 min. Then, 100 µL of each aliquot was performed to antibacterial activity.

2.5.2. pH Sensitivity

Precipitated bacteriocins were adjusted to pH 3, 4, 5, 6, 7, and 9 with hydrochloric acid (5 N HCl) or sodium hydroxide (5 N NaOH). After 2 h of incubation at room temperature the residual activity was assayed.

2.5.3. Enzyme Treatments

To test enzyme sensitivity, precipitated bacteriocins were treated with the following enzymes (obtained from Sigma) at a final concentration of 1 mg/mL: proteinase K in the storage buffer (0.05 M Tris hydrochloride, pH 7.5, 0.01 M $CaCl_2$, 50 mL glycerol, adding of Milli Q water until 100 mL), α-chymotrypsin in 0.1 M potassium phosphate buffer (pH 7.0), α-amylase in 0.1 M potassium phosphate buffer (pH 7.0) and catalase (C-100 bovine liver). After incubation at 37˚C for 1 h, the test tubes containing proteinase K were heated at 80˚C for 10 min to inactivate the enzymes and the antimicrobial activities were assayed as described above.

3. Results

3.1. Antibacterial Activity of Crude Bacteriocins Extract

The two lactic acid bacteria strains At1BE22 and At34E21 were identified as *Pediococcus acidilactici* based on morphological, physiological and API 50 CH profiles. Susceptibilities of various Gram-positive and Gram-negative bacteria to growth inhibition by the bacteriocins from both strains were presented in **Table 1**. The crude bacteriocins inhibited the growth of *Lact. delbrueckii* F/31, *L. innocua* ATCC 33090, *Ent. faecalis*, *Ent. faecalis* ATCC 29212, *Streptococcus* sp, *Ent. faecalis* CIP 105042 and *Ent. faecium* ATCC 51558. But, they did not exhibit inhibitory activity towards the other Gram-positive indicator bacteria and Gram-negative indicator microorganisms such as *Ps. aeruginosa* ATCC 27853, *E. coli* ATCC 25922 and *Salm. typhimurium* ATCC 5066. *Lactobacillus delbrueckii* F/31 showed more sensivity to bacteriocins with an inhibition zone of 16 - 17 mm while *Ent. faecalis* and *Ent. faecalis* CIP 105042 had the smallest inhibition diameter (9 - 11 mm). Although bacteriocins from both *Ped. acidilactici* strains inhibited the same indicator bacteria, they did not show the same inhibition diameter. Thus, growth inhibition of *L. innocua* ATCC 33090 was higher with bacteriocin from *Ped. acidilactici* At34E21 (14 mm) than with bacteriocin from *Ped. acidilactici* At1BE22 (11.5 mm). Similarly, bacteriocin from the strain At1BE22 was found to be more active against *Ent. faecalis* ATCC 29212 than the one from strain At34E21.

3.2. Minimum Inhibitory Concentration

The minimum inhibitory concentration (MIC) of bacteriocins produced by LAB At1BE22 and At34E21 was tested by serial dilutions of precipitates in sterile phosphate buffer pH 7. As shown in **Figure 1**, there was a decrease in the antimicrobial effectiveness of bacteriocins when dilution factor increased. Antibacterial activity in unit-activity per milliliter (AU/mL) was therefore determined as the inverse of the lowest dilution causing inhibition of bacterial indicator. The MIC of bacteriocins produced by these strains was shown in **Table 1**. The MICs for the sensitive bacteria were between 800 AU/mL and 6400 AU/mL for *Ped. acidilactici* At1BE22 and between 400 AU/mL and 6400 AU/mL for *Ped. acidilactici* At34E21. Results showed also that MICs were in comparable ranges for both bacteriocins concerning strains *Ent. faecalis* LNSP and *Ent. faecalis* ATCC29212. But for the other sensitive indicator strains, MICs values were different. For example, the MIC for bacteriocin

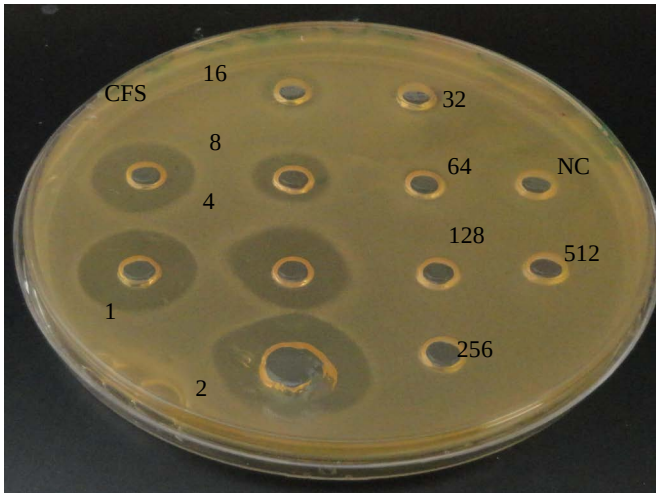

Figure 1. Antibacterial activity of bacteriocin produced by *Ped. acidilactici* At1BE22 against *L. innocua* ATCC 33090. CFS: cell-free supernatant neutralized and treated with catalase; 1: first diluted crud concentrated bacteriocin; 2, 4, 8, 16, 32, 64, 128, 256 and 512: dilutions 2, 4, 8, 16, 32, 64, 128, 256 and 512; NC: negative control.

from strain At34E21 against *Ent. faecalis* CIP 105042 was 400 AU/mL while it was 800 AU/mL for bacteriocin from strain At1BE22. In addition, MICs values demonstrated the particular sensitivity of *Ent. faecalis* CIP 105042 to bacteriocin from strain At34E21, but also to bacteriocin from strain At1BE22, as the MIC was the lowest obtained through this study (400 AU/mL). For bacteriocin from *Ped. acidilactici* At34E21, the less sensitive indicator strains were *L. innocua* and *Lact. delbrueckii*. Their MICs values were 6400 AU/mL. As for bacteriocin from *Ped. acidilactici* At1BE22, *Ent. faecium* ATCC 51558 was the less sensitive tested strain.

3.3. Stability of Bacteriocin Activity to Heat and pH

Figure 2 and **Figure 3** showed the effect of temperature on bacteriocin activities in terms of inhibition zones. The inhibitory compounds produced by *Ped. acidilactici* strains were considered to be heat stable. In fact, results demonstrated that inhibitory activities of bacteriocins from both strains were not affected by heating at 60˚C for 15 min and 30 min. At the same temperature and after heat treatment for 60 min, bacteriocin from *Ped. acidilactici* At1BE22 lost its activity against *Ent. faecalis* and *Ent. faecalis* CIP 105042 while bacteriocin from *Ped. acidilactici* At34E21 lost its activity against all tested strains of *Ent. faecalis*. When bacteriocins were heat at 80˚C, although a partial loss in their activities was observed with a continuous increase in inactivation time, they remained stable against all sensitive indicator bacteria except against *Ent. faecalis* strains. After heating at 100˚C or 121˚C for 30 min, bacteriocin produced by *Ped. acidilactici* At34E21 was considered to be the most heat stable, as the activity remained stable against three indicator strains (*Lact. dekbrueckii* F/31, *L. innocua* 33090 and *Ent. faecalis* ATCC 29213) while bacteriocin from *Ped. acidilactici* At1BE22 remained stable against two strains at the same time.

The pH stability was studied in the range of pH 3 to 9. Results were presented in **Figure 4** and **Figure 5**. The bacteriocin was found active in a wide range of pH with the maximum activity observed at pH 5 and 6 for both *Ped. acidilactici* strains against all indicator bacteria. But, bacteriocin produced by strain At34E21 was more stable at acidic pH than basic pH.

3.4. Enzyme Treatments of Substances Produced by *Pediococcus acidilactici* At1BE22 and At34E21

Results of enzyme treatments showed that inhibitory substances produced by *Ped. acidilactici* strains At34E21 and At1BE22 were inactivated by proteolytic enzymes (proteinase K, α-chymotrypsin). On the other hand, catalase and α-amylase had no effect on their activity (**Figure 6**). This indicates that the inhibitory substances are of proteinaceous nature, a general characteristics of bacteriocins.

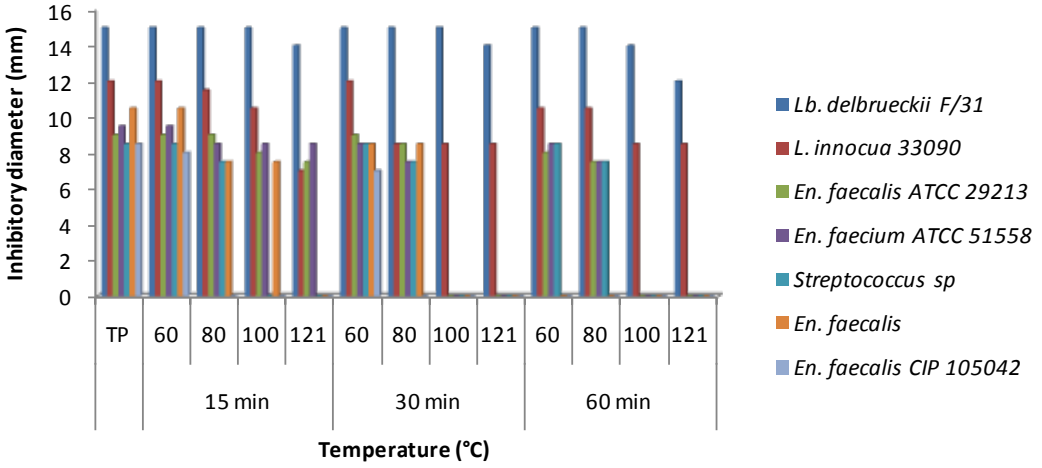

Figure 2. Effect of temperature on antimicrobial activity of bacteriocin produced by *Ped. acidilactici* At1BE22. TP: positive control.

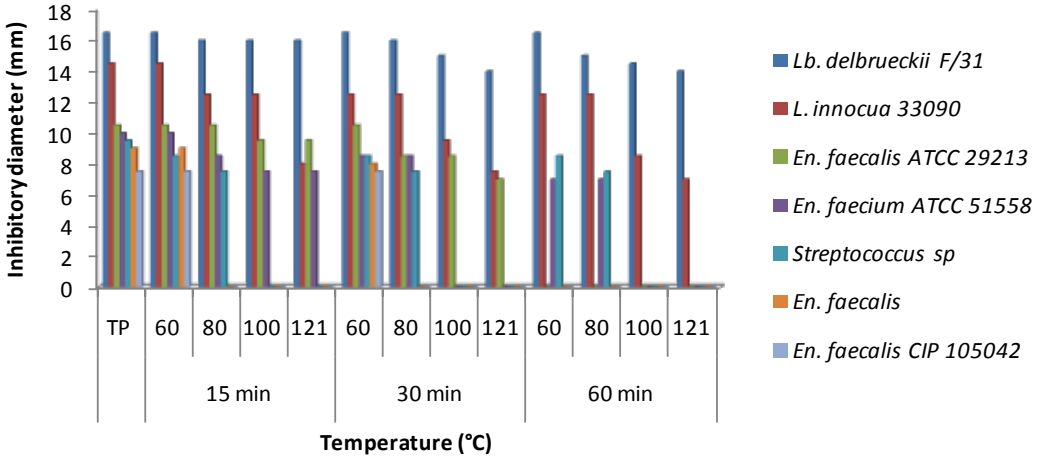

Figure 3. Effect of temperature on antimicrobial activity of bacteriocin produced by *Ped. acidilactici* At34E21. TP: positive control.

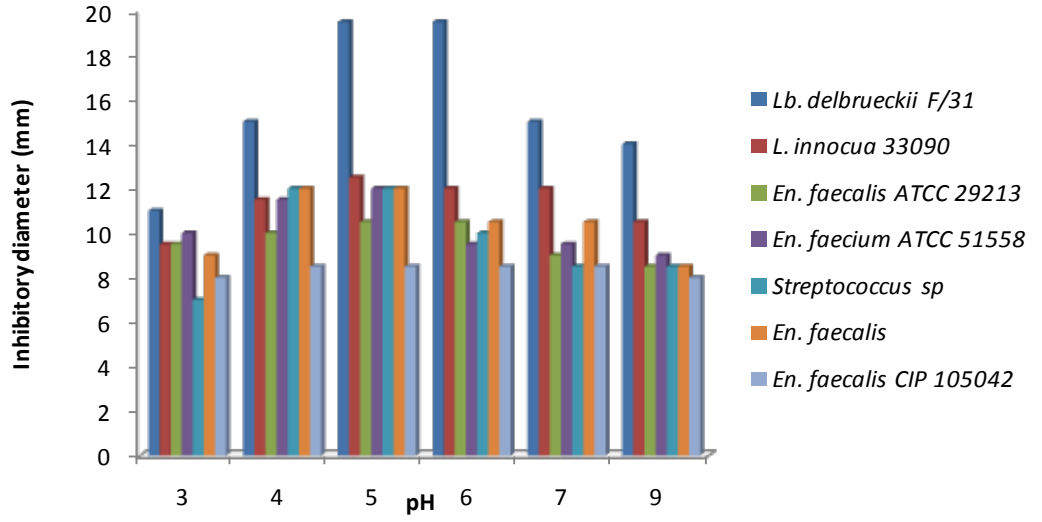

Figure 4. Effect of pH on antimicrobial activity of bacteriocin produced by *Ped. acidilactici* At1BE22.

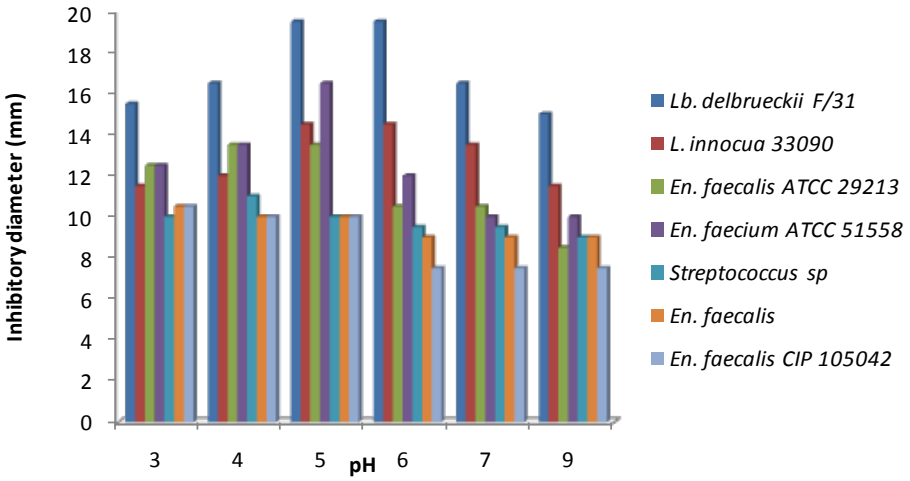

Figure 5. Effect of pH on antimicrobial activity of bacteriocin produced by *Ped. acidilactici* At34E21.

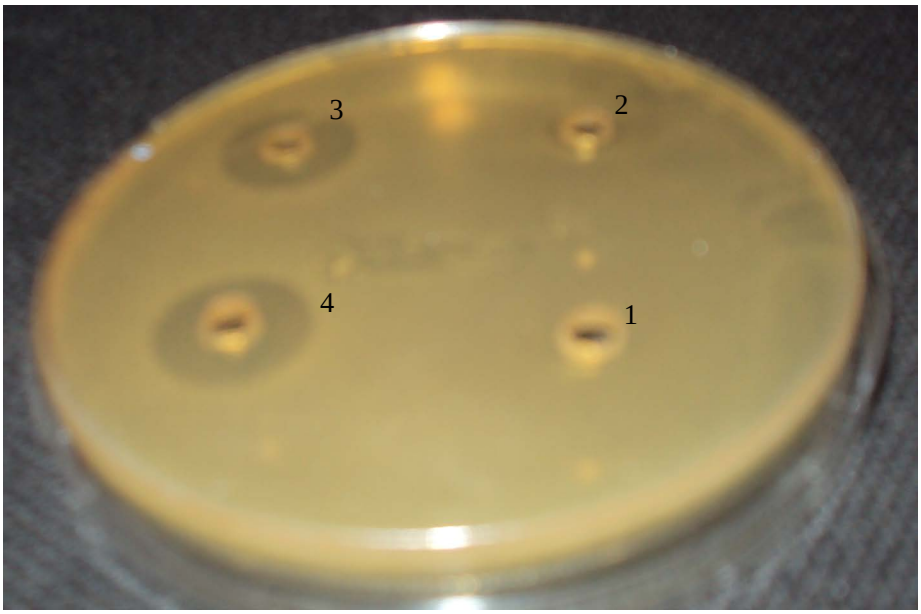

Figure 6. Effect of enzymes on bacteriocin from *Ped. acidilactici* At34E21 against *Lact. delbrueckii* F/31. 1: α-chymotrypsin; 2: proteinase K; 3: catalase; 4: α-amylase.

4. Discussion

Several types of bacteriocins from food associated LAB have been identified and characterized [7] [19] [20]. Because of the increasing demand for more natural and microbiologically safe food products, there is a need for bio preservation methods. Bacteriocins have considerable potential for food preservation, as well as for human therapy as potential supplements or replacements for currently used antibiotics. In this study, two *Ped. acidilactici* strains (At1BE22 and At34E21) isolated during *tchapalo* production showed antimicrobial properties due to bacteriocins production. These bacteriocins were active against *Lact. delbrueckii* F/31 and *L. innocua* ATCC 33090 which are food spoilage organisms. Both bacteriocins were also active against *Ent. faecalis*, *Ent. faecalis* ATCC 29212, *Streptococcus* sp, *Ent. faecalis* CIP 105042 and *Ent. faecium* ATCC 51558 which are opportunistic food borne pathogens [21] [22].

Earlier reports revealed the presence of bacteriocins in LAB strains and they have inhibitory effect against several bacteria [8] [11] [19] [20] [23]. Todorov and Dicks [5] found that bacteriocin produced by *Lactobacillus*

plantarum ST194BZ, a strain isolated from *boza*, a Balkan traditional drink, inhibits the growth of *Lactobacillus casei*, *Lactobacillus sakei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Enterococcus faecalis*, *Escherichia coli*, *Enterobacter cloacae* and *Pseudomonas aeruginosa*. Mandal *et al*. [6] had isolated *Pediococcus acidilactici* LAB5 from vacuum-packed fermented meat product which bacteriocin inhibited also *Ent. faecalis*, *L. innocua*, *Streptococcus* sp and other food borne pathogens such as *L. monocytogenes* and *Staph. aureus*. But, bacteriocins produced by *Pediococcus acidilactici* isolated in our study did not inhibit *S. aureus.* They had only inhibitory effect against closely related species. Other reported *Ped. acidilactici* producing bacteriocin isolated from fecal samples of healthy human volunteers with inhibition capacities against *Helicobacter pylori* causing peptic ulcer disease [24] [25]. This finding can be explained by the fact that these different strains of *Ped. acidilactici* do not come from the same substrate and they do not play the same role.

Moreover, there was a decrease in bacteriocins antimicrobial effectiveness with an increase of dilution factor. This means that inhibition zone diameter was proportional to the bacteriocin concentration as previously indicated by Najim *et al.* [26]. Bacteriocins from both *Ped. acidilactici* strains were stable after heat treatment at 60˚C for 30 minutes for all indicator bacteria, but their activities were lost as temperature increased. Only *Lact. delbrueckeii* F/31 and *L. innocua* 33090 remained sensitive to bacteriocins from both tested strains after heat treatment at 121˚C for 60 minutes. Temperature stability is an important factor if bacteriocins must be used as food preservative because many procedures of food preparation involve a heating step [23] [27]. Therefore, these strains could be used as potential biopreservatives during *tchapalo* production as temperature of fermentation of sweet wort into *tchapalo* does not exceed 40˚C [28]. Šeatovic *et al*. [28] also mentioned that heat stability is a major feature of low-molecular-weight bacteriocins; however, some bacteriocins produced by *Lactobacillus* strains were inactivated by 10 to 15 min treatment at 60˚C - 100˚C. Bacteriocin produced by *Pediococcus acidilactici* strains showed stability at a broader pH range from 3 to 9 with a maximum activity observed at pH 5 and 6. So these strains may be used as potential probiotic to produce *tchapalo* and lots of other fermented cereal beverages. Indeed, many bacteriocins and bacteriocin-like substances are considerably more tolerant to acid than alkaline pH values [23] [26] [29]. Similar results were reported by Mandal *et al.* [6]. They found that bacteriocin produced by *Pediococcus acidilactici* LAB5 was stable to a wide range of acidic pH but not in the high alkaline condition, due to alkali lysis. Bacteriocins produced by *Lact. plantarum* and *Lb. brevis* OGI retained their antimicrobial activity in an acidic pH range from 2 to 6, while inactivation occurred at pH 8 to 12 [18].

Treatment with catalase and α-amylase indicated that antibacterial activity of bacteriocin was not due to H_2O_2 or carbohydrates. The fact that bacteriocins were inactivated by proteinase K and α-chymotrypsin indicated its proteinaceous nature as the general characteristic of bacteriocins. These results were similar to some previously reported studies [5] [6] [25].

5. Conclusion

Bacteriocins produced by *Pediococcus acidilactici* strains in this study inhibited the growth of spoilage and food borne opportunistic pathogens. They showed stability over a wide range of pH and temperature. These strains could be used as potential biopreservatives starter cultures to produce sweet wort, *tchapalo* and other beverages. Further studies will be focused on purification and molecular characterization of these bacteriocins.

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References

[1] Tamang, J.P., Tamang, B., Schillinger, U., Franz, C.M.A.P., Gores, M. and Holzapfel, W.H. (2005) Identification of Predominant Lactic Acid Bacteria Isolated from Traditionally Fermented Vegetable Products of the Eastern Himalayas. *International Journal of Food Microbiology*, **105**, 347-356. http://dx.doi.org/10.1016/j.ijfoodmicro.2005.04.024

[2] Diop, M.B., Dubois-Dauphin, R., Tine, E., Destain, J., Thonart, P. and Ngom, A. (2007) Bacteriocin Producers from Traditional Food Products. *Biotechnology, Agronomy, Society and Environment*, 11, 275-281.

[3] Aka, S., N'Guessan, K.F., Nanga, Y.Z., Loukou, Y.G., Mazabraud, A.I. and Djè, K.M. (2010) Characterization of *Lactobacillus* Species Isolated from Mash, Sour Wort and *tchapalo* Produced in Côte d'Ivoire. *Food*, **4**, 49-54.

[4] Savadogo, A., Ouattara, C., Bassole, I. and Traore, A. (2004) Antimicrobial Activities of Lactic Acid Bacteria Strains Isolated from Burkina Faso fermented Milk. *Pakistan Journal of Nutrition*, **3**, 174-179. http://dx.doi.org/10.3923/pjn.2004.174.179

[5] Todorov, S.D. and Dicks, L.M.T. (2005) Effect of Growth Medium on Bacteriocin Production by *Lactobacillus plantarum* ST194BZ, a Strain Isolated from Boza Bacteriocin Production by *L. plantarum* ST194BZ. *Food Technology and Biotechnology*, **43**, 165-173.

[6] Mandal, V., Sen, S.K. and Mandal, N.C. (2008) Optimized Culture Conditions for Bacteriocin Production by *Pediococcus acidilactici* LAB 5 and Its Characterization. *Indian Journal of Biochemistry and Biophysics*, **45**, 106-110.

[7] Şanlibaba, P., Akkoç, N. and Akçelik, M. (2009) Identification and Characterisation of Antimicrobial Activity of Nisin A Produced by *Lactococcus lactis* subsp. *lactis* LL27. *Czech Journal of Food Sciences*, **27**, 55-64.

[8] Meera, N.S. and Devi, M.C. (2012) Partial Characterization and Optimization of Parameters for Bacteriocin Production by Probiotic Lactic Acid Bacteria. *Journal of Microbiology and Biotechnology Research*, **2**, 357-365.

[9] Cleveland, J., Montiville, T.J., Nes, I.F. and Chikindas, M.L. (2001) Bacteriocins: Safe, Natural Antimicrobials for Food Preservation. *International Journal of Food Microbiology*, **71**, 1-20. http://dx.doi.org/10.1016/S0168-1605(01)00560-8

[10] Ivanova, I., Kabadjova, P., Pantev, A., Danova, S. and Dousset, X. (2000) Detection, Purification and Partial Characterization of a Novel Bacteriocin Substance Produced by *Lactoccous lactis* Subsp. *Lactis* b14 Isolated from Boza-Bulgarian Traditional Cereal Beverage. *Biocatalysis Fundamentals & Applications*, **41**, 47-53.

[11] Sifour, M., Tayeb, I., Haddar, H.O., Namous, H. and Aissaoui, S. (2012) Production and Caracterization of Bacteriocin of *Lactobacillus plantarum* F12 with Inhibitory Activity against *Listeria monocytogenes*. *The Online Journal of Science and Technology*, **2**, 55-61.

[12] Bromberg, R., Moreno, I., Zaganini, C.L., Delboni, R.R. and De Oliveira, J. (2004) Isolation of Bacteriocin Producing Lactic Acid Bacteria from Meat and Meat Products and Its Spectrum of Inhibitory Activity. *Brazilian Journal of Microbiology*, **35**, 137-144. http://dx.doi.org/10.1590/S1517-83822004000100023

[13] Vaughan, A., Eijsink, V.G.H., O'Sullivan, T.F., O'Hanlon, K. and Van Sinderen, D. (2001) An Analysis of Bacteriocins Produced by Lactic Acid Bacteria Isolated from Malted Barley. *Journal of Applied Microbiology*, **91**, 131-138. http://dx.doi.org/10.1046/j.1365-2672.2001.01365.x

[14] Gollop, N., Zakin, V. and Weinberg, Z.G. (2005) Antibacterial Activity of Lactic Acid Bacteria Included in Inoculants for Silage and in Silages Treated with These Inoculants. *Journal of Applied Microbiology*, **98**, 662-666. http://dx.doi.org/10.1111/j.1365-2672.2004.02504.x

[15] Djè, M.K., Aka, S., Nanga, Y.Z., Yao, K.C. and Loukou, Y.G. (2009) Predominant Lactic Acid Bacteria Involved in the Spontaneous Fermentation Step of *Tchapalo* Process, a Traditional Sorghum Beer of Côte d'Ivoire. *Research Journal of Biological Sciences*, **4**, 789-795.

[16] Aka, S., Camara, F., Nanga, Y.Z., Loukou, Y.G. and Dje, K.M. (2008) Evaluation of Organic Acids and Sugars Contents during the Production of *Tchapalo*, a Traditional Sorghum Beer in Côte d'Ivoire. *Journal of Food Technology*, **6**, 189-195.

[17] Arici, M., Bilgin, B., Sagdic, O. and Ozdemir, C. (2004) Some Characteristics of *Lactobacillus* Isolates from Infant Faeces. *Food Microbiology*, **21**, 19-24. http://dx.doi.org/10.1016/S0740-0020(03)00044-3

[18] Ogunbanwo, S.T., Sanni, A.I. and Onilude, A.A. (2003) Characterization of Bacteriocin Produced by *Lactobacillus plantarum* F1 and *Lactobacillus brevis* OG1. *African Journal of Biotechnology*, **2**, 219-227.

[19] Sankar, N.R., Priyanka, V.D., Reddy, P.S., Rajanikanth, P., Kumar, V.K. and Indira, M. (2012) Purification and Characterization of Bacteriocin Produced by *Lactobacillus plantarum* Isolated from Cow Milk. *International Journal of Microbiology Research*, **3**, 133-137.

[20] Fatima, D. and Mebrouk, K. (2013) Characterization and Determination of the Factors Affecting Anti-Listerial Bacteriocins from *Lactobacillus plantarum* and *Pediococcus pentosaceus* Isolated from Dairy Milk Products. *African Journal of Food Science*, **7**, 35-44. http://dx.doi.org/10.5897/AJFS12.037

[21] Teixeira, L.M. and Merquior, V.L.C. (2013) *Enterococcus*. In: De Filippis, I. and McKee, M.L., Eds., *Molecular Typing in Bacterial Infections*, Springer Science Business Media, New York, 17-26. http://dx.doi.org/10.1007/978-1-62703-185-1_2

[22] Trang, V.T., Thanh, L.X., Sarter, S., Shimamura, T. and Takeuchi, H. (2013) Study of the Antimicrobial Activity of Aminoreductone against Pathogenic Bacteria in Comparison with Other Antibiotics. *International Journal of Biological Science and Engineering*, **7**, 675-678.

[23] Adebayo, F.A., Afolabi, O.R. and Akintokun, A.K. (2014) Antimicrobial Properties of Purified Bacteriocins Produced from *Lactobacillus casei* and *Lactobacillus fermentum* against Selected Pathogenic Microorganisms. *British Journal of Medicine and Medical Research*, **4**, 3415-3431. http://dx.doi.org/10.9734/BJMMR/2014/8584

[24] Kaur, B., Garg, N., Sachdev, A., Kumar, B., Mittu, B. and Chauhan, A. (2012) Isolation and Molecular Characterization of Anti-*Helicobacter pylori*. Bacteriocin Producing *Pediococcus acidilactici* BA28. *Open Access Scientific Reports*, **1**, 1-6.

[25] Kaur, B. and Garg, N. (2013) Characteristics of Bacteriocin BA28 Produced by *Pediococcus acidilactici* BA28. *Mintage Journal of Pharmaceutical and Medical Sciences*, **2**, 17-20.

[26] Najim, H.N., Zuhair, A.M. and Zina, S.K. (2012) The Antibacterial Activity of Bacteriocin Produced by *Lactobacillus acidophilus* Isolates against Sensitive Reference Strain *Lactobacillus acidophilus* R0052 and Its Stability to Different pH, Heating and Storage Temperatures. *Proceeding of the Eleventh Veterinary Scientific Conference*, 274-279.

[27] Malini, M. and Savitha, J. (2012) Heat Stable Bacteriocin from *Lactobacillus paracasei* Subsp. *Tolerans* Isolated from Locally Available Cheese: An *in Vitro* Study. *E3 Journal of Biotechnology and Pharmaceutical Research*, **3**, 28-41.

[28] Djè, M.K., N'Guessan, K.F., Djeni, T.N. and Dadié, T.A. (2008) Biochemical Changes during Alcoholic Fermentation in the Production of *Tchapalo*, a Traditional Sorghum Beer. *International Journal of Food Engineering*, **4**, 1-15. http://dx.doi.org/10.2202/1556-3758.1408

[29] Šeatovic, S.L., Novakovic, J.S.J., Zavišic, G.N., Radulovic, Ž.C., Gavrovic-Jankulovic, M.Đ. and Jankov, R.M. (2011) The Partial Characterization of the Antibacterial Peptide Bacteriocin G Produced by the Probiotic Bacteria *Lactobacillus plantarum* G2. *Journal of the Serbian Chemical Society*, **76**, 699-707. http://dx.doi.org/10.2298/JSC100605060S

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