J. Food Sci. Nutr.
Vol. 1, No. 2, p. 269~277(1996)

Bacteriocins: Assay, Biochemistry, and Mode of Action

-Review-

Hyun-Dong Paik

Dept. of Food Engineering, Kyungnam University, Masan 631-701, Korea

Abstract

Bacteriocins are proteins produced by a heterogeneous group of bacteria that have a bactericidal effect on closely related organisms. Recently, bacteriocins from lactic acid bacteria and other food-related organisms have been the subject of much research because of their potential as food biopreservatives. Various modifications of agar plate diffusion assays are the most widely used methods even though the limitations of such assays are generally recognized. The ability to obtain a concentrated crude preparation of bacteriocin by optimizing production parameters greatly simplifies recovery of bacteriocin on subsequent purification steps. Some studies performed to optimize bacteriocin production have used commercial media to provide a rich supply of growth nutrients. Many bacteriocins have been purified to homogeneity, and the amino acid sequences of many of these purified bacteriocins have been determined. Obtaining characterization data on purified bacteriocin will minimize the risk of overlapping of research and confusion in identification of these compounds. Several mechanisms leading to cell death have been hypothesized. These include depletion of the proton motive force(PMF) across the cell membrane; RNase and/or DNase activity within the sensitive cell; and pore formation and lysis of sensitive cells at the cell membrane.

Key words: bacteriocin, assay, biochemistry, mode of action

INTRODUCTION

Biopreservatives are antimicrobial compounds of animal, plant, and microbial origin and have long been used in food without any known adverse effects on human health. Use of biopreservatives can enhance the safety and extend the shelf life of food(1). Tagg et al.(2) defined bacteriocins as bactericidal proteins with a narrow spectrum of activity targeted toward species related to the producer culture. Because bacteriocins are proteins and natural, there is tremendous interest in their use as a novel means to ensure the safety of food. This literature review will summarize recent information on assay methods, production of bacteriocins, purification, characterization and mode of action of bacteriocins.

ASSAY METHODS FOR BACTERIOCINS

Assay methods to assess bactericidal activity, one criterion in the definition of a bacteriocin, have been used for many years. Direct or deferred antagonism are methods generally used to detect bacteriocin activity. These methods include 1) spotting culture supernatants on indicator lawns; 2) cross-streaking bacteria; 3) overlapping colonies of the producer strain with an indicator lawn; 4) agar well diffusion of culture supernatant; 5) the flip plate method(3).

Various modifications of agar plate diffusion assays are the most widely used methods even though the limitations of such assays are generally recognized(4). For example, it cannot be determined if cells are killed or only inhibited from growing. Generally, the inhibition zone size is determined by diffusion of antimicrobial compounds including bacteriocin, and the growth rate of the indicator strain(5). Large zones of inhibition are usually formed when the indicator strain is slow growing(6). Addition of Tween 80 is likely to increase the sensitivity of modified well-diffusion assay for propionicin PLG-1(7).

Methods of quantitatively estimating the activity of a bacteriocin have been based on the critical dilution of antagonistic activity(8). Briefly, diluted culture supernatant(including bacteriocin) is spotted on an indicator lawn and activity is quantitated subjectively in arbitrary units(AU) of bacteriocin activity. Generally, the AU is the reciprocal of the dilution of bacteriocin that last caused inhibition(9). Disadvantages of this method are the frequently subjective judgment of inhibition and differences in assay sensitivity because of inconsistent procedures among laboratories(8). The titration of bacteriocin activity is subject to error depending upon the

reproducibility of the indicator cell concentration and the ability of the investigator to determine the last dilution showing complete inhibition. Therefore, the AU value is only an approximate rather than a precisely quantitative measure of bacteriocin activity(3).

An enzyme-linked immunosorbent assay(ELISA) using polyclonal antiserum for nisin detection was used with commercial cheese samples(10). This method had a limit of detection of 1.9×10^{-2} i.u./ml and yielded results that correlated well with results of the bioassay that measured inhibition of *Micrococcus flavus* NCIB 8166. Skytta and Mattila-Sandholm(11) developed a quantitative method using automated turbidometry to assess the antimicrobial efficacy of bacteriocin-like inhibitors produced by *Pediococus dammosus* and *Pediococcus pentosaccus*. Growth of the test strain(*Pseudomonas aeruginosa* and other organisms) was kinetically monitored and various growth curve parameters were used as quantitative indicators of inhibition.

Recently, several investigators have developed new detection methods using microdilution wells(12), hydrophobic grid membrane filters(13), and other simplified techniques(14). These methods are very convenient, rapid, and sensitive for screening bacteriocin-producing bacteria.

PRODUCTION OF BACTERIOCIN

Most studies of bacteriocins begin with detection of inhibitory activity on an agar medium. However, further characterization of bacteriocins is facilitated by their production in liquid medium. The ability to obtain a concentrated crude preparation of bacteriocin by optimizing production parameters greatly simplifies recovery of bacteriocin in subsequent purification steps, because severe losses of activity may occur during the course of protein purification(3). Most studies performed to optimize bacteriocin production have used commercial media to provide a rich supply of growth nutrients.

The effects of several factors on production of nisin by *Lactococcus lactis*, pediocin AcH by *Pediococcus acidilactici*, leuconocin Lcm1 by *Leuconostoc carnosum* Lm1 and sakacin A by *Lactobacillus sake* LB 706 were studied(15). Production of a bacteriocin in a simple medium can be increased by growing the cells at optimum pH and supplementing with nutrients specific for the particular species/strain. Also, conditions that provide high cell density resulted in high bacteriocin production. Economical media such as trypticase glucose yeast- extract(TGE) or TGE buffer broths with food-grade ingredients could be used to obtain high bacteriocin yields.

Effect of growth medium

Production of an unnamed bacteriocin by *Streptococcus mutans* was shown to be influenced by the growth medium(16). A medium containing Trypticase(BBL), yeast extract, sodium chloride, potassium phosphate, and agar was the most effective for bacteriocin production.

The effect of several inorganic and organic acids on nisin production was studied(17). Potassium chloride and calcium chloride increased production of nisin. Of the organic salts studied, sodium citrate, sodium acetate and sodium lactate increased nisin production, while sodium oxalate depressed it. Vuyst and Vandamme(18) reported that carbon source regulation appears to be a major control mechanism for nisin production. The influence of different phosphorous and nitrogen sources on *Lactococcus lactis* subsp. *lactis* NIZO 22186 growth and nisin production was studied by Vuyst and Vandamme(19). Potassium dihydrogen phosphate (KH_2PO_4) was found to be the best phosphorous source for nisin production. A complex medium with cotton seed meal as nitrogen source also gave very high activity.

Addition of some ingredients to the medium was necessary to improve the yields of bacteriocin. Yeast extract(16,20,21) and beef extract(22) increased bacteriocin activity. Addition of Tween 80 is likely to increase production of some bacteriocins such as pediocin AcH (23), lactococcin G(24), jenseniin G(25), enterocin 1146 (21) and curvaticin FS47(26). This increase in measured activity against an indicator strain could be caused by increased production of the bacteriocin or by improved diffusion of the bacteriocin in the assay system.

Several studies of inexpensive media have been reported. Liao et al.(20) showed that whey permeate complemented with yeast extract supported growth and bacteriocin production by *Pediococcus acidilactici* PO2; the medium contained all the minerals and trace elements required for growth. Barber et al.(27) developed a molasses fermentation medium for the industrial production of bacteriocin by *Clostridium acetobutylicum*. Biswas et al.(23) reported that high levels of pediocin AcH could be produced by *Pediococcus acidilactici* H in a simple medium(TGE broth) consisting of relatively

inexpensive, food-grade ingredients. Hsieh et al.(7) developed combination medium of beet molasses and corn steep liquor for the industrial production of propionicin PLG-1 by *Propionibacterium thoenii* P127.

Effect of culture conditions

Bacteriocin production is also influenced by culture conditions such as pH, temperature, and growth phase. The pH of the medium is particularly important. For example, Parente et al.(28) reported that pH was an important factor in the production of lactococcin 140 by *Lactococcus lactis* 140NWC. A maximum activity of 1.54×10^4 AU/ml was obtained at pH 5.5. In contrast, the optimal pH for growth and lactic acid production was between 6.0 and 6.5. Maximum production of piscicolin 61 by *Carnobacterium piscicola* LV61 was obtained at pH 6.5(29); of bavaricin MN by *Lactobacillus bavaricus* MN at pH 6.0(22); of mesenterocin 5 by *Leuconostoc mesenteroides* subsp. *mesenteroides* UL5 at pH 5.0(30); of leuconocin S by *Leuconostoc* strain OX at pH 6.5~7.0(31); of lactacin B by *Lactobacillus acidophilus* N2 at pH 6.0(32); and of enterocin 1146 by *Enterococcus faecium* DPC1146 at pH 5.5~6.5(21). In contrast, production of acidocin 8912 by *Lactobacillus acidophilus* TK8912 was not affected in the pH range 5 to 7; rather, the incubation temperature seemed to be more important in affecting acidocin 8912 production in this study(33).

Optimal production of bacteriocin can occur at different growth phases. Some bacteriocins such as lactococcin 140 and nisin are produced during the exponential phase(18,28). During the late exponential and early stationary phase of growth, many bacteriocins, such as nisin(34), helveticin J(35), lactocin S(36), pediocin AcH(23), propionicin PLG-1(37), and pediocin SJ-1(38) are produced extracellularly. This suggests that these bacteriocins are secondary metabolites.

Although most bacteriocins are studied in batch culture, continuous culture has been used for bavaricin MN production(22). The level (6,400 AU/ml) of bavaricin MN produced during continuous culture was twice that seen in batch fermentations with the same medium, pH, agitation rate, and inoculum size. This level was maintained, independent of growth rate($0.058\sim0.205$ h^{-1}), for 345h.

Recently, Jones et al.(39) studied lactoferricin, a new antimicrobial peptide derived from acid-pepsin digestion of bovine lactoferrin. This lactoferricin is an example of production of a more effective or new bacteriocin by protein modification or engineering from a mother compound.

Genetic determinants for production

The genetic determinants of bacteriocin production and immunity to bacteriocins have great potential as genetic markers.

Bacteriocin production and immunity are frequently associated with plasmid DNA(40). Production of lactocin S by *Lactobacillus sake* L45 and of acidocin 8912 by *Lactobacillus acidophilus* TK8912 was demonstrated to be encoded by plasmids of 50kb(32.9 MDa; pCI M1) and 10.5 MDa(pLA 103)(33,36), respectively. Gonzalez and Kunka(41) reported the association of a 6.2-MDa plasmid(pSRQ11) and pediocin PA-1 production. Production of pediocin SJ-1 was associated with a 4.6 MDa plasmid(38). Other plasmids encoding bacteriocin production and immunity are the 22-kb(14.5 MDa) plasmid of *Carnobacterium piscicola* LV61 (29), the 40- and 49-MDa plasmids of *Carnobacterium piscicola* LV17 (42), the 10-kb(6.6 MDa) plasmid of *Lactococcus lactis* subsp. *lactis* ADRIA 85LO30(43), and the 63 kb(41.5 Mda) plasmid of *Lactococcus lactis* DPC3147(44).

On the other hand, there are several reports of chromosomal location of genetic determinants of bacteriocin production. For instance, production of helveticin J by *Lactobacillus helveticus* 481 was shown to be associated with the chromosome(35). Two bacteriocins produced by propionibacteria, propionicin PLG-1 from *Propionibacterium thoenii* P127(37) and jenseniin G from *Propionibacterium jensenii* P126(45), were also reported to be chromosomally located.

PURIFICATION METHODS FOR BACTERIOCINS

Several techniques have been used to obtain purified or partially purified bacteriocins. For a brief review of purification and characterization of bacteriocins, see Paik and Oh(46). The purification scheme may be varied for some applications. Highly purified preparations would be needed for determination of a bacteriocin's amino acid composition and sequence. However, high yields of active bacteriocin will be the focus of a food biopreservative system. The ability to assay for the target

protein during purification steps is important. Target bacteriocins can be assayed by determination of biological activity and by other analytical methods, including SDS-PAGE(sodium dodecyl sulfate-polyacrylamide gel electrophoresis).

Most purifications start with a method that concentrates bacteriocins from culture supernatants, because bacteriocins usually are extracellular products. Ammonium sulfate precipitation is well established as an initial step in the purification process. Ammonium sulfate fractionation is an effective method because variations in the distribution of hydrophobic and hydrophilic regions allow specific proteins to precipitate over a narrow range of salt concentrations(3).

Dialysis and ultrafiltration are valuable methods of concentrating and purifying bacteriocins. By using membranes of specific pore size, the researcher can retain proteins above a particular size and allow smaller proteins to pass through.

Several methods of chromatography, such as gel filtration, ion exchange, and/or hydrophobic interaction chromatography, have been recommended for achieving significant purification of bacteriocins. Especially, reverse-phase chromatography has been frequently used as a final step for several bacteriocins, including pediocin PA-1(47), curvacin A(48), sakacin A(49), plantaricin A(50), bavaricin A(51), piscicolin 61(52), plantaricin S(53), acidocin J1132(54), and enterocin A(55).

High performance liquid chromatography(HPLC) as well as hydrophobic interaction chromatography have also been used to obtain more highly purified bacteriocin preparations. Separation on reversed-phase supports in HPLC has been used to obtain highly purified preparations of leucocin A-UAL 187(56), lactacin F(57), mesentericin Y105(58), lacticin 481(59), salivaricin A(60), curvaticin FS47(26), staphylococcin 1580(61), propionicin PLG-1(62) and piscicolin 126(63). The hydrophobic nature of these bacteriocins allows their purification by reversed-phase HPLC.

In reversed-phase chromatography, the weak mobile phase is usually 0.1%(v/v) trifluoroacetic acid(TFA), while the eluting mobile phase is an organic solvent such as 2-propanol or acetonitrile. Once the sample is injected onto the column in the weak mobile phase, each protein is retained until the appropriate concentration of organic solvent displaces it from the support. The sample peak shape(resolution) of the bacteriocin in the chromatogram is often very sharp, as a result of displacement elution. However, because of the acidity and the organic solvents needed to elute bacteriocins from the extremely hydrophobic reverse phase chromatography stationary phase, protein denaturation and loss of bacteriocin activity often occur. In many cases, bacteriocin activity cannot be retrieved after the tertiary structure is disrupted. Therefore, reversed-phase HPLC systems can be used as a preparative technique only for those bacteriocins that are stable in organic solvents, or for bacteriocins that can renature after unfolding occurs during the elution process(64). Many of the completely purified bacteriocins obtained by using reversed-phase HPLC or chromatography were small highly hydrophobic molecules that apparently could easily renature.

AMINO ACID COMPOSITION AND N-TERMINAL SEQUENCE OF BACTERIOCINS

Amino acid composition analysis provides an important quantitative parameter in the characterization of purified bacteriocins. The most important step in obtaining an unambiguous N-terminal sequence is to purify suitable quantities of the bacteriocin in a manner compatible with automated or manual Edman degradation procedures. Matsudaira(65) suggested several requirements for obtaining sequences of unknown samples. First, the sample should be relatively pure(>80%). Second, the sample should be free of contaminants such as Tris, glycine, sodium dodecyl sulfate(SDS), or acrylamide, which will either affect the performance of the sequencing machine or clutter the chromatograms with large artifact peaks. Third, a sufficient quantity of sample should be available for analysis. Most sequencing facilities request 10~100pmol of bacteriocin for N-terminal sequence analysis. If no sequence is obtained from 100pmole, then one would suspect that the bacteriocin has a blocked N-terminus. If the N-terminus of a bacteriocin is blocked, then it must be cleaved chemically(CNBr cleavage) or enzymatically (proteolytic digestion) to generate internal peptides with unblocked N-termini. In this case, a 5-fold increase in sample size is necessary to do chemical or enzymatic cleavage(65).

MODE OF ACTION OF BACTERIOCINS

Studies on the mode of action of bacteriocins started

with the colicins, antimicrobial proteins produced by *E. coli*. The general lethal action of the colicins was suggested to occur in three stages: binding to a specific receptor on the cell surface; insertion into or transport across the sensitive cell's membrane; and killing of the cell(66). Several mechanisms leading to cell death have been hypothesized. These include depletion of the proton motive force(PMF) across the cell membrane; RNase and/or DNase activity within the sensitive cell; and lysis of sensitive cells at the cell membrane(66). For a review of this early work, see Montville and Kaiser(66). The present literature review will mainly focus on recent studies.

Bruno and Montville(67) studied the influence of four bacteriocins(pediocin PA-1, leuconocin S, lactacin F, and nisin) from lactic acid bacteria on the PMF of sensitive cells. They suggested that the bacteriocins of lactic acid bacteria all have the same mechanism, namely, depletion of PMF. Pediocin PA-1(20μg/ml), leuconocin S(48.5μg/ml), and nisin(5μg/ml) mediated total or major PMF dissipation of energized *Listeria monocytogenes* Scott A, while lactacin F(13.5μg/ml) mediated 87% depletion of the PMF of energized *Lactobacillus delbrueckii* ATCC 4797 cells. Pediocin PA-1, leuconocin S, and lactacin F acted in an energy-independent manner, whereas the activity of nisin was energy-dependent.

By using liposomes and proteoliposomes, Gao et al. (68) showed that nisin depolarized membranes and dissipated the membrane potential($\Delta\psi$) and the pH gradient(ΔpH) in a voltage-dependent manner. The basal PMF and the influence of nisin on the PMF were studied in *L. monocytogenes* Scott A by Bruno et al.(69), who showed that addition of nisin($\geq$ 5μg/ml) completely dissipated the PMF in cells at external pH values of 5.5 and 7.0. With 1μg/ml of nisin, ΔpH was completely dissipated, but $\Delta\psi$ decreased only slightly. The action of nisin on the PMF in *L. monocytogenes* Scott A was both time-dependent and concentration-dependent.

van Belkum et al.(70) reported that purified lactococcin A specifically increased permeability of lactococcal cytoplasmic membranes in a voltage-independent, protein-mediated manner and dissipated the membrane potential. Lactococcin A also inhibited the PMF-driven leucine uptake and leucine counterflow in membrane vesicles of a sensitive strain but not in membrane vesicles of a strain immune to the bacteriocin. The specificity of lactococcin A may be mediated by a recepter protein associated with the cytoplasmic membrane. From the foregoing information, it appears likely that lactococcin A induces the formation of pores in the cytoplasmic membrane of *L. lactis* and these pores allow free diffusion of ions and amino acids. The efflux of essential compounds can explain the growth inhibition and ultimate death of lactococcal cells exposed to lactococcin A.

Venema et al.(71) demonstrated that purified lactococcin B(Lcn B) experts its bactericidal effect on sensitive *L. lactis* cells by dissipating the PMF and thereby causing leakage of intracellular substrates. The Lcn B induces formation of pores in the cytoplasmic membrane of sensitive cells in the absence of a PMF. At low concentrations of Lcn B, efflux of some ions and amino acids that are taken up by PMF-driven systems was found. On the other hand, a 150-fold higher Lcn B concentration was required for efflux of glutamate, previously taken up via a unidirectional ATP-driven transport system. In immune *L. lactis* cells, the PMF was not dissipated, and no leakage of intracellular substrates was detected.

The effect of pediocin JD, a bacteriocin produced by *Pediococcus acidilactici* JD 1-23, on the PMF and proton permeability of *L. monocytogenes* Scott A was studied(72). The pH gradient of cells exposed to pediocin JD was rapidly dissipated, while control cells maintained a pH gradient and a membrane potential of 0.65 pH unit and 75mV, respectively. The inhibitory action of pediocin JD against *L. monocytogenes* is directed at the cytoplasmic membrane and may be caused by the collapse of one or both of the individual components of the PMF.

Pediocin PA-1, a bacteriocin produced by *P. acidilactici* PAC1.0, showed a bactericidal effect on sensitive *Pediococcus* cells, in which it acted on the cytoplasmic membrane(73). Pediocin PA-1 dissipated the transmembrane electrical potential and inhibited amino acid transport in sensitive *Pediococcus* cells. Release of ions and small molecules from the target cells led to cell death, with or without lysis.

Schved et al.(74) monitored alterations induced by pediocin SJ-1 in the cytoplasmic membrane of sensitive *Lactobacillus plantarum* cells by using a 1-anilino-8-naphthalenesulphonic acid(ANS) fluorescent probe. The addition of pediocin SJ-1 to the sensitive strain showed an increase in fluorescence intensity of ANS. Pediocin SJ-1 neutralized charges located on the hydrophilic portion of membrane phospholipids. Furthermore, pe-

diocin SJ-1 was likely to create pores in the cytoplasmic membrane, which could explain the leakage of low molecular weight compounds and depolarization of the cytoplasmic membrane.

Changes in membrane permeability of *L. monocytogenes* and mitochondria caused by mesentericin Y105 were reported(75). Mesentericin Y105 dissipated the plasma membrane potential of *L. monocytogenes* and inhibited the transport of leucine and glutamic acid. Also, this bacteriocin uncoupled mitochondria by increasing state 4 respiration and decreasing state 3 respiration, apparently by inducing pore formation in the energy-transducing membranes.

Lactacin F is bactericidal against *Lactobacillus delbrueckii, L. helveticus*, and *Enterococcus faecalis*. Inhibitory activity against *L. delbrueckii* was contributed by two peptides, LafA and LafX, which are encoded within the lactacin F operon(76). The mode of action of lactacin F against *E. faecalis* ATCC 19443 was studied(77). Lactacin F caused an immediate loss of cellular K^+, depolarization of the cytoplasmic membrane, and hydrolysis of internal ATP. The ATP hydrolysis was due not to dissipation of the PMF but most likely to efflux of inorganic phosphate, resulting in a shift of the ATP hydrolysis equilibrium. From these results, it appears that possible mechanisms are interaction of lactacin F with cytoplasmic membranes and formation of poration complexes.

Lactobacillus acidophilus JCM 1132 produces a heat-stable, two component bacteriocin designated acidocin J1132. Acidocin acted bactericidally rather than bacteriolytically on sensitive cells and mediated total PMF dissipation of sensitive cells in a concentratoin-dependent manner and finally caused the leakage of essential compounds by the formation of pores in the cytoplasmic membrane.

The bactericidal effect of plantaricin C, a bacteriocin of Lactobacillus plantarum LL441, was studied(78). Like other previous bacteriocins, plantaricin C dissipated PMF, and inhibited amino acid transport in sensitive cells, and was a pore-forming bacteriocin. The intracellular ATP levels and the glycolytic rate of sensitive cells was significantly reduced when plantaricin C was added.

CONCLUSIONS

During the past decade, bacteriocins have become a primary focus of research because of their potential use as nontoxic biopreservatives. To date, many bacteriocins have been optimized for production, purified to homogeneity, characterized and compared with other bacteriocins. Future efforts directed toward molecular characterization of the structure, function, and regulation of purified bacteriocin will accelerate efforts to engineer innovative antimicrobial peptides with enhanced capabilities and diverse applications(3). Manipulation of genes for bacteriocin production and immunity is expected to provide the opportunity for drastic improvement of bacteriocin production, and expansion of the inhibitory spectrum. If a bacteriocin is to be used commercially, rapid tests to detect and quantitate it would be useful. At it's molecular weight, it is probably large enough to elicit antibody production and thus be detectable by immunological assays such as ELISA. Rather than using purified bacteriocin in foods, it may be possible to preserve some fermented foods by adding live cultures of bacteriocin producer either alone or in combination with other desirable strains(79).

REFERENCES

1. Ray, B. : Cells of lactic acid bacteria as food biopreservatives. In "*Food biopreservatives of microbial origin*" Ray, B. and Daeschel, M.(eds.), CRC Press, Inc., Boca Raton, p.81(1992)
2. Tagg, J. R., Dajani, A. S. and Wannamaker, L. W. : Bacteriocins of gram-positive bacteria. *Bacteriol. Rev.*, **40**, 722(1976)
3. Muriana, P. M. and Luchansky, J. B. : Biochemical methods for purification of bacteriocins. In "*Bacteriocins of lactic acid bacteria*" Hoover, D. G. and Steenson, L. R.(eds.), Academic Press, Inc., San Diego, p.41(1993)
4. Tagg, J. R. and McGiven, A. R. : Assay system for bacteriocins. *Appl. Microbiol.*, **21**, 943(1971)
5. Linton, A. H. : Theory of antibiotic inhibition zone formation, disc sensitivity methods and MIC determinations. In "*Antibiotics : Assessment of antimicrobial activity and resistance*" Denver, A. D. and Quesnel, L. B.(eds.), Academic Press, Inc., London, p.19(1983)
6. Piddock, L. J. V. : Techniques used for the determination of antimicrobial resistance and sensitivity in bacteria. *J. Appl. Bacteriol.*, **68**, 307(1990)
7. Hsieh, H., Paik, H. D. and Glatz, B. A. : Improvement of detection and production of propionicin PLG-1, a bacteriocin produced by *Propionibacterium thoenii*. *J. Food Prot.*, **59**, 734(1996)
8. Hoover, D. G. and Harlander, S. K. : Screening methods for detecting bacteriocin activity. In "*Bacteriocins of lactic acid bacteria*" Hoover, D. G. and Steenson, L. R. (eds.), Academic Press, Inc., San Diego, p.23(1993)

9. Mayr-Harting, A., Hedges, A. J. and Berkeley, R. C. W. : Methods for studying bacteriocins. In "*Methods in microbiology*" Bergen, T. and Norris, J. R.(eds.), Academic Press, Inc., London, Vol. 7A, p.315(1972)
10. Falahee, M. B., Adams, M. R., Dale, J. W. and Morris, B. A. : An enzyme immunoassay for nisin. *Int. J. Food Sci. Technol.*, **25**, 590(1990)
11. Skytta, E. and Mattila-Sandholm, T. : A quantitative method for assessing bacteriocins and other food antimicrobials by automated turbidometry. *J. Microbiol. Methods.*, **14**, 77(1991)
12. Toba, T , Samant, S. K. and Itoh, T. : Assay system for detecting bacteriocin in microdilution wells. *Lett. Appl. Microbiol.*, **13**, 102(1991)
13. Ryser, E. T. and Richard, J. A. : Detection of bacteriocin activity in bacteria using hydrophobic grid membrane filters. *Lett. Appl. Microbiol.*, **14**, 104(1992)
14. Benkerroum, N., Ghouati, Y., Sandine, W. E. and Tantaoui-Elaraki, A. : Methods to demonstrate the bactericidal activity of bacteriocins. *Lett. Appl. Microbiol.*, **17**, 78(1993)
15. Yang, R. and Ray, B. : Factors influencing production of bacteriocins by lactic acid bacteria. *Food Microbiol.*, **11**, 281(1994)
16. Rogers, A. H. : Effect of the medium on bacteriocin production among strains of *Streptococcus mutans*. *Appl. Microbiol.*, **24**, 294(1972)
17. Kalra, M. S. and Dudani, A. T. : Effect of different inorganic and organic salts on nisin production in a broth culture. *Indian J. Dairy Sci.*, **27**, 109(1974)
18. Vuyst, L. D. and Vandamme, E. J. : Influence of the carbon source on nisin production in *Lactococcus lactis* subsp. *lactis* batch fermentations. *J. Gen. Microbiol.*, **138**, 571(1992)
19. Vuyst, L. D. and Vandamme, E. J. : Influence of the phosphorous and nitrogen source on nisin production in *Lactococcus lactis* subsp. *lactis* batch fermentations using a complex medium. *Appl. Microbiol. Biotechnol.*, **40**, 17 (1993)
20. Liao, C., Yousef, A. E., Richter, E. R. and Chism, G. W. : *Pediococus acidilactici* PO2 bacteriocin production in whey permeate and inhibition of *Listeria monocytogenes* in foods. *J. Food Sci.*, **58**, 430(1993)
21. Parente, E. and Hill, C. : A comparison of factors affecting the production of two bacteriocins from lactic acid bacteria. *J. Appl. Bacteriol.*, **73**, 290(1992)
22. Kaiser, A. L. and Montville, T. J. : The influence of pH and growth rate on production of the bacteriocin, bavaricin MN, in batch and continuous fermentations. *J. Appl. Bacteriol.*, **75**, 536(1993)
23. Biswas, S. R., Ray, P., Johnson, M. C. and Ray, B. : Influence of growth conditions in the production of a bacteriocin, pediocin AcH, by *Pediococcus acidilactici* H. *Appl. Environ. Microbiol.*, **57**, 1265(1991)
24. Nissen-Meyer, J., Holo, H., Havarstein, L. S., Sletten, K. and Nes, I. F. : A novel lactococcal bacteriocin whose activity depends on the complementary action of two peptides. *J. Bacteriol.*, **174**, 5686(1992)
25. Barefoot, S. F. : unpublished data(1995)
26. Garver, K. I. and Muriana, P. M. : Purification and partial amino acid sequence of curvaticin FS47, a heat-stable bacteriocin produced by *Lactobacillus curvatus* FS47. *Appl. Environ. Microbiol.*, **60**, 2191(1994)
27. Barber, J. M., Robb, F. T., Webster, J. R. and Woods, D. R. : Bacteriocin production by *Clostridium acetobutylicum* in an industrial fermentation process. *Appl. Environ. Microbiol.*, **37**, 433(1979)
28. Parente, E., Ricciardi, A. and Addario, G. : Influence of pH on growth and bacteriocin production by *Lactococcus lactis* subsp. *lactis* 140NWC during batch fermentation. *Appl. Microbiol. Biotechnol.*, **41**, 388(1994)
29. Schillinger, U., Stile, M. E. and Holzapfel, W. H. : Bacteriocin production by *Carnobacterium piscicola* LV 61. *Int. J. Food Microbiol.*, **20**, 131(1993)
30. Daba, H., Lacroix, C., Huang, J. and Simard, R. E. : Influence of growth conditions on production and activity of mesentericin 5 by a strain of *Leuconostoc mesenteroides*. *Appl. Microbiol. Biotechnol.*, **39**, 166(1993)
31. Lewus, C. B., Sun, S. and Montville, T. J. : Production of an amylase sensitive bacteriocin by an atypical *Leuconostoc paramesenteroides* strain. *Appl. Environ. Microbiol.*, **58**, 143(1992)
32. Barefoot, S. F. and Klaenhammer, T. R. : Purification and characterization of the *Lactobacillus acidophilus* bacteriocin lactacin B. *Antimicrob. Agents Chemother.*, **26**, 328(1984)
33. Kanatani, K., Tahara, T., Yoshida, K., Miura, H., Sakamoto, M. and Oshimura, M. : Plasmid-associated bacteriocin production by and immunity of *Lactobacillus acidophilus* TK8912. *Biosci. Biotech. Biochem.*, **56**, 648(1992)
34. Hurst, A. : Nisin. *Adv. Appl. Microbiol.*, **27**, 85(1981)
35. 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. *J. Bacteriol.*, **167**, 439(1986)
36. Mortvedt, C. I. and Nes, I. F. : Plasmid-associated bacteriocin production by a *Lactobacillus sake* strain. *J. Gen. Microbiol.*, **136**, 1601(1990)
37. Lyon, W. J. and Glatz, B. A. : Partial purification and characterization of a bacteriocin produced by *Propionibacterium thoenii*. *Appl. Environ. Microbiol.*, **57**, 701(1993)
38. Schved, F., Lalazar, A., Henis, Y. and Juven, B. J. : Purification, partial characterization and plasmid-linkage of pediocin SJ-1, a bacteriocin produced by *Pediococcus acidilactici*. *J. Appl. Bacteriol.*, **74**, 67(1993)
39. Jones, E. M., Smart, A., Bloomberg, G., Burgess, L. and Millar, M. R. : Lactoferricin, a new antimicrobial peptide. *J. Appl. Bacteriol.*, **77**, 208(1994)
40. Klaenhammer, T. R. : Bacteriocins of lactic acid bacteria. *Biochimie*, **70**, 337(1988)
41. Gonzalez, C. F. and Kunka, B. S. : Plasmid-associated bacteriocin production and sucrose fermentation in *Pediococcus acidilactici*. *Appl. Environ. Microbiol.*, **53**, 2534 (1987)
42. Ahn, C. and Stiles, M. E. : Plasmid-associated bacteriocin production by a strain of *Carnobacterium piscicola* from meat. *Appl. Environ. Microbiol.*, **56**, 2503(1990)
43. Dufour, A., Thuault, D., Boulliou, A., Bourgeois, C. M. and Pennec, J. L. : Plasmid-encoded determinants for bacteriocin production and immunity in a *Lactococcus*

lactis strain and purification of the inhibitory peptide. *J. Gen. Microbiol.*, **137**, 2423(1991)
44. Ryan, M. P., Rea, M. C., Hill. C. and Ross, R. P. : An application in cheddar cheese manufacture for a strain of *Lactococcus lactis* producing a novel broad-spectrum bacteriocin, lacticin 3147. *Appl. Environ. Microbiol.*, **62**, 612(1996)
45. Grinstead, D. A. and Barefoot, S. F. : Jenseniin G, a heat-stable bacteriocin produced by *Propionibacterium jensenii* P126. *Appl. Environ. Microbiol.*, **58**, 215(1992)
46. Paik, H. D. and Oh, D. W. : Purification, characterization, and comparison of bacteriocins. *J. Microbiol. Biotechnol.*, **6**, 151(1996)
47. Lozano, J. C. N., Nissen-Meyer, J., Sletten, K., Pelaz, C. and Nes, I. F. : Purification and amino acid sequence of a bacteriocin produced by *Pediococcus acidilactici*. *J. Gen. Microbiol.*, **138**, 1985(1992)
48. Tichaczek, P. S., Nissen-Meyer, J., Nes. I. F., Vogel, R. F. and Hammes, W. P. : Characterization of the bacteriocins curvacin A from *Lactobacillus curvaticus* LTH1174 and sakacin P from L. sake LTH673. *System. Appl. Microbiol.*, **15**, 460(1992)
49. Holck, A. L., Axelsson, L., Birkeland, S. -E., Aukrust, T. and Blom, T. : Purification and amino acid sequence of sakacin A, a bacteriocin from *Lactobacillus sake* Lb 706. *J. Gen. Microbiol.*, **138**, 2715(1992)
50. Nissen-Meyer, J., Larsen, A. G., Sletten, K., Daeschel, M. and Nes, I. F. : Purification and characterization of plantaricin A, a *Lactobacillus plantarum* bacteriocin whose activity depends on the action of two peptides. *J. Gen. Microbiol.*, **139**, 1973(1993)
51. Larsen, A. G., Vogensen, F. K. and Josephsen, J. : Antimicrobial activity of lactic acid bacteria isolated from sour doughs : Purification and characterization of bavaricin A, a bacteriocin produced by *Lactobacillus bavaricus* M1401. *J. Appl. Bacteriol.*, **75**, 113(1993)
52. Holck, A. L., Axelsson, L. and Schillinger, U. : Purification and cloning of piscicolin 61, a bacteriocin from *Carnobacterium piscicola* LV61. *Curr. Microbiol.*, **29**, 63(1994)
53. Jimenez-Diaz, R., Ruiz-Barba, J. L., Cathcart, D. P., Holo, H., Nes, I. F., Sletten, K. H. and Warner, P. J. : Purification and partial amino acid sequence of plantaricin S, a bacteriocin produced by *Lactobacillus plantarum* LPCO 10, the activity of which depends on the complementary action of two peptides. *Appl. Environ. Microbiol.*, **61**, 4459(1995)
54. Tahara, T., Oshimura, M., Umezawa, C. and Kanatani, K. : Isolation, partial characterization, and mode of action of acidocin J1132, a two-component bacteriocin produced by *Lactobacillus acidophilus* JCM 1132. *Appl. Environ. Microbiol.*, **62**, 892(1996)
55. Aymerich, T., Holo, H., Havarstein, L. S., Hugas, M., Garriga, M. and Nes, I. F. : Biochemical and genetic characterization of enterocin A from *Enterococcus faecium*, a new antilisterial bacteriocin in the pediocin family of bacteriocins. *Appl. Environ. Microbiol.*, **62**, 1676(1996)
56. Hastings, J. W., Sailer, M., Johnson, K., Roy, K. L., Vederas, J. C. and Stiles, M. E. : Characterization of leucocin A-UAL 187 and cloning of the bacteriocin gene from *Leuconostoc gelidum*. *J. Bacteriol.*, **173**, 7491(1991)
57. Muriana, P. M. and Klaenhammer, T. R. : Purification and partial characterization of lactacin F, a bacteriocin produced by *Lactobacillus acidophilus* 11088. *Appl. Environ. Microbiol.*, **57**, 114(1991)
58. Hechard, Y., Derijard, B., Leteller, F. and Cenatiempo, Y. : Characterization and purification of mesentericin Y105, an anti-Listeria bacteriocin from *Leuconostoc mesenteroides*. *J. Gen. Microbiol.*, **138**, 2725(1992)
59. Piard, J.-C., Muriana, P. M., Desmazeaud, M. J. and Klaenhammer, T. R. : Purification and partial characterization of lacticin 481, a lanthionine-containing bacteriocin produced by *Lactococcus lactis* subsp. *lactis* CNRZ 481. *Appl. Environ. Microbiol.*, **58**, 279(1992)
60. Ross, K. F., Ronson, C. W. and Tagg, J. R. : Isolation and characterization of the lantibiotic salivaricin A and its structural gene salA from *Streptococcus salivarius* 20P3. *Appl. Environ. Microbiol.*, **59**. 2014(1993)
61. Sahl, H. · Staphylococcin 1580 is identical to the lantibiotic epidermin : Implications for the nature of bacteriocins from gram-positive bacteria. *Appl. Environ. Microbiol.*, **60**, 752(1994)
62. Paik, H. D. and Glatz, B. A. : Purification and partial amino acid sequence of propionicin PLG-1, a bacteriocin produced by *Propionibacterium thoenii* P127. *Lait*, **75**, 367(1995)
63. Jack, R. W., Wan, J., Gordon, J., Harmark, K., Davidson, B. E., Hillier, A. J., Wettenhall, R. E. H., Hickey, M. W. and Coventry, M. J. : Characterization of the chemical and antimicrobial properties of piscicolin 126, a bacteriocin produced by *Carnobacterium piscicola* JG126. *Appl. Environ. Microbiol.*, **62**, 2897(1996)
64. Chicz, R. M. and Regnier, F. E. : High-performance liquid chromatography : Effective protein purification by various chromatographic modes. In "*Guide to protein purification*" Deutscher, M. P.(ed.), Academic Press, Inc., San Diego, p. 392(1990)
65. Matsudaira, P. : Limited N-terminal sequence analysis. In "*Guide to protein purification*" Deutscher, M. P.(ed.), Academic Press, Inc., San Diego(1990)
66. Montville, T. J. and Kaiser, A. L. : Antimicrobial proteins : Clasification, nomenclature, diversity, and relationship to bacteriocins. In "*Bacteriocin of lactic acid bacteria*" Hoover, D. G. and Steenson, L. R.(eds.). Academic Press, Inc., San Diego, p.1(1993)
67. Bruno, M. E. C. and Montville, T. J. : Common mechanistic action of bacteriocins from lactic acid bacteria. *Appl. Environ. Microbiol.*, **59**, 3003(1993)
68. Gao, F. H., Abee, T. and Konings, W. N. : Mechanism of action of the peptide antibiotic nisin in liposomes and cytochrome C oxidase-containing proteoliposomes. *Appl. Environ. Microbiol.*, **57**, 2164(1991)
69. Bruno, M. E. C., Kaiser, A. and Montville, T. J. : Depletion of proton motive force by nisin in *Listeria monocytogenes* cells. *Appl. Environ. Microbiol.*, **58**, 2255(1992)
70. van Belkum, M. J., Kok, J., Venema, G., Holo, H., Nes, I. F., Konings, W. N. and Abee, T. : The bacteriocin lactococcin A specifically increases permeability of lacto-

coccal cytoplasmic membranes in a voltage-independent, protein-mediated manner. *J. Bacteriol.*, **173**, 7934(1991)
71. Venema, K., Abee, T., Haandrikman, A. J., Leenhouts, K. J., Kok, J., Konings, W. N. and Venema, G. : Mode of action of lactococcin B, thio-activated bacteriocin from *Lactococcus lactis*. *Appl. Environ. Microbiol.*, **59**, 1041 (1993)
72. Christensen, D. P. and Hutkins. R. W. : Collapse of the proton motive force in *Listeria monocytogenes* caused by a bacteriocin produced by *Pediococcus acidilactici*. *Appl. Environ. Microbiol.*, **58**, 3312(1992)
73. Chikindas, M. L., Garcia-Garcera, M. J., Driessen, A. J. M, Ledeboer. A. M., Nissen-Meyer, J., Nes, I. F., Abee, T., Konnings, W. N. and Venema, G. : Pediocin PA-1, a bacteriocin from *Pediococcus acidilactici* PAC1.0, forms hydrophilic pores in the cytoplasmic membrane of target cells. *Appl. Environ. Microbiol.*, **59**, 3577(1993)
74. Schved. F., Lindner, P. and Juven, B. J. : Interaction of the bacteriocin pediocin SJ-1 with the cytoplasmic membrane of sensitive bacterial cells as detected by ANS fluorescence. *J. Appl. Bacteriol.*, **76**, 30(1994)
75. Maftah, A., Renault, D., Vignoles, C., Hechard, Y., Bressolher, P., Ratinaud, M. H., Cenatiempo, Y. and Julien, R. : Membrane permeabilization of *Listeria monocytogenes* and mitochondria by the bacteriocin mesentericin Y105. *J. Bacteriol.*, **175**, 3232(1993)
76. Klaenhammer, T. R. : Genetics of bacteriocin produced by lactic acid bacteria. *FEMS Microbiol. Rev.*, **12**, 39(1993)
77. Abee, T., Klaenhammer, T. R. and Letellier, L. : Kinetic studies of the action of lactacin F, a bacteriocin produced by *Lactobacillus johnsonii* that forms poration complexes in the cytoplasmic membrane. *Appl. Environ. Microbiol.*, **60**, 1006(1994)
78. Gonzalez, B., Glaasker, E., Kunji, E. R. S., Driessen, A. J. M., Suarez, J. E. and Konings, W. N. : Bactericidal mode of action of plantaricin C. *Appl. Environ. Microbiol.* **62**, 2701(1996)
79. Paik, H. D. : Enhanced production, purification, and characterization of propionicin PLG-1, a bacteriocin produced by *Propionibacterium thoenii* P127. *Ph. D. dissertation*, Iowa State Univ., Ames(1995)

(Received November 19, 1996)