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Research Article

Characterization and Evaluation of Antimicrobial Activity of Bacteriocins from *Lactobacillus Curvatus* and *Pediococcus Pentosaceus*

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Abstract

Lactobacillus curvatus M3 and *Pediococcus pentosaceus* N2, isolated from fermented beef and infant faeces, respectively, exhibited antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Escherichia coli* ATCC25922, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Salmonella typhi*. The activity was not due to H_2O_2 as similar inhibition was obtained by supernatants produced under aerobic or anaerobic growth conditions. The active ingredients were identified as bacteriocins of molecular mass of 3-4 kDa. The two bacteriocins were sensitive to proteinase-k and pepsin but were not affected by α-amylase, and experienced little reduction in activity on heating to 100˚C for 30min but were destroyed by autoclaving. Both bacteriocins exhibited highest inhibitory activity at pH 5.0. The bacteriocin from *Lb. curvatus* M3 was bactericidal while that from N2 was bacteriostatic to *Staph. aureus*. Kinetics of growth and bacteriocin production showed that maximal inhibitory activity coincided with the phase of optimal growth and with a drop in the pH of the growth medium to 4.2-4.5.

Keywords: *Lactobacillus curvatus*, *Pediococcus pentosaceus*, Bacteriocins

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Introduction

Food consumers are nowadays concerned about the synthetic chemicals used as preservatives in food, with a noticeable inclination toward less processed foods, but it has to be remembered that untreated foods can harbor dangerous pathogens [1].

One of the oldest food processing techniques known to man is food fermentation. Since the dawn of civilization, methods for the fermentation of milk, meat and vegetables have been known [2]. Fermentations resulted in the development of foods with good keeping qualities and organoleptically desirable characteristics. The fermentation processes were generally artisan in nature, unaware of the role of microorganisms [3].

By the middle of the 19th century, the roles of the main microbial groups responsible for these fermentations, such as the Lactic Acid Bacteria (LAB), were being understood, and their activities started to be controlled and manipulated. Although fermentations originally aimed at preserving the fermented food items, other attributes of the process, such as imparting of unique flavors, textures and aromas, and enhancement of the keeping quality and microbiological safety, have gained prominence [3].

LAB have an important role in the inhibition of food-borne pathogenic and spoilage microorganisms such as *Listeria monocytogenes*, *Clostridium* sp and *Staphylococcus* sp [4]. LAB display a wide range of antimicrobial activities, important among which is the production of lactic and acetic acids. Moreover, certain strains of LAB are further known to produce bioactive molecules such as ethanol, formic acid, fatty acids, hydrogen peroxide, diacetyl, reuterin and reutericyclin [5]. In addition, many strains also produce bacteriocins and bacteriocin-like molecules that display antibacterial activity [6].

Screening for bacteriocins during the last few decades has yielded a myriad of bacteriocins with different properties, indicator species and producer organisms [7]. This has prompted researchers worldwide to pursue exploration in this area of natural and safe antimicrobial compounds. The aim of this study was to: (i) screen for the presence of bacteriocion-producing LAB in fermented beef and newborn infant faeces, (ii) characterize the bacteriocins for their heat and pH stability, enzyme action and their inhibitory spectrum against a wide range of bacteria.

Materials and Methods

Isolation and characterization of the bacteriocinogenic bacteria

Two bacterial isolates, coded M3 and N2, were isolated from traditional fermented Sudanese beef (sharmoot) and newborn infant's faeces, respectively. They were subsequently purified by streaking on de Man, Rogosa, Sharpe (MRS) agar [8], and the streaked plates were incubated anaerobically at 30°C for 2-3 days using an anaerobic jar system (GasPak; BBL Microbiology Systems, Cockeysville, Maryland, USA) with a gas-generating kit (BR0038B, Oxoid, Hampshire, UK). The two isolates were first identified to the generic level based on their physiological and biochemical characteristics making use of accepted schemes of classification [9-12]. They were initially

examined for Gram reaction, catalase production, cell morphology using phase contrast microscopy, gas production from glucose, hydrolysis of arginine, growth at pH 4.4 and 9.6, salt tolerance and growth at 10°C and 45°C.

Gas production from glucose was determined in modified MRS broth containing inverted Durham tubes with diammonium citrate replaced by ammonium sulphate [13]. Glucose was sterilized separately (115°C for 10min) and was aseptically added to the medium. Hydrolysis of arginine was tested in MRS broth without glucose or meat extract but containing 0.3% arginine and 0.2% sodium citrate instead of ammonium citrate. Ammonia was detected using Nessler's reagent [14]. Growth at pH 4.4 and 9.6 was tested in MRS broth adjusted to these pH values using 1N HCl. Salt tolerance was tested in MRS broth supplemented with 6.5% NaCl. Growth at different temperatures was observed in MRS broth after incubation for 3 days at 15°C and 45°C.

Ultimately, the two isolates were identified to specific level through biochemical profiling using the KB009 HiCarbohydrate identification kit (HiMedia Laboratories, Mumbai, India), which makes use of the patterns of utilization of 35 sugars.

Screening for antagonistic activity

The antagonistic activities of the two isolates against four indicator bacteria (*Staphylococcus aureus* ATCC43306, *Bacillus subtilis* NCTC8236, *Enterococcus faecalis* ATCC10541 and *Escherichia coli* ATCC25922) were initially screened by the direct spot-on-lawn method [15] which was afterwards confirmed by the agar-well diffusion method [16].

In the spot-on-lawn method, aliquots of about 20µL of 48-h old MRS broth cultures of each isolate were spotted on the surface of MRS agar plates (1.5% agar) and were aerobically incubated at 35°C for 24h. Twenty-four-h old physiological saline-washed cells of indicator bacteria [17] to be tested for sensitivity were inoculated (0.2mL of a 10^5CFU/mL bacterial suspension) into 7.0mL of soft Nutrient Agar medium (0.8% agar). The seeded soft agar was poured as an overlay onto the MRS plates on which the test bacteria had grown for 24h (deferred antagonism). The antagonism was detected by the formation of a growth inhibition halo of the indicator microorganism around bacteriocinogenic test isolates.

In the agar-well diffusion method, cell-free supernatants of M3 and N2 were used to inhibit growth of the indicator bacteria. The cell-free supernatants were prepared following the method of Çadirci and Çitak (2005) [17] in which 24-h old cultures were used to inoculate MRS broth (2%) in test tubes. The inoculated tubes were incubated at 30°C for 24, 48 and 72h without shaking. At the end of the incubation period the broth cultures were centrifuged at 6000g for 15min in a centrifuge (EBA20, Zentrifugen, Tuttlingen, Germany). The cell-free supernatants were carefully decanted, filter-sterilized (pore size 0.22µm, Millipore, Bedford, Mass., USA) and were kept at 4°C for use in the determination of their antagonistic activities against the four indicator bacteria, and for their characterization.

The test for spectrum of inhibition was later extended to cover another six bacteria, namely, *Staph. aureus* ATCC25923, *E. coli* (local isolate), *Pseudomonas aeruginosa* ATCC27853, *Klebsiella pneumoniae* ATCC10031, *Proteus vulgaris* ATCC6380 and *Salmonella typhi* ATCC1319106. To test for antagonism, molten Nutrient Agar (45-48°C) (Oxoid,) medium was first seeded with washed cells of the indicator bacteria, and the inoculated medium was immediately poured into sterile Petri dishes. After solidification, the medium was allowed to dry for at least 30minutes at room temperature. Four wells of uniform diameter (about 5mm) were aseptically bored in the agar using a sterile Pasteur pipette. Fifty µL of the cell-free supernatants of each test isolate were dispensed into each of three wells (replicates), while sterile MRS broth was poured into the fourth well to serve as a control treatment. Plates were left to stand for at least five h at room temperature to allow diffusion of the cell-free extracts. The plates were then incubated at 30°C for 24h. At the end of the incubation period, diameters of the resulting inhibition zones, if any, were measured and the results recorded in mm.

Characterization of the cell-free supernatants

Partial purification of the supernatants: For partial purification of the cell-free supernatants, a modification of the method of Brink et al., [18] was adopted, in which 50mL of *n*-butanol and 5g NaCl were added to 50mL of cell-free supernatant, mixed well and the mixture was centrifuged (6000g) for 10minutes. The *n*-butanol was then evaporated at 45°C and the residue was resuspended in 50mL saline (9g NaCl/L). The partially purified supernatants were used for determination of their characteristics and their antagonistic activities against *Staphylococcus aureus* ATCC43306.

Sensitivity of the partially purified supernatants to enzymes

Sensitivity of the partially purified supernatants to the effects of various enzymes, including proteolytic ones, was conducted as suggested by Barefoot and Klaenhammer (1983) [16]. The partially purified supernatants were adjusted to pH 6.0 with 1N NaOH. One mL of each supernatant was incubated at 37°C for 1h in the presence of the proteolytic enzymes proteinase-K (Vivantis Technologies Sdn Bhd, Revongen Corporation Center, Subang Taya, Selangor DE, Malaysia), pepsin (Sigma Chemical Co., St. Louis, Mo.), and α-amylase (Mühlenchemie GmbH, Ahrensburg, Germany). Enzymes were added to a final concentration of 2.0mg/mL. Enzyme-free supernatants, as well as medium with no supernatant or enzyme were used as controls. Residual antimicrobial activity was assayed by the agar well-diffusion method using *Staphylococcus aureus* ATCC 43306 as the indicator organism.

Heat and pH stability

The effect of heat on the partially purified supernatants was tested, as described by Ivanova et al., [19], by heating the supernatants to 40, 60 and 100°C for 10, 30 and 60min at each temperature in a water bath (No. Y22, Grant Instruments, Cambridge, UK). Moreover, an additional treatment of autoclaving the supernatants at 121°C for 15minutes was included. The residual antagonistic activity was assayed as above. The pH stability was also tested by the method of Ivanova et al., [19]. Supernatants were adjusted to pH 3.0, 5.0, 7.0 and 9.0 with 1M HCl or 1M NaOH. The supernatants were then incubated at 37°C for 4h after which the pH values were re-adjusted to 6.0 for all treatments. Inclusion of the pH values of 7.0 and 9.0 also served for exclusion of the effect of any acids that might be produced by the test isolates. Residual antimicrobial activity was also determined as above.

Production of the inhibitory factor during anaerobic growth

To exclude any possible inhibition due to production of hydrogen peroxide (H_2O_2) by the test isolates, the technique of Lewus et al., [20] was followed. Each isolate was grown under anaerobic conditions for 24 h at 35°C in an anaerobic jar system (GasPak; BBL). At the end

of the incubation period, cell-free supernatants were prepared as described above. Antagonistic activities of these supernatants against the indicator bacterium were assayed by the agar well-diffusion method.

Molecular mass determination

The active peptides in the cell-free filter-sterilized supernatants of the two bacterial isolates were first precipitated by addition of 40% (w/v) ammonium sulfate, centrifuged (6000rpm, 15min), and their pellets and pellicles were concentrated and used for protein separation by Tricine- sodium dodecyl sulfate polyacrylamide gel electrophoresis (Tricine-SDS-PAGE) using 15% acrylamide. The gel electrode assembly was placed in a Mini PROTEAN II electrophoresis chamber (Biorad Laboraties, Hercules, CA), and the proteins were electrophoresed at 100volts for about 2h. A protein molecular mass marker (MoBiTec GmbH, Rastatt Germany) with a molecular weight range of 14.0 to 116.0kDa was included. Staining of the gels was done by covering with Coomassie blue stain overnight. The Coomassie blue stain was composed of 5.75mL glacial acetic acid, 0.157g Coomassie blue dye, 28.5mL methanol and 62.5mL deionized water. The preparations were destained with a buffer composed of 10.0mL glacial acetic acid, 50.0mL methanol and 100mL deionized water.

Mode of action of the partially-purified supernatants

To find out whether the antagonistic effects of the supernatants were bactericidal or bacteriostatic in nature, we followed a procedure similar to that described by Faye et al., [21] and Nilsen et al., [22] in which 4-mL aliquots of the supernatant were added to 20mL of a suspension of physiological-saline-washed 24-h old *Staphylococcus aureus* ATCC43306 cells (ca. 10^6CFU/mL) in Nutrient Broth in a McCartney bottle. *S. aureus* cells were first allowed to grow for four h before addition of the supernatant and their optical densities (OD_{540}) were recorded at hourly intervals. Thenceforth, optical densities were measured at hourly intervals for five more hours. The investigation was terminated by plating aliquots on Nutrient Agar plates and incubating at 35°C for 24h to detect growth.

Kinetics of growth and bacteriocin production

The procedure followed to determine the kinetics of growth and production of the antagonistic activity was similar to that described by Ghrairi et al., [23]. MRS broth (250mL) was inoculated with 1% of an overnight culture of each strain and incubated at 30°C without agitation under uncontrolled pH conditions. Samples were removed at hourly intervals up to 13h, and then at 24, 25 and 26h from start of the investigation. Measurement of biomass by absorbance at 600nm, pH measurement and determination of the antibacterial activity were carried out by assaying the effect of serial two-fold dilutions of partially purified bacteiocins on *Staphylococcus aureus* ATCC 43306 by the well-diffusion method. The antimicrobial titer was expressed in arbitrary units (AU/mL). One arbitrary unit was defined as the reciprocal of the highest dilution showing a clear inhibition zone around the well [24].

Results and Discussion

Spectrum of antibacterial activity

The two bacterial isolates were characterized as follows: Isolate M3 was a Gram-positive, facultatively anaerobic none-spore-forming catalase-negative rod which was identified as *Lactobacillus curvatus*, while isolate N2 was a Gram-positive facultatively anaerobic catalase-negative coccus identified as *Pediococcus pentosaceus*. On initial screening, both *L. curvatus* M3 and *P. pentosaceus* N2 could produce wide inhibition zones against all four indicator bacteria (*Staph. aureus*, *Bacillus subtilis, Enterococcus faecalis* and *E. coli*) by the spot-on-lawn method as well as the agar well-diffusion test. Table 1 shows results of inhibition of the four indicator bacteria by cell-free extracts obtained from the two isolates after incubation for 24, 48 and 72h. Plate 1 depicts zones formed by extracts from *P. pentosaceus* N2 against three of the indicator bacteria.

Screening for the spectrum of inhibitory activity showed that seven of the 10 test bacteria were inhibited by both bacteriocins, but they could not inhibit *Pseudomonas aeruginosa*, *Salmonella typhi* or the local *E. coli* isolate (Table 2). Production by *Lb. curvatus* of different bacteriocins has been reported by many authors. For instance, *Lb. curvatus* LTH1174 produced curvaticin A [25,26], *Lb. curvatus* ACU-1 produced sakacin Q [27], *Lb. curvatus* FS47 produced curvaticin FS47 [28], *Lb. curvatus* L442 produced curvaticin L442 [29], and *Lb. curvatus* CRL705 produced lactocin 705 [30,31]. It has been shown that curvaticin LB65 produced by *Lb. curvatus* was inhibitory to *Listeria monocytogenes*, *Staph. aureus, Enterococcus faecalis*, *Micrococcus leuteus*, *Klebsiella pnumoniae*, *E. coli* and many LAB [32].

Bacteriocins produced by pediococci are known to have a relatively broad spectrum of activity, and are produced by *P. pentosaceus* and *P. acidilactici* [22]. *P. pentosaceus* has been reported to produce a variety of bacteriocins such as pediocin N5p [33], pediocin ST18 [34] and pediocin PA-1 which is produced by *P. pentosaceus* TISTR536 [35]. Bacteriocins from P. pentosaceus have been shown to inhibit many bacterial genera. Pediocin A, produced by Pediococcus pentosaceus FBB61, exhibited inhibition against species of *Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Staphylococcus, Enterococcus, Listeria* and *Clostridium* [36]. It has also been reported that a bacteriocin from Pediococcus pentosaceus ST44AM was active against lactic acid bacteria, *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Listeria innocua, Listeria ivanovii subsp. ivanovii* and *Listeria monocytogenes* [37].

Sensitivity to enzymes

No inhibition of the indicator bacterium (*Staphylococcus aureus)* was produced by the partially-purified supernatants in presence of the protein-digesting enzymes (proteinase-k and pepsin) indicating complete digestion of the inhibitory substance in the supernatants, and asserting its proteinaceous nature; while no reduction in the inhibitory activity was observed in the presence of the carbohydrate-degrading α-amylase. No inhibitory activity was shown in the uninoculated medium containing no enzyme (negative control) (Table 3). The inhibition by supernatants from both isolates was not due to production of H_2O_2 as the diameters of inhibition zones due to supernatants produced during aerobic or anaerobic growth conditions were similar (data not shown). These results are enough evidence of the bacteriocin nature of the active ingredient in these supernatants since bacteriocins are usually defined as ribosomally synthesized proteinaceous compounds released extracellularly by bacteria that can be shown to interfere with the growth of other bacteria, typically including some that are closely related to the producing bacterium and to which the producer cell expresses a degree of specific immunity [38].

Test strain	*Staph. aureus*			*B. subtilis*			*Ent. faecalis*			*E. coli*		
	24h	48h	72h	24h	48h	72h	24h	48h	72h	24h	48h	72h
***L.curvatus* M3**	++	++	++	++	++	++	+	++	+	++	++	++
***P. pentosaceus* N2**	+++	+++	+++	++	++	++	+	+	+	+	+	+

Table 1: Inhibition zones produced against four indicator bacteria by cell-free extracts obtained after 24, 48 and 72h of growth.

+ = 6-12 mm zone diameter; ++ = 13-19 mm; +++ = > 20 mm

Test strain	*Ent.faecalis*	*B. subtilis*	*Staph. aureus*	*Staph. aureus*	*P. aeruginosa*	*K. pneumoniae*	*Proteus vulgaris*	*S. typhi*	*E. coli* ATCC 25922	*E. coli* (local)
***L.curvatus* M3**	13	16	10	15	0.0	9	10	0.0	6	0.0
***P. pentosaceus* N2**	12	14	15	18	0.0	6	11	0.0	10	0.0

Table 2: Spectrum of inhibitory activity against ten indicator tbacteria (zone diameters, mm).

Test strain	Proteinase-K	Pepsin	α -amylase	Enzyme-free supernatants	Medium with no enzyme or supernatant
***L.curvatus* M3**	0.0	0.0	13	13	0.0
***P. pentosaceus* N2**	0.0	0.0	13	14	0.0

Table 3: Effect of enzymes on activity of cell-free supernatants obtained from two LAB isolates against *Staph. aureus* ATCC 43306 (Inhibition zone diameters in mm).

Heat and pH stability

No reduction was observed in the activity of the bacteriocin from of *L. curvatus* M3 on heating to 40°C for 10minutes (Figure 1a), but a reduction of 17.6% was observed on heating for 30 or 60minutes. When the supernatant was heated at 60°C, a reduction of 35.3% was observed whether heated for 10, 30 or 60minutes. On heating to 100°C, a reduction of 17.6% was observed after 10minutes, with further reduction on extending the heating period to 30 or 60minutes. On the other hand, no reduction in activity was observed on heating the bacteriocin from *P. pentosaceus* N2 to 40, 60 or 100°C for 10 or 30minutes, but on heating at these temperatures to 60minutes, a reduction of 54% was recorded, and no activity was detected in autoclaved supernatant even after just 10 minutes (Figure 1b). It was found that the bacteriocin produced by *Pediococus pentosaceus* (VTCC-B-601) was stable as even heating at 100°C, for 30minutes or autoclaving at 121°C for 15minutes [39].

As for the effect of pH on stability, *L. curvatus* bacteriocin showed highest activity at pH 5.0 and a decline at pH 3.0, 7.0 and 9.0. However, the reduction in inhibitory activity was not great, amounting only to 16.7% at pH 9.0 (Figure 2). The highest inhibitory activity by the *P. pentosaceus* bacteriocin was recorded at pH 5.0, decreasing slightly at pH 7.0 and pH 3.0, but dropping sharply at pH 9.0 (a decrease of 41% from that at pH 5.0) as shown at figure 2. Temperature and pH have been shown to have a significant effect on bacteriocin [40]. A bacteriocin from *L. acidophilus* could withstand heating at 75°C for 15 min [41]. Moreover, lactocin RN 78 produced by *L. casei* RN 78 withstood heating up to 121°C for °C 15min [42]. The bacteriocin of *L. acidophilus* NCIM5426 was found to be heat-stable (12°C for 15min) and active over a wide pH range of 4.0-10.0. It showed stability (60%) for 30 days at room temperature. Heat and pH stability are important characteristics that would allow bacteriocins to act over a wide range of environmental conditions.

Molecular weight determination

Plate 2 shows the bands produced by the electrophoretic separation of the two bacteriocins in comparison to a marker of standard protein molecular weights. The molecular weights of both proteins were in the approximate range of 3 - 4 kDa. Similar molecular masses have been reported for curvacin A (4.3 kDa) from *Lactobacillus curvatus* LTH 1174 isolated from fermented sausage [43], curvaticin FS47 (4.07 kDa) from *Lactobacillus curvatus* FS47 isolated from ground beef [28], curvaticin L442 (4.5kDa) from *Lactobacillus curvatus* L442, isolated from Greek traditional fermented sausage [44] and curvaticin LB65 from *Lactobacillus curvatus* LB65 [32].

Interestingly, a very wide variation is seen in the molecular weight of the inhibitory substances produced by pediococci. For instance, pediocin A, produced by *P. pentosaceus* FBB61 had a molecular weight of 80 kDa [36], while *P. pentosaceus* K34 produced a bacteriocin (bacPPK34) of molecular weight of 2.5- 6.2 kDa [45], and *P. pentosaceus* IE-3 produced a non-bacteriocin chemical peptide of only 1.7 kDa [46]. Moreover, *P. pentosaceus* T1 produced an anti-listerial substance with an active fraction of molecular weight of 23 kDa [15]. The latter are more likely Bacteriocin-Like Substances (BLIS).

Mode of action of the two bacteriocins

A strong inhibition of the growth of *Staphylococcus aureus* ATCC 43306, already growing for four h, was obtained due to addition of the *Lactobacillus curvatus* M3 bacteriocin, where an increase of only 10% in optical density was obtained 5hours after addition of the bacteriocin as compared to an increase of 290% in the untreated control broth (Figure 3a). On re-culturing of the treated broth culture on fresh medium, no growth was obtained indicating bactericidal activity. It has been reported that curvaticin from *Lactobacillus curvatus* was bactericidal [32,47]. On the other hand, on addition of the *Pediococcus pentosaceus* N2 bacteriocin, growth of the indicator bacterium was greatly retarded. In the following five hours of incubation, an increase of only 45.6% in the optical density of the broth treated with the bacteriocin was recorded, as compared to an increase of 255.5% in the untreated broth (Figure 3b). However, on streaking of aliquots from the treated broth culture on fresh Nutrient Agar plates, growth was resumed after 24hours of incubation at 30°C indicating bacteriostatic activity. A similar finding on the bacteriostatic nature of pediocin has been reported [48].

Production kinetics

Figure 4a depicts the growth and bacteriocin production of *L. curvatus* M3 which showed an early increase in growth that continued up to 24h from inoculation, after which a gradual decline

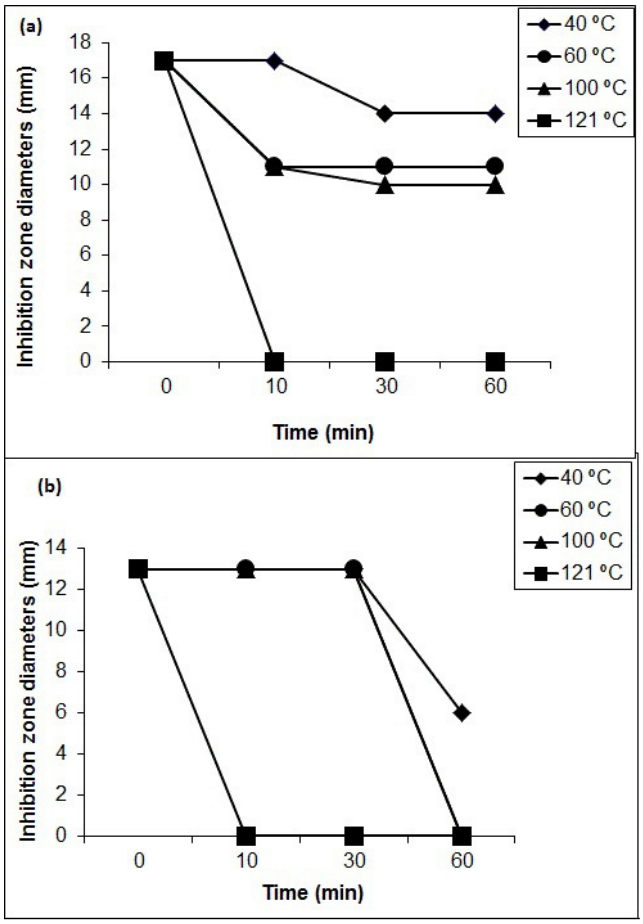

Figure 1: Effect of heating on activity of *Lb. curvatus* M3 bacteriocin.

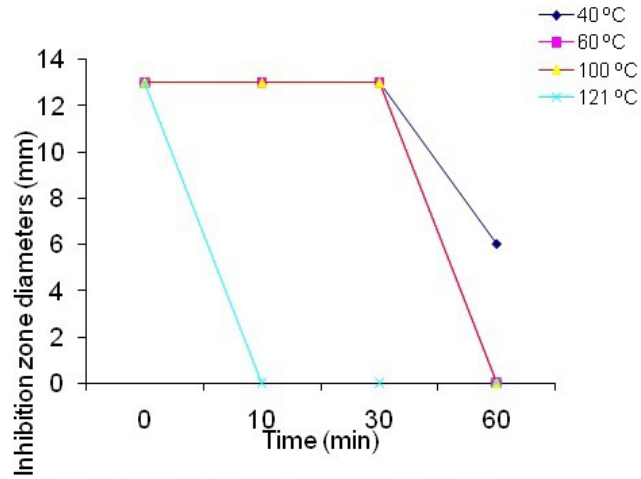

Figure 2: Effect of heating on activity of *P. pentosaceus* N2 bacteriocin.

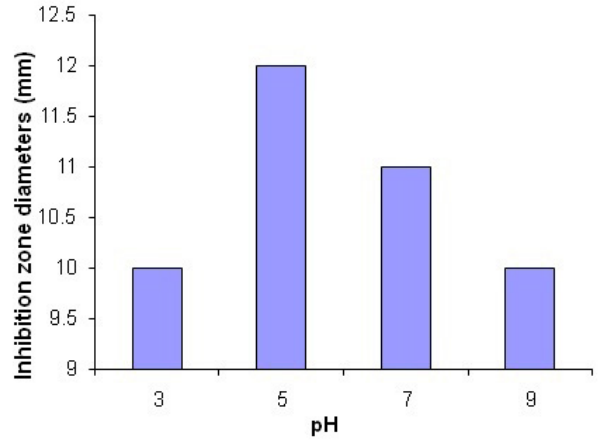

Figure 3a: Effect of pH on activity of bacteriocin from *Lb. curvatus* M3.

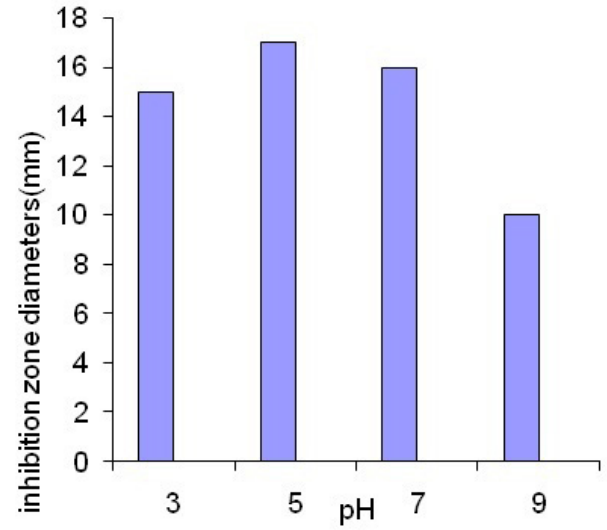

Figure 3b: Effect of pH on activity of bacteriocin from *P. pentosaceus* N2.

could be seen. The inhibitory activity could be detected as early as sixh from inoculation, with a gradual increase up to 12h, after which a steady state in activity was obtained with no decline up to 26h. A similar trend in bacteriocin production by *L. curvatus* LB65 has been reported [32], but the inhibitory activity declined within 24h. Maximum growth and activity were obtained at the pH value of 4.2 (Figure 4b).

Figure 5 shows that increase in growth of *P. pentosaceus* N2 was detectable since the start of the investigation, but the active growth phase seemed to start after 8h from inoculation and continued up to the 25th hour after which it started to decline. On the other hand, no antagonistic activity could be detected against *Staph. aureus* up to eight h of growth. Thenceforth, antagonistic activity began to be detected, and showed a steady increase up to 26h of growth when the investigation was terminated. Maximal activity appeared to coincide with optimal growth, but also coincided with the drop in pH to the range of 4.5 to 4.2 cell growth and bacteriocin production are

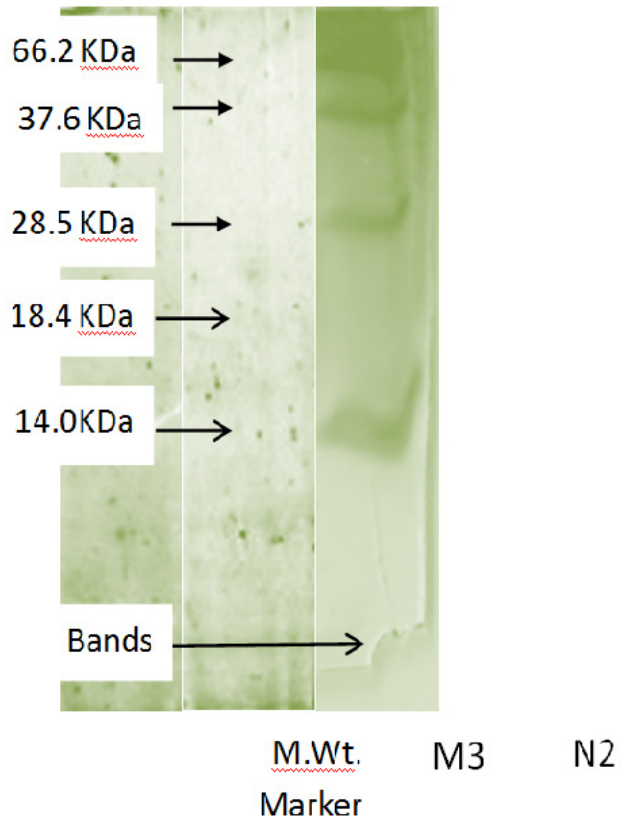

Figure 4a: Gel electrophoresis (SDS-PAGE) of the inhibitory moieties of *L. curvatus* M3 and *P. pentosaceus* N2 stained with Coomassie blue.

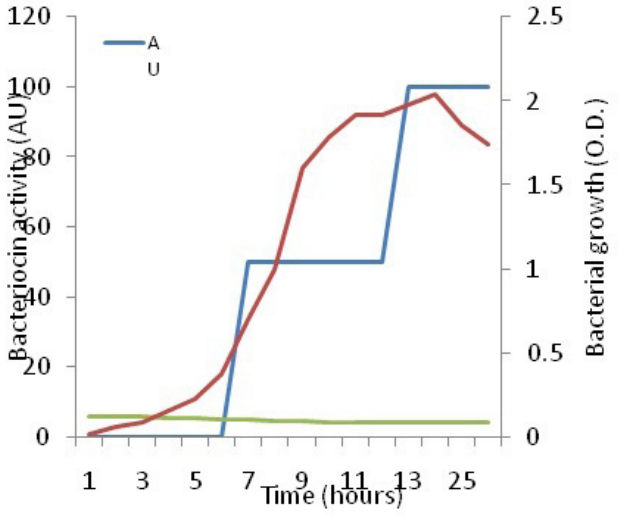

Figure 4b: Kinetics of growth and bacteriocin production by *Lb. curvatus* M3.

influenced by temperature and pH as has been shown by many authors [49]. These authors showed that the highest bacteriocin activity occurred at a low constant temperature of 28°C and a constant pH of 5.4. The present results are in line with the finding that bacteriocin production is stimulated under unfavorable growth conditions such as low pH [13].

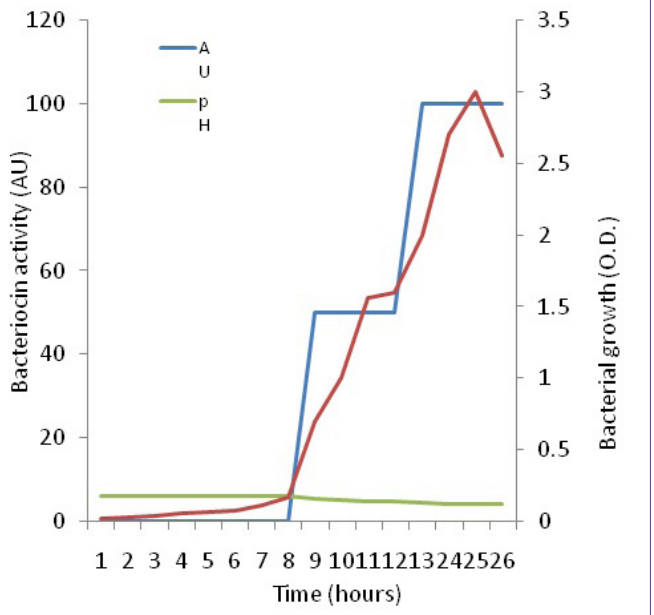

Figure 5: Kinetics of growth and bacteriocin production by P. pentosaceus N2.

Conclusion

This study determined the bacteriocinogenic capabilities of *Lactobacillus curvatus* M3 and *Pediococcus pentosaceus* N2 isolated from traditional fermented Sudanese beef (sharmoot) and newborn infant's faeces, respectively. Results of the study indicated the possible food preservation (M3) and clinical (N2) benefits of the two bacteria, and their possible utilization in these two aspect.

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