Turkish Journal of Biology

Volume 22 | Number 2 Article 10

1-1-1998

Purification and Characterization of Pediocin F, ABacteriocin Produced By Pediococcus acidilacticiF

Özlem OSMANAGAOĞLU

Ufuk GÜNDÜZ

Yavuz BEYATLI

Cumhur ÇÖKMÜŞ

Tr. J. of Biology
22 (1998) 217-228

Purification and Characterization of Pediocin F, A Bacteriocin Produced By *Pediococcus acidilactici* F

Özlem OSMANAĞAOĞLU
Gazi University, Faculty of Arts and Science, Department of Biology Teknikokullar, 0650 Ankara -TURKEY

Ufuk GÜNDÜZ
Middle East Technical University, Faculty of Arts and Science, Department of Biology, Ankara-TURKEY

Yavuz BEYATLI
Gazi University, Faculty of Arts and Science, Department of Biology, Teknikokullar, 06500 Ankara-TURKEY

Cumhur ÇÖKMÜŞ
Ankara University, Faculty of Science, Department of Biology, 06100 Ankara-TURKEY

Received: 15.04.1997

Abstract: In this work, *Pediococcus acidilactici* F isolated from fermented sausage was reported to produce an extracellular antimicrobial peptide, termed Pediocin F, that was protein in nature and effective against many bacteria associated with food spoilage and food related health hazards. Pediocin F is reported here to be sensitive to proteolytic enzymes, resistant to heat and organic solvents, and active over a wide range of pH. We have also reported that the extracellular antimicrobial protein produced by *P. acidilactici* F was really a small peptide. Coomassie-blue stained SDS-PAGE technique allowed us to estimate the molecular weight of this small peptide to be about 4460 dalton.

Key Words: *Pediococcus acidilactici*, Pediocin, characterization, SDS-PAGE

Pediococcus acidilactici F Tarafından Üretilen Pediocin F' in Saflaştırılıp Karakterize Edilmesi

Özet: Bu çalışmada fermente sosisden izole edilen *Pediococcus acidilactici* F' in protein yapısında olan, gıdalarla ilgili sağlığı tehtit eden ve gıdaların bozulmasına sebep olan bir çok bakteriye karşı Pediocin F olarak adlandırılan hücre dışı bir antimikrobiyal peptit ürettiği rapor edilmiştir. Pediocin F, proteolitik enzimlere karşı hassas, ısıya ve organik çözücülere karşı dayanıklı ve geniş bir pH skalasında aktiftirler. Coomassie-blue ile boyanmış SDS-PAGE tekniği bakterinin antimikrobiyal etkisi ile ilgili protein bandının tanımlanması amacı ile kullanılmış ve bu çalışma sonucunda *acidilactic*i F tarafından üretilen ve Pediocin F olarak adlandırılan bu proteinin 4460 dalton moleküler ağırlığına sahip oldukça küçük bir peptit olduğu bulunmuştur.

Anahtar Sözcükler: *Pediococcus acidilactici*, Pediocin, karakterizasyon, SDS-PAGE

Introduction

Several species and strains of Pediococci have been used as starter cultures in the fermentation of vegetables (26), meats (9,28), sausage products (29) and cheddar cheese (11). Pediococci are lactic acid bacteria which help to preserve fermented foods and contribute in flavor development (11, 22). Bacteriocins from Pediococcus species, designated as Pediocins, were shown to be protein in nature and inhibitory to several other bacteria, many of which are associated with food spoilage and health hazards of food origin (4, 7, 10, 12, 13, 15, 19, 24, 27, 32). Several unique properties such as activity over a wide pH range and after high- and low-temperature treatment make them suitable as biological preservatives to extend the shelf-life of refrigerated semipreserved foods and canned foods (1, 21, 23). Some of these studies reported antimicrobial activity against different bacterial species, but little is known about the chemical and physical nature of these antimicrobial substrances. Gonzalez and Kunka (12) reported that *P. acidilactici* PAC 1.0 produced a bacteriocin, Pediocin PA1, which has a moleculer weight of 16500 daltons determined by gel-filtration and proteinaceous in nature as indicated by sensitivity to various proteolytic enzymes. Bhunia *et al.* (4) reported the proteinaceous nature of Pediocin AcH produced by *P. acidilactici* strain H and have also reported that this antimicrobial substance was effective against many bacteria associated with food spoilage and food related health hazards. They have also reported the molecular weight of this extracellular antimicrobial protein to be about 2700 dalton on SDS-PAGE gel. Recently, the molecular weight of Pediocin AcH, as determined by the amino acid composition and sequence, was reported to be 4628 Dalton (25). In this study, a new heat-stable Pediocin F, produced by a sausage isolate of *P. acidilactici* F, was purified and characterized.

Materials and Methods

Bacterial Cultures and Media:

The bacterial strains used in this study are shown in Table 1. Cultures are maintained at -70 °C as frozen stock cultures in equal volumes of 10% non-fat dry milk and 25% glycerol. All lactic acid bacteria were grown in TGE (trypticase glucose yeast extract) broth (3) whereas non lactic acid bacteria were grown in TS (tryptic soy) broth (5) supplemented with 0.5 % yeast extract at 1 % level and propagated at least twice before use. *Pediococcus acidilactici* F, (PAF 1.0) which has been isolated from fermented sausage used for production of Pediocin F and the cultures were grown at 37 °C for 20 hr in TGE broth. As necessary, 1.5 % agar was added to TGE broth to make a solid medium. The soft agar, used for overlaying TGE hard agar in some studies, was prepared by adding 0.75 % agar to the broth.

Antimicrobial Activity Assay of Bacteriocin:

An aliquot of a test culture broth was heated in boiling water for 15 min and serially diluted (1:10 to 1:250), and 5µl from each dilution was spotted onto a lawn of *Lactobacillus plantarum* NcDO 955 in an assay plate. The assay plate had a bottom layer of TGE hard agar and a top layer of TGE soft agar seeded with about 10^6 *L. plantarum* NcDO 955 cells. The plates were incubated at 37 °C for overnight, and the highest dilution that produced a distinct zone was mul-

tiplied by 200 (1ml/5µl) to obtain the activity units per milliliter.

Table 1. Bacterial strains and growth conditions

Strain	Growth medium	Growth conditions
P. acidilactici H (LB42-923)	37 °C, 20 hr	TGE
P. acidilactici F	37 °C, 20 hr	TGE
L. plantarum NcDO 955	30 °C, 18 hr	TGE
L.. mesenteroides Ly	30 °C, 18 hr	TGE
E. feacalis MB1	30 °C, 18 hr	TGE
P. acidilactic Ped L	37 °C, 20 hr	TGE
L. lactis ssp lactis OZ1	30 °C, 24 hr	TGE
L. innocua	30 °C, 18 hr	TGE
L. innovaii	30 °C, 18 hr	TGE
B. cereus	20 °C, 24 hr	TS
B. subtilis	20 °C, 24 hr	TS
S. aureus	20 °C, 24 hr	TS
E. coli	20 °C, 24 hr	TS

Extraction and Purification of Bacteriocin:

For extraction and purification of Pediocin F, a novel method of Yang *et al* (31) was applied. *Pediococcus acidilactici* F was grown to early stationary phase in TGE broth. Broth culture was heated at 72 °C for 30 minutes to kill the cells after its pH adjusted to 6.5. The cells were harvested by centrifugation at 15000 rpm for 15 minutes. After the cells had been washed with 5mM sodium phosphate (pH 6.5), the cells were then resuspended in milli-Q-dH_2O, and 0.1 M NaCl was added and the pH of the mixture was adjusted to 2.0. The mixture was kept at 4 °C for 2 hr by mixing with stirrer. Cell suspensions were then centrifuged at 29000 rpm for 20 minutes, and the supernatants were dialyzed in 1000 MW cut-off spectra/for dialysis membrane against dH_2O at 4 °C for 24 hr and then freeze-dried.

Trypsin treatment of Pediocin F for SDS-PAGE:

The trypsin treatment was done as follows. 100ml 8mM phosphate buffer, pH 7.0, containing 20µg of trypsin (T-8128, Sigma) was added to 100µl of dialysate (material inside the dialysis membrane), and the mixture was incubated at 37 °C for 1 hr and treated as mentioned before.

Table 2. Effect of enzymes on antimicrobial activity of Pediocin F produced by *Pediococcus acidilactici* F

Treatment	Activity*
Catalase	+
Chymotrypsin	-
DNase	+
Ficin	-
Lipase	+
Lysozyme	+
Papain	-
Protease IV	-
Protease XIV	-
Protease XXIV	-
Protease K	-
Ribonuclease A	+
Trypsin	-

*Disc assay method using lactobacillus plantarum NcDO 955 as an indicator organismm;

+, presence; -, loss of activity

SDS-PAGE and Identification of the Activity Band:

Freeze-dried preparation was analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The method of Bhunia *et al* (4) used 10 cm layer of 16.5 % acrylamide resolving gel and a 2 cm layer of 10 % acrylamide spacer gel. Moleculer Weight standart (Novex, Mark 12), Pediocin AcH, Pediocin F and Pediocin F treated with trypsin were dissolved (1 mg/ml) in sample buffer (4.6 % SDS, 10% ß-mercaptoethanol, 20 % glycerol, 1.5 % Tris, 1% bromophenol blue, pH 6.8). Two parallel SDS- PAGE gel were run under same conditions and electrophoresis was performed at 40 mA for approximately 2 hr and then 80 mA for another 2 hr. At the end of electrophoresis, one gel containing the Molecular weight standart and Pediocin F was stained with coomassie brilliant blue and the other gel containing Pediocin F and Pediocin F treated with trypsin was used for identification of the bacteriocin band(s) by using growth inhibition of *L. plantarum* NcDO 955. For growth inhibition, the gel was fixed immediately for 2 hr in solution of 20 % isopropanol and 10% acetic acid and washed in dH_2O for overnight at room temperature. This gel was then placed on a TGE agar prepoured plate, and overlaid with TGE soft agar seeded with *L.plantarum* NcDO 955. The plate was incubated at 30 °C for 20 hr, examined for the location of the zone of growth of inhibition by comparing with the bands in the stained gel and photographed. The other stained gel was destained and photographed.

Effect of heat treatment on antimicrobial activity:

For thermal stability determination of Pediocin F, 2 ml (10mg/ml) of freeze-dried Pediocin F preparation was boiled for 15 minutes. Another sample was autoclaved (121 °C) for 15 minutes, and all samples were cooled and assayed for activity.

Effect of enzymes on antimicrobial activity:

To determine the sensitivity of Pediocin F to proteolytic and other enzymes, the enzymes listed in Table 2 were dissolved in sterile 4 mM/l phosphate buffer, pH 7.0, at a concentration of 200 µg/ml. Freeze-dried Pediocin F was added to the enzyme solutions at a concentration of 10 mg/ml and the samples were incbated at 37 °C for 1 hr. The activity of the sample was then determined by disc assay method (4)

Effect of pH on antimicrobial activity:

A freeze-dried Pediocin F preparation was dissolved in deionized water at a concentration of 50 mg/ml. Samples from this were adjusted with sterile 10 mM/l NaOH or 10 mM/l HCl to different pH levels between 3 to 12. Samples were maintained for: (1) 2 hr at 25 °C; (2) 24 hr at 25 °C, or (3) 20 min at 100 °C. The samples were then adjusted to pH 7.0 with sterile 4 mM/l phosphate buffer and assayed for activity.

Effect of organic solvents on antimicrobial activity:

Some of the organic solvents listed in Table 4 were used to show the sensitivity of Pediocin F to them. Freeze-dried pediocin F preparations were dissolved in organic solvents at a concentration of 10 mg/ml. The samples were incubated at 25 °C for 1 hr and the solvents were evaporated in a centrifugal concentrator. Dried samples were then dissolved in sterile deionized water to a concentration of 10 mg/ml and assayed for activity.

Table 3. Effect of pH on antimicrobial activity of pediocin F produced by Pediococcus acidilactic F

	Activity* after		
pH	2 hr at 25 °C	24 hr at 25 °C	15 min at 121 °C
3.0	+	+	+
4.0	+	+	+
5.0	+	+	+
6.0	+	+	+
7.0	+	+	+
8.0	+	+	+
9.0	+	+	+
10.0	+	-	-
12.0	-	-	-

*Disc assay method using Lactobacillus plantarum NcDO 955 as an indicator organism;

+, presence, -, loss of activity

Table 4. Effect of heat and organic solvents on antimicrobial activity of Pediocin F produced by *Pediococcus acidilactic*i F

Treatment	Activity*
Acetone	+
Acetonitrile	+
Chloroform	+
Ethylalcohol	+
Formaldehyde	+
Hexane	+
Isopropanol	+
121 °C/15 min	+
93 °C/15 min	+

*Disc assay method using Lactobacillus plantarum NcDO 955 as an indicator organism;

+, presence; -, loss of activity

Spectrum of antimicrobial activity:

The disc assay method (4) was used to determine the antimicrobial activity of Pediocin F against several cells of gram positive bacteria of many genera that include nonpathogenic species and strains of *Lactobacillus, Lactococcus, Leuconostocs, Pediococcus, Enterococcus* and *Listeria* species other than *L. monocytogenes*, many of which are associated with spoilage of meat and dairy products as well as pathogenic bacteria (Table 5). Incubation of the plates was at the optimum temperature for the particular indicator organism.

Results and Discussion

The bacteriocins produced by different species and strains of lactic acid bacteria have potential uses as biological food preservatives. In order to use them in the most effective ways, it will be important to obtain relatively large quantities of these peptides in a pure and concentrated from, and determine their physical and chemical characteristics as well as mode of inhibitory effect against food spoilage and food borne bacteria. For extraction and purification of Pediocin F, a novel purification method of Yang *et al* (31) was used. This method was based on pH dependancy of bacteriocins to adsorb to and release from the cell surface of gram positive bacteria and produces dry preparations of bacteriocins with high potency and in a concentrated and pure form. As compared to ammonium sulphate precipitation of the bacteriocins from cell-free culture liquor which does not yield a good product because many other proteins from the medium can also be precipitated and the yield is not very high; for extraction and purification of lactic

acid bacteria, the total loss in the novel method of Yang *et al* is quite low and it is an ecomonical procedure to produce large quantities of bacteriocins from lactic acid bacteria to be used as food biopreservatives.

Table 5. Sensitivity of indicator strains to Pediocin F produced by *Pediococcus acidilactici* F

Bacterial Strains	Sensitivity to Pediocin F
L. plantarum NcDO 955	+
L. mesenteroides Ly1	+
E. feacalis MB1	+
P. acidilactici Ped L	+
L. innocua	+
L. innovaii	+
L lactis ssp. lactis OZ1	+
B. cereus	+
B. subtilis	+
E. coli	+
S. aureus	+

Sensitivity (+) to Pediocin F was determined from the presence or absence of zone of growth inhibition against 5µl of each preparation by disc assay method.

One characteristic of classical bacteriocins is a narrow spectrum of activity (29). Well-defined bacteriocins produced by lactobacilli usually have inhibitory activities restricted to closely related species (2, 18, 20, 30). On the other hand, bacteriocins produced by Pediococcus spp. appear to have a relatively broad spectrum of activity, that include non-lactobacillaceae gram positive bacterial species (7, 15).

Pediococcus acidilactici F isolated from fermented sausage produced an antimicrobial peptide so called Pediocin F which were protein in nature and inhibitory to a variety of spoilage and pathogenic microorganisms as well as lactic acid bacteria often encountered in foods. These include non-pathogenic species and strains of *Lactobacillus, Lactococcus, Leuconostocs, Enterococcus, Pediococcus* and *Listeria* species other than *L. monocytogenes,* many of which are associated with spoilage of meat and dairy products and pathogenic strains of *Bacillus cereus, Bacillus subtilis, Escherichia coli*, and *Staphylococcus aureus*, and by disc assay method of Bhunia *et al*, (4) purified Pediocin F was found to be highly active against these bacteria. The antimicrobial activity of *P. acidilactici* F reported here was not due to organic acids, hydrogen peroxide or bacteriophage as the cell-free media remained active following neutralization to pH 7.0, treatments with catalase and DNase or autoclaving. The loss of antimicrobial activity following treatment

with proteolytic enzymes indicated that the active component secreted extracellularly by *P. acidilactici* F was proteinaceous.

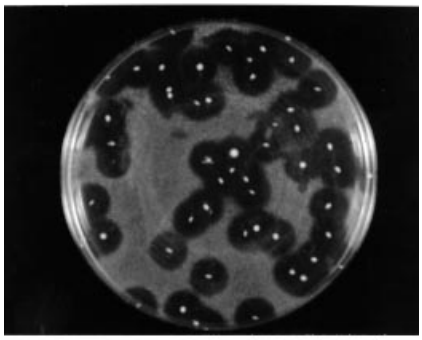

Figure 1. Pediocin F of *Pediococcus acidilactici* F colonies produced zone of growth inhibition on a lawn of *Lactobacillus plantarum* NcDO 955 used as indicator

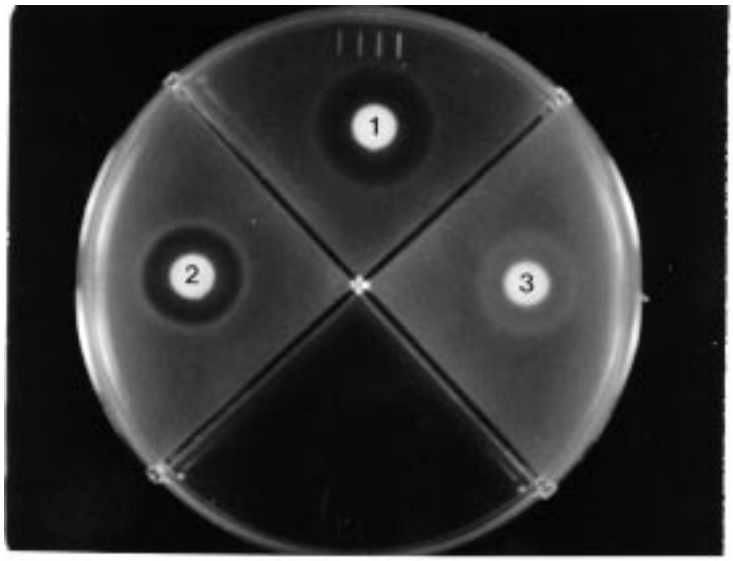

Figure 2. Disc assay of Antimicrobial activity of antimicrobial peptide from *P. acidilactici* F against *L. plantarum* NcDO 955 (1) untreated, (2) Pediocin F after heating at 121 °C for 15 min, (3) Pediocin F after treatment with trypsin

Treatment with protease destroyed activity indicating that the antimicrobial compounds could be heat stable proteins and most probably bacteriocins (29). Heat treatment at 93 °C and 121 °C for 15 min did not destroy its activity. However, prior treatment of the peptide with trypsin caused loss of activity. Treatment with lipase and organic solvents did not cause any loss of activ-

ity, probably because of the absence of lipid moieties in the molecule. Purified Pediocin F was found to be stable over a wide pH range between 3 and 9. Activity was lost at pH 10 and above after 24 hr at 25 °C and heat treatment (Table 3). The loss of activity at higher pH could be due to degradation of the molecule. This is similar to the properties reported for bacteriocins produced by other lactic acid bacteria such as Pediocin AcH (4); Pediocin A (7); Pediocin PA-1 (12); Lactacin (2); Lactacin 27 (30); Acidolin (14); Nisin (1, 16, 17) and Diplococcin (8).

In SDS-PAGE electrophoresis, Pediocin F preparation showed only 3 major bands after coomassie-blue staining (Figure 3). In the gel that was used to determine the antibacterial property (Figure 4), the zone of growth inhibition of *Lactobacillus plantarum* NcDO 955 correspond with the band above the lowermost band in the stained gel indicating that the band with a molecular weight of 4460 dalton contained the antimicrobial activity whereas the other protein bands did not show any activity. The antimicrobial activity was absent in the Pediocin F sample treated with trypsin. There are reports (4) that indicate the molecular weight of Pediocins with different methods. The molecular weight of these proteins differed greatly, about 2700 dalton in strain H (4) and about 16599 dalton in PAC1.0 (12). Our results are almost similar to that of Bhunia *et al.* (4). The differences in results could be due to differences in the techniques used since Gonzalez and Kunka (12) used gel filtration and may have encountered aggregation of the peptides. Çökmüş and Yousten (6) determined the bacteriocin production of mosquito pathogenic strains of *Bacillus sphaericus* in nondenaturing 6% polyacrylamide gels (PAGE) by overlaying gels with NY (Nutrient Broth Yeast Extract) soft agar seeded with the indicator bacterial strains. Our work is currently in progress to determine the mode of action of Pediocin F.

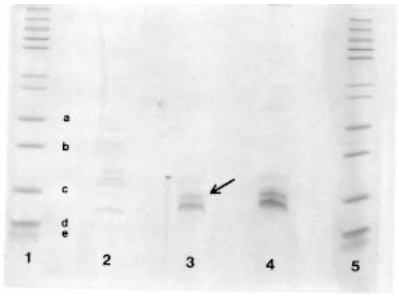

Figure 3. Coomassie-Blue stained SDS-PAGE gel I: lane 1 and 5 contain molecular weight standart a:21.5 KDa, b: 14.4 KDa, c: 6.0KDa, d: 3.5KDa, e: 2.5KDa. Lane 2 contains Pediocin AcH (10µl) produced by *P. acidilactici* strain H, obtained from Dr. Bibek Ray, University of Wyoming, lane 3 and 4 contain Pediocin F in different amounts; 10.0µl and 15.0µl respectively

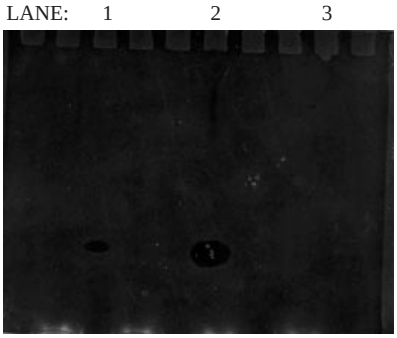

Figure 4. SDS-PAGE gel II: overlaid with indicator bacteria to determine which band corresponds to the antimicrobial activity. Lane 1 contains control Pediocin AcH (from Dr. Bibek Ray), lane 2 contains Pediocin F, showing the zone of growth inhibition corresponding the band molecular weight of 4460 dalton. Other bands did not show antimicrobial activity. Lane 3 contains trypsin treated Pediocin F and showed no growth inhibition zone

Acknowledgements:

The studies reported here were supported by a NATO-A2 type schoolarship from TUBİTAK, the scientific and technical research of council of Turkey. The authors gratefully acknowledge Dr. Bibek Ray and Dr. Kurt Miller of University of Wyoming-Laboratory of Food Microbiology and Laboratory of Molecular Biology respectively, for their guidance in the theoritical field, their assistance, general support and creative discussions. We also thank Yanling Chen for her advises in purification and SDS-PAGE techniques.

References

1. Bailey, F. J., and Hurst, A., Preparation of a highly active from of nisin form Streptococcus lactis. Canadian Journal of Microbiology, 17: 61-67, 1971.
2. Barefoot, S. F., and Klaenhammer, T. R., Detection and activity of Lactacin B, a bacteriocin produced by Lactobacillus acidophilus. Applied and Enviromental Microbiology, 45: 1808-1815, 1983.
3. Biswas, S. R., Ray, P., Johnson, M. C., and Ray, B., Influence of growth conditions on the production of a bacteriocin, pediocin AcH, by Pediococcus acidilactici H. Applied and Enviromental Microbiology, 57: 1265-1267, 1991.
4. Bhunia, A. K., Johnson, M. C., and Ray, B., Direct detection of an antimicrobial peptide of Pediococcus acidilactici in sodium dodecyl sulfate polyacrylamide gel electrophoresis. Journal of Industrial Microbiology, 2: 319-322, 1987.
5. Bhunia, A. K., Johnson, M. C., and Ray, B., and Kalchayanad, N., Mode of action of pediocin AcH, a bacteriocin from Pediococcus acidilactici. Journal of Applied microbiology, 70: 25-33, 1991.
6. Çökmüş, C., and Yousten, A. A., Bacteriocin production by Bacillus sphaericus. Journal of Invertebrate Pathology, 61: 323-325, 1993.

7. Daeschel, M. A., and Klaenhammer, T. R., Association of 13.6 megadalton plasmid in Pediococcus pentosaseus with bacteriocin activity. applied and Enviromental Microbiology, 50: 1538-1541, 1985.

8. Davey, G. P., and Richardson, B. C., Purification and some properties of diplococcin from Streptococcus cremoris 346. Applied and Enviromental Microbiology, 41: 84-89, 1981.

9. Diebel, R. H., Wilson, G. D., and Niven, C. F., Microbiology of meat curing. IV A lyophilized Pediococcus cerevisiae starter culture for fermented sausage. Applied Microbiology, 9: 239-243, 1961.

10. Fleming, H. P., Etchells, J. L., and Costilow, R. N., Microbial inhibition by an isolate of Pediococcus from cucumber brines. Applied Microbiology, 30: 1040-1043, 1975.

11. Franklin, J. G., and Sharpe, M. E., The incidence of bacteria in cheese milk and cheddar cheese and their association with flavor. Journal of Dairy Research, 30: 87-99, 1963.

12. Gonzalez, C. F., and Kunka, B. S., Plasmid-associated bacteriocin production and sucrose fermentation in Pediococcus acidilatici. Applied and Enviromental Microbiology, 50: 532-534, 1985.

13. Graham, D. C., and McKay, L. L., Plasmid DNA in strains of Pediococcus cerevisia and Pediococcus pentosaceus. Applied and Enviromental Microbiology, 50: 532-534, 1985.

14. Hamden, I. Y., and Mikolajcik, E. M., Acidolin: an antibiotic produced by Lactobacillus acidophilus. Journal of Antibiotics, 27: 631-636, 1974.

15. Hoover, D. G., Walsh, M., Kolaetis, K. M., and Daly, M. M. A bacteriocin produced by Pediococcus spp. associated with a 5.5 Md plasmid. Journal of Food Protection, 51: 29-31, 1988.

16. Hurst, A., Biosynthesis of the antibiotic nisin by whole Streptococcus lactis organism. Journal of general Microbiology, 44: 209-220, 1966.

17. Hurst, A., Function of nisin and nisin-like basic proteins in the growth cycle of Streptococcus lactis. Nature, 214: 1232-1234, 1967.

18. Joerger, M. C., and Klaenhammer, T. R., Characterization and purification of helveticin J and evidence for a chromosomally determined bacteriocin produced by Lactobacillus helveticus 481. Journal of Bacteriology, 167: 439-446, 1986.

19. Juven, B. J., Meinersmann, R. J., and Stern, N. J., Antagonistic effect of Lactobacilli and Pediococci to control intestinal colonization by human enteropathogen in live poultry. Journal of Applied Bacteriolgy, 70: 95-103, 1991.

20. Juven, B. J., Schved, F., and Lindner, P., Antagonistic compound produced by a chicken intestinal strain of Lactobacillus acidophilus. Journal of Food Protection, 55: 157-161, 1992.

21. Keen, J. H., Preparation and chemical properties of colicin 1. Canadian Journal of Microbiology, 12: 425-426, 1966.

22. Keen, A. R., Walker, N. J., and Peberdy, M. F., The formation of 2-butanone and 2-butanol in cheddar cheese. Journal of Dairy Research, 41: 249-257, 1974.

23. Lowry, O. H., Rosenbrough, N. J., and Randall, R. J., Protein measurement with the folin-phenol reagent. Journal of Biological Chemistry, 193: 265-275, 1951.

24. Motlagh, A. M., Johnson, M. C., and Ray, B., Viability loss of foodborne pathogens by starter culture metabolites. Journal of Food Protection, 54: 873-878, 1991.

25. Motlagh, A. M., Bhunia, A. K., Szostek, F., Hansen, T. R., Johnson, M. C., Ray, B., Nucleotide and amino acid sequence of pap-gene (Pediocin AcH production) in Pediococcus acidilactici H. Letters in Applied Microbiology, 15: 45-48, 1992.

26. Pederson, C. S., The genus Pediococcus. Bacteriological Reviews, 13: 225-232, 1949.

27. Pucci, M. J., Vedanuthu, E. R., Kunka, B. S., and Vanderbergh, P. A., Inhibition of Listeria monocytogenes by using bacteriocin PA-1 produced by Pediococcus acidilactici PAC1.0. Applied and enviromental Microbiology, 54: 2349-2353, 1988.

28. Smith, J. L., and Palumbo, S. A., Microorganisms as food additives. Journal of Food Protection, 44: 936-955, 1981.

29. Tagg, J. R., Dajani, A. S., and Wannamaker, L. W., Bacteriocins of gram-positive bacteria. Bacteriological Reviews, 40: 722-756, 1976.

30. Upreti, G. C., and Hinsdill, R. D., Production and mode of action of Lactocin 27: Bacteriocin from a homofermentative Lactobacillus. Antimicrobial Agents and Chemotherapy, 7: 139-145, 1975.

31. Yang, R., Johnson, M. C., and Ray, B., Novel method to extract large amounts of bacteriocins from lactic acid bacteria. Applied and Enviromental Microbiology, 58: 3355-3359, 1992.

32. Yousef, A. E., Luchansky, J. B., Degnan, A. J., and doyle, M. P., Behavior of Listeria monocytogenes in wiener exudates in the presence of Pediococcus acidilactici H or Pediocin AcH during storage at 4 °C or 25 °C. Applied and Enviromental Microbiology, 57: 1461-1467, 1991.