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Differences in Susceptibility of *Listeria monocytogenes* Strains to Sakacin P, Sakacin A, Pediocin PA-1, and Nisin

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Two hundred strains of *Listeria monocytogenes* collected from food and the food industry were analyzed for susceptibility to the class IIa bacteriocins sakacin P, sakacin A, and pediocin PA-1 and the class I bacteriocin nisin. The individual 50% inhibitory concentrations (IC_{50}) were determined in a microtiter assay and expressed in nanograms per milliliter. The IC_{50} of sakacin P ranged from 0.01 to 0.61 ng ml^{-1}. The corresponding values for pediocin PA-1, sakacin A, and nisin were 0.10 to 7.34, 0.16 to 44.2, and 2.2 to 781 ng ml^{-1}, respectively. The use of a large number of strains and the accuracy of the IC_{50} determination revealed patterns not previously described, and for the first time it was shown that the IC_{50} of sakacin P divided the *L. monocytogenes* strains into two distinct groups. Ten strains from each group were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of whole-cell proteins and amplified fragment length polymorphism. The results from these studies essentially confirmed the grouping based on the IC_{50} of sakacin P. A high correlation was found between the IC_{50} of sakacin P and that of pediocin PA-1 for the 200 strains. Surprisingly, the correlation between the IC_{50} of the two class IIa bacteriocins sakacin A and sakacin P was lower than the correlation between the IC_{50} of sakacin A and the class I bacteriocin nisin.

Awareness of the food-borne pathogen *Listeria monocytogenes* has increased in recent years. At the same time, lactic acid bacteria (LAB) have been considered and used as biopreservatives (10, 53). Many LAB produce bacteriocins—small, heat-stable, membrane-active antimicrobial peptides. The class I bacteriocin nisin, which is commercially available as a food preservative (14), and bacteriocins of class IIa (pediocin-like) are of special interest as inhibitors of *Listeria* spp. (19, 47). The addition of bacteriocinogenic LAB or purified bacteriocins to inhibit the growth of *Listeria* spp. in food products has been studied (8, 22, 35–37, 43; for a review see reference 10).

Intrinsic properties of food have a significant influence on the effect of bacteriocins on the target cells (2, 5, 27, 39). However, knowledge about the susceptibility of the target organism is also necessary when bacteriocins are added to food as biopreservatives. The susceptibilities of different strains of *L. monocytogenes* to both nisin and pediocin-like bacteriocins differ (22, 38, 42, 47). Identical sensitivity patterns have been reported (20, 47) for different strains of *L. monocytogenes* in response to different pediocin-like bacteriocins. But for comparison of the potencies of the different bacteriocins, quantitative MICs or 50% inhibitory concentrations (IC_{50}) are needed (9, 18).

Resistance by *L. monocytogenes* strains to nisin and class IIa bacteriocins has been reported (29, 30, 38, 43, 47, 57, 59). The term "resistance" is often not defined clearly, so strains able to grow at the highest bacteriocin concentration available have been defined as resistant. In model systems it has been shown that high-level-resistant mutants arise spontaneously when bacteriocin-sensitive *L. monocytogenes* strains grow in the presence of high concentrations of a class IIa bacteriocin (29). In appropriate media the MICs for these mutants are 1,000 times higher than those for the wild-type strains (15, 29). The mode of action of class IIa bacteriocins has been elucidated recently, together with studies of mutant strains with resistance to high levels of these bacteriocins (12, 13, 15, 29, 31, 33, 34, 46, 49, 55, 61). In order to be active, class IIa bacteriocins need a target molecule at the surface of a sensitive cell, and mannose phosphotransferase system (PTS) permease is the proposed target molecule (33, 34). Gravesen et al. (29) suggest that high-level resistance to class IIa bacteriocins in *L. monocytogenes* is caused by prevention of synthesis of a mannose-specific PTS permease (EII_t^{Man}) and up-regulation of the synthesis of a putative β-glucoside-specific PTS enzyme II (EII^{Bgl}) and a phospho-β-glucosidase. The up-regulations are probably a consequence of abolished expression of *mptACD* (mannose permease 2, encoding a putative mannose PTS permease) (29). Membrane adaptation, by increased levels of desaturated and short-acyl-chain phosphatidylglycerols, has also been suggested as part of a resistance mechanism (55).

Various methods are used to determine differences in susceptibility to bacteriocins (6, 16, 18, 38, 40, 42, 47, 52, 60). To achieve high discrimination between strains with different susceptibilities, a standardized microtiter plate assay, performed with bacteriocin solutions of known concentrations, was used to provide data necessary to determine the potencies of the bacteriocins sakacin P, pediocin PA-1, sakacin A, and nisin against *L. monocytogenes.* The distributions of IC_{50} were determined, and correlation coefficients for the IC_{50} were calculated, providing new information regarding the pattern of susceptibility to bacteriocins for this species.

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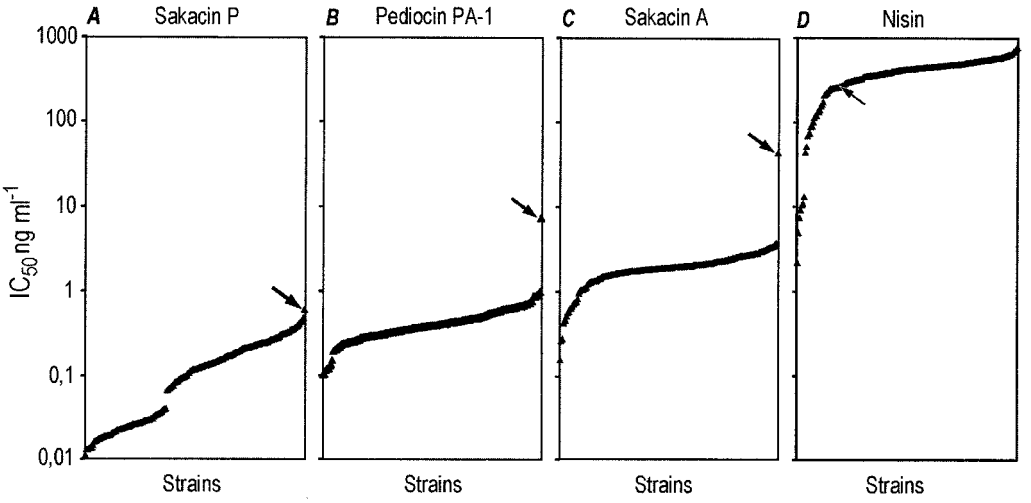

FIG. 1. IC_{50} (measured as the concentration, in nanograms per milliliter, needed to achieve 50% inhibition of growth in a microtiter plate assay), on a logarithmic scale, for 200 strains of *L. monocytogenes*. The strains are sorted according to ascending IC_{50} for each of the bacteriocins: sakacin P (A), pediocin PA-1 (B), sakacin A (C), and nisin (D). Arrows indicate the IC_{50} of each of the bacteriocins for strain L1040; see the text for details.

MATERIALS AND METHODS

Bacterial strains and growth conditions. In this study 200 *L. monocytogenes* strains, originating from food and food industry environments, were investigated. The strains either originated from different sources or had been shown to differ by multilocus enzyme electrophoresis and/or restriction enzyme analysis (1; L. M. Rørvik, personal communication). *Listeria ivanovii* Li4 (4) was used as an indicator for the determination of bacteriocin concentrations. The *Listeria* strains were kept at −80°C and grown in brain heart infusion medium (Oxoid Ltd., Basingstoke, United Kingdom) at 30°C overnight prior to IC_{50} determination. For sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of whole-cell proteins and for fatty acid analysis, the cells were grown for 24 h at 28°C on plates containing 30 g of Trypticase soy broth (BBL, Cockeysville, Md.) and 15 g of Bacto Agar (Difco, Detroit, Mich.) per liter of distilled water. The bacteriocin-deficient *Lactobacillus sakei* strain Lb790(pSAK20) (3, 4), grown in MRS medium (Oxoid Ltd.), was used as a host for the production of sakacin P, sakacin A, and pediocin PA-1 by introducing the isogenic plasmids pSPP2 (4), pSAK17B (3), and pPED2 (4), respectively. A bacteriocin-negative supernatant was obtained by introducing the isogenic plasmid pLPV111 (3) into the same *Lactobacillus sakei* strain.

Preparation of bacteriocin stock solutions and a bacteriocin-negative control supernatant for IC_{50} determinations. Purified nisin (a gift from Danisco Innovation, Beaminster, United Kingdom) was dissolved in 0.02 N HCl with 0.1% Tween 80, the pH was adjusted to 6.2, and the solution was sterilized by filtration (pore size, 0.2 μm; Millex GS; Millipore, Molsheim, France). This nisin stock solution (12.5 μg ml^{-1}) was stored at 4°C and used within 14 days. Cell-free supernatants (CFS) of sakacin P, sakacin A, and pediocin PA-1, as well as a bacteriocin-negative control, were produced by heterologous expression in *Lactobacillus sakei* Lb790 as previously described (3, 4). Cultures were grown overnight at 30°C with the appropriate antibiotics and then reinoculated in fresh MRS medium without antibiotics before further incubation at 25°C. The CFS was prepared from a dense bacterial culture (optical density at 600 nm [OD_{600}], 3.5) by removal of cells by centrifugation at 15,000 × *g* for 15 min, followed by pH adjustment (pH 6.5) and heat treatment (80 to 90°C for 20 min) to eliminate protease activity. Aliquots of the CFS (stock solutions) of sakacin P, sakacin A, pediocin PA-1, and the bacteriocin-negative control were stored at −20°C. For induction of high-level resistance as described by Gravesen et al. (28), the sakacin P CFS was subjected to catalase treatment instead of heat treatment.

Production of purified bacteriocins of known concentrations. Sakacin P, sakacin A, and pediocin PA-1 were produced as described above and purified by ammonium sulfate precipitation, cation-exchange chromatography, hydrophobic interaction chromatography, and reverse-phase chromatography as previously described (18). The concentrations of the purified bacteriocins were assessed by measuring UV absorption at 280 nm (18). Purified bacteriocins, in appropriate predilutions in *Listeria* enrichment broth (LEB) (Oxoid Ltd.) with 0.1% Tween 80 (Sigma), were stored at −20°C until use.

Bacteriocin assay. *L. ivanovii* Li4 was used as an indicator in the microtiter plate assay for determining the concentrations of bacteriocins in the different stock solutions (CFS) essentially as previously described (4). One hundred microliters of a standardized inoculum was added to the wells (approximately 10^5 CFU/well) containing 100 μl of culture medium with bacteriocin at twofold dilutions. The turbidity was measured before and after incubation at 25°C for 17 h. Concentrations of bacteriocins in the various CFS were determined by correlating the activity of the CFS with the activity of the corresponding purified bacteriocin solution of known concentration applied on the same plate. Each concentration reported for the CFS is the mean of at least three different measurements of both purified bacteriocins and stock solutions. The concentrations of sakacin P, sakacin A, and pediocin PA-1 in the CFS were determined to be 2.7, 3.8, and 0.9 μg ml^{-1}, respectively.

IC_{50} determinations for 200 *L. monocytogenes* strains. Screening of the susceptibilities of the 200 strains to the bacteriocins sakacin P, sakacin A, pediocin PA-1, and nisin was performed as a modification of the bacteriocin assay described above. The IC_{50} are the calculated concentrations of a bacteriocin needed to inhibit growth by 50%, compared to uninhibited growth (i.e., average growth of the same strain in a medium without bacteriocin in eight wells on the same plate). All wells on a plate were filled with 100 μl of LEB containing 0.1% Tween 80 as in the bioassay. Duplicates of twofold dilutions of the stock solutions of sakacin P, sakacin A, pediocin-PA-1, and nisin were prepared in one microtiter plate. In addition, the probability of inhibition by bacteriocin-free CFS was tested for each strain. The inoculum for the IC_{50} test was prepared by diluting overnight cultures to an OD_{600} of 0.002 in LEB with 0.1% Tween 80. A 100-μl inoculum was added to wells containing bacteriocin as well as to the control wells containing only medium. The turbidity was measured, and the plates were incubated at 25°C until control wells for uninhibited growth reached an OD_{600} of 0.23 to 0.3 (approximately 17 h). The OD_{600} values were then used for the calculation of IC_{50}, and the averages of parallel IC_{50} with a coefficient of variation less than 15% are presented (Fig. 1).

Determination of correlation coefficients. Correlation coefficients between the IC_{50} of the four bacteriocins for the 200 *L. monocytogenes* strains were calculated by using Excel (Microsoft Office XP Professional, version 2002; Microsoft Corporation).

AFLP analysis. The primers and adapters used for amplified fragment length polymorphism (AFLP) analysis have been described by Vos et al. (58). Genomic DNAs from various strains of *Listeria* were prepared by using the Qiagen (Valencia, Calif.) tissue kit according to the manufacturer's instructions. Genomic DNA (approximately 0.5 μg) was incubated overnight at 37°C with 12 U of *Eco*RI (Promega, Madison, Wis.) and 4 U of *Mse*I (New England Biolabs,

Beverly, Mass.) in 1× NEB buffer 2 (New England Biolabs) with 1 ng of bovine serum albumin μl^{-1} in a total volume of 20 μl. To 5.5 μl of the digestion mix, 5.5 μl of a solution containing 2 μM *Eco*RI adapters and 2 μM *Mse*I adapters in 1× T4 DNA ligase buffer (Promega), 50 mM NaCl, and 1 ng of bovine serum albumin μl^{-1} with 12 U of *Eco*RI, 4 U of *Mse*I, and 0.5 U of T4 DNA ligase was added. Incubation was continued overnight at room temperature. After ligation, the reaction mixture was diluted 50-fold in TE buffer (10 mM Tris-HCl–0.1 mM EDTA [pH 8.0]) and stored at 4°C. The diluted ligation mix (5.0 μl) was then used as a template for PCR by adding 5.0 μl of 1× AmpliTaqGold DNA polymerase buffer, $MgCl_2$ to 5 mM, deoxynucleoside triphosphates (Promega) to 200 μM, 30 ng of primer AFML4 (5′ GAT GAG TCC TGA GTA AC 3′) and 5 ng of primer AFEC4 (5′ GAC TGC GTA CCA ATT CC 3′), and 2.5 U of AmpliTaqGold DNA polymerase in a total volume of 50 μl. PCRs were performed on a Perkin-Elmer 9700 thermal cycler. After a 10-min activation of the polymerase at 94°C, the following cycle profile was used: cycle 1, denaturation for 2 min at 94°C, annealing for 20 s at 66°C, and elongation for 2 min at 72°C; cycles 2 to 10, 2 min at 94°C, 20 s at an annealing temperature 1°C lower than the previous cycle, starting at 65°C, and 2 min at 72°C; cycles 11 to 21, 2 min at 94°C, 20 s at 56°C, and 2 min at 72°C; cycle 22, 30 min at 60°C. Two microliters of the amplified product was added to 25 μl of loading buffer (24 μl of deionized formamide and Gene Scan 500 TAMRA standard [Applied Biosystems]). All samples were denatured for 5 min at 95°C and then rapidly cooled on ice prior to electrophoresis. Amplified fragments were separated on an ABI Prism Genetic Analyzer 310. Fragment profiles were analyzed by using GelCompare II with Pearson product moment correlation (*r*) and the unweighted pair group method with arithmetic mean (UPGMA; Applied Maths, Sint-Martens-Latem, Belgium).

SDS-PAGE of whole-cell proteins. Whole-cell protein extracts were prepared, and SDS-PAGE was performed as described by Pot et al. (45). Duplicate protein extracts were prepared to check the reproducibility of the growth conditions and the preparation of the extracts. Registration of the protein patterns, normalization of the densitometric traces, and pattern storage were performed using GelCompar software (version 4.2; Applied Maths). The strains were grouped by using GelCompar software (version 4.2) with the Pearson product moment correlation coefficient (*r*) and UPGMA cluster analysis as previously described (45).

Fatty acid analysis. Cells were saponified, methylated to fatty acid methyl esters, and extracted by following the Sherlock Microbial Identification System (version 3.0; MIDI, Inc., Newark, Del.). Fatty acid methyl esters were then separated on an Agilent 6890A series gas chromatograph with a 7683 autoinjector and an autosampler tray module (Agilent Technologies Inc., Wilmington, Del.). The separation was achieved with a fused-silica capillary column (25 m by 0.2 mm) with cross-linked 5% phenylmethyl silicone (film thickness, 0.33 μm; HP Ultra 2). H_2 was used as the carrier. Peak integration and identification were performed by using a Chemstation (Hewlett-Packard GmbH, Waldbronn, Germany) and the Sherlock software (MIDI, Inc.).

Development of high-level resistance to class IIa bacteriocins. Overnight cultures of *L. monocytogenes* were serially diluted in peptone saline before 5 μl was spotted onto tryptic soy agar (TSA) plates (Oxoid Ltd.) with or without 30% fermentate containing sakacin P as previously described (28, 29). After incubation at 30°C for 2 days, spontaneously resistant colonies on TSA plates with sakacin P and colonies from TSA plates (control without fermentate) were picked. The frequencies of mutations could be calculated, and the IC_{50} of sakacin P, sakacin A, pediocin PA-1, and nisin were determined for both mutants and wild-type strains.

RESULTS

IC_{50}. Figure 1 shows the differences in the susceptibilities of the 200 different *L. monocytogenes* strains to sakacin P, sakacin A, pediocin PA-1, and nisin. The IC_{50} of sakacin P were in the lowest range, from 0.01 to 0.61 ng ml^{-1}. For pediocin PA-1, sakacin A, and nisin, the IC_{50} were 0.10 to 7.34, 0.16 to 44.2, and 2.2 to 781 ng ml^{-1}, respectively.

The IC_{50} of sakacin P divided the collection of *Listeria* strains into two clearly distinct groups based on susceptibility (Fig. 1). For 37% of the strains, the IC_{50} were equal to or less than 0.041 ng of sakacin P ml^{-1}; for the remaining 63%, the IC_{50} were equal to or above 0.066 ng of sakacin P ml^{-1}. Ten of the strains for which the IC_{50} were immediately above 0.066 ng ml^{-1} and 10 strains for which the IC_{50} were below 0.041 ng ml^{-1} were analyzed further by SDS-PAGE of whole-cell proteins, fatty acid analysis, and AFLP. Both the AFLP and SDS-PAGE studies grouped the 20 strains into two distinct groups (Fig. 2) that essentially correlated with the grouping obtained by using the IC_{50} of sakacin P. Such a grouping was not obtained by fatty acid analysis.

One of the 200 strains, *L. monocytogenes* L1040, had a special susceptibility pattern. It was the most tolerant to all three class IIa bacteriocins (IC_{50}, 0.61, 7.34, and 44.2 ng ml^{-1} for sakacin P, pediocin PA-1, and sakacin A, respectively [Fig. 1A through C]). It was, however, among the 40 strains most sensitive to nisin (IC_{50}, 257 ng ml^{-1}).

Correlation coefficients. There was a high correlation between the IC_{50} of sakacin P and that of pediocin PA-1 for the 200 strains. The correlation coefficient between the IC_{50} of sakacin A and that of nisin was higher than the correlation coefficient between the IC_{50} of sakacin A and that of sakacin P (Table 1).

Spontaneous high-level resistance. Four strains (two from each sakacin P IC_{50} group) as well as *L. monocytogenes* strain L1040 were spotted onto sakacin P agar plates, and mutants were obtained. With these four strains, the IC_{50} of sakacin P, pediocin PA-1, and sakacin A for the mutants were 1,000 times higher than those for the wild-type strains. The sakacin P IC_{50} for mutants of *L. monocytogenes* L1040 also was 1,000 times higher than that for the wild-type strain. The stock solutions of sakacin A and pediocin PA-1 were not concentrated enough to determine proper IC_{50}, but the IC_{50} of sakacin A and pediocin PA-1 for the *L. monocytogenes* L1040 mutants were at least 64 times higher than those for the wild-type strain. Sakacin P-resistant mutants were developed with frequencies of approximately 10^{-5} for all five strains. The mutations did not influence the strains' susceptibility to nisin (data not shown).

DISCUSSION

Reports on the potency of bacteriocins against *L. monocytogenes* often suffer from the investigation of a small number of indicator strains (9, 18, 20), and it is generally difficult to compare studies on susceptibility to bacteriocins due to differences in methodology and terminology. In the present study, the susceptibility of as many as 200 different strains of *L. monocytogenes* to four bacteriocins, expressed as individual IC_{50} in nanograms per milliliter, were determined in order to obtain the detailed information needed to draw conclusions about differences in the potencies of these bacteriocins against *L. monocytogenes* and to reveal possible differences in susceptibility patterns between strains.

The nature of bacteriocin solutions is important in susceptibility testing. The complete purification of three class IIa bacteriocins to the amounts required for all IC_{50} tests was considered a laborious task. It was therefore decided to use heterologously expressed bacteriocins from the same host strain, a system already available and used (4, 5). Since the background would be constant, this was considered far better than using different (wild-type) production strains with different and unknown backgrounds. Still, there is a theoretical possibility that some compound(s) produced by the host strain with no inhibitory activity of its own (as shown by the control

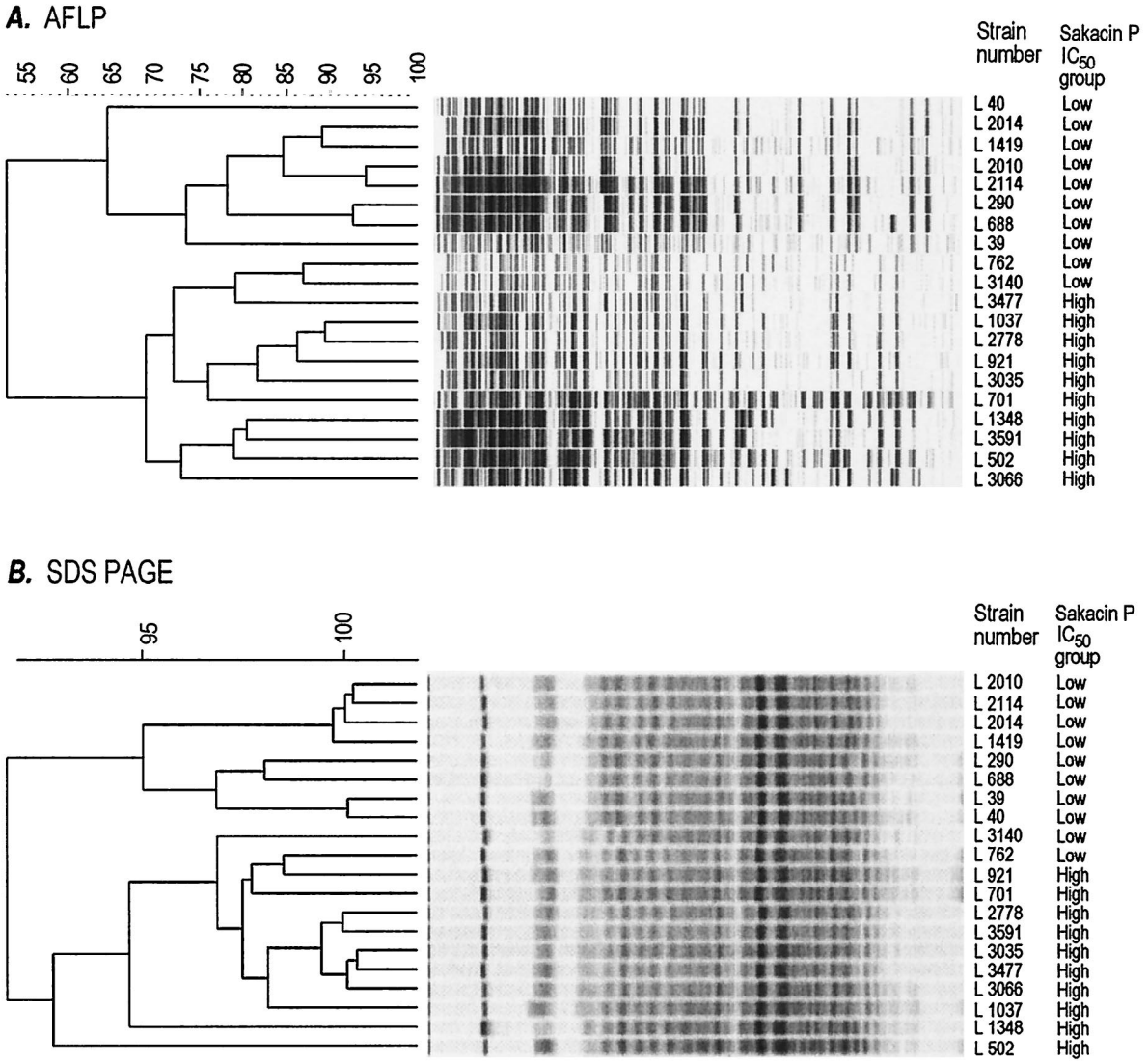

FIG. 2. Dendrograms created with GelCompare II (see the text for details) based on AFLP analysis (A) and SDS-PAGE analysis (B) of 20 *L. monocytogenes* strains. Scales above the dendrograms show the percent similarity level in the cluster analysis. The strains' susceptibilities to sakacin P are measured as 50% inhibition of growth in a microtiter plate assay and are designated either Low (sakacin P IC_{50}, ≤ 0.041 ng ml^{-1}) or High (sakacin P IC_{50}, ≥ 0.066 ng ml^{-1}).

test) could affect the three class IIa bacteriocins differently. This would nevertheless not affect one of the main subjects of this study, namely, the differences in the susceptibilities of *L. monocytogenes* strains to each bacteriocin. Regarding the potency of the bacteriocins, the differences observed in this study have also been confirmed with pure bacteriocins for some of the *L. monocytogenes* strains (G. Fimland, personal communication). This suggests that the influence of the background supernatant on the IC_{50} results is very small.

All 200 strains of *L. monocytogenes* were sensitive to all bacteriocins tested, and the IC_{50} of nisin, sakacin P, sakacin A, and pediocin PA-1 demonstrate the natural differences in susceptibility to these bacteriocins. As can be seen in Fig. 1, there were large strain-to-strain differences in susceptibility for each of the bacteriocins used. This is in accordance with previous reports (6, 20, 22, 47, 54), and such strain-to-strain differences have to be considered when bacteriocins are to be used as biopreservatives.

In Fig. 1, the IC_{50} for the 200 strains are sorted in ascending order for each of the bacteriocins. The sakacin P response (Fig. 1A) differs from the effects of the other bacteriocins in that the bacterial strains were divided into two groups based on the sakacin P IC_{50}. For all of the sakacin P-sensitive strains in the lower group (which contains 74 strains), the IC_{50} of sakacin P were below the minimum IC_{50} determined for any of the other bacteriocins (Fig. 1). The discriminatory power of the method used in this study is greater than that obtained by determination of the highest of twofold dilutions inhibiting growth (20) or by susceptibility determinations based on plating on a limited number of bacteriocin concentrations in agar plates (47). The discovery of such a grouping of strains as is seen in Fig.

TABLE 1. Correlation coefficients between IC_{50} of sakacin P, sakacin A, pediocin PA-1, and nisin for 199 *L. monocytogenes* strains[a]

Bacteriocin	Correlation coefficient with:			
	Sakacin P	Sakacin A	Pediocin PA-1	Nisin
Sakacin P	1.00			
Sakacin A	0.49	1.00		
Pediocin PA-1	0.84	0.68	1.00	
Nisin	0.26	0.65	0.35	1.00

[a] One strain displayed an extreme susceptibility pattern relative to those of the 199 others. The IC_{50} for this strain were removed from the data set before the correlations were calculated.

1A shows the importance of the discrimination obtained in this study by determinations of individual IC_{50}.

To obtain additional information about the characteristics of strains in the "low" and "high" sakacin P IC_{50} group, 10 strains from each group were subjected to AFLP, fatty acid analyses, and SDS-PAGE of whole-cell proteins. Statistical analysis of the fatty acid composition of the membranes from these strains did not give any distinct grouping (data not shown). Fatty acid composition has previously been correlated to induced resistance to bacteriocins (55) but may not be involved in natural differences in susceptibility. All strains were grouped equally into two major groups based on results from both AFLP and SDS-PAGE, although the grouping based on SDS-PAGE occurred at a much higher similarity level and was not clearly apparent by visual examination. As can be seen in Fig. 2, the correlation between the sakacin P grouping and the grouping based on AFLP and SDS-PAGE was high. Further investigations are needed to explain the relation between sakacin P susceptibility and other classifications, as well as the exceptions to this correlation, i.e., strains L762 and L3140 (Fig. 2). Another feature of the two sakacin P IC_{50} groups was connected to the distribution of serotypes, as the group for which sakacin P IC_{50} were low contained 31 of 33 serotype-4 *L. monocytogenes* strains. Since the serotype describes a surface property, we speculate that surface properties in serotype-4 strains are partly responsible for the high potency of sakacin P against these strains.

Rasch and Knøchel (47) have reported the natural differences in the susceptibilities of 350 *L. monocytogenes* strains to nisin and pediocin PA-1 based on growth characteristics on agar containing three different concentrations of the bacteriocins (given in arbitrary units). Susceptibility to bavaricin A (another name for sakacin P) was determined for 22 of the strains, and for these strains the resistance patterns for bavaricin A and pediocin PA-1 are reported to be in complete agreement (47). However, these investigators' use of arbitrary units and the lack of individual MIC or IC_{50} make a comparison to the results obtained in this study difficult. Identical susceptibility patterns for class IIa bacteriocins have also been reported for a lower number of *L. monocytogenes* strains (20), based on a method not as highly discriminatory as the method used in this study. Identical susceptibility patterns for all the strains would have returned very high, and equal, correlation coefficients between the IC_{50} of the class IIa bacteriocins. We conclude that the patterns are not identical for class IIa bacteriocins, as the correlation coefficients (Table 1) vary from 0.49 to 0.84. These findings are due to the improved discrimination in determination of IC_{50} combined with the high number of different strains investigated.

Figure 1 shows that the IC_{50} for nisin is higher than the IC_{50} for the class IIa bacteriocins, as reported earlier (9). This is perhaps due to the different modes of action of the lantibiotics (nisin) and class IIa bacteriocins (34). It is therefore surprising that the correlation between nisin and sakacin A is higher than the correlation between sakacin P and sakacin A. Class IIa bacteriocins are generally known to be highly effective against *Listeria* spp. (18, 21, 44, 50). This is the first report of individual IC_{50} of sakacin P, sakacin A, and pediocin PA-1, expressed in nanograms per milliliter, for this high number of *L. monocytogenes* strains, and sakacin P is shown to be the most potent of the class IIa bacteriocins. For approximately half of the strains, the sakacin P IC_{50} were lower than the lowest pediocin PA-1 IC_{50}, and nearly all of the sakacin A IC_{50} were higher than the maximum sakacin P IC_{50} (Fig. 1). This agrees with the observations of Fimland (23), who reports that sakacin P is more potent than pediocin PA-1 against many *L. monocytogenes* strains. Important characteristics of class IIa bacteriocins are their cysteine content and their ability to make disulfide bridges. Pediocin PA-1/AcH, enterocin A, divercin V41, sakacin G, and plantaricin 423 are known to possess two disulfide bridges, whereas the other class IIa bacteriocins contain a single disulfide bridge (18, 21, 26, 51, 56). Structure-function analyses of pediocin-like bacteriocins (24–26) show that a less flexible mutant of sakacin P, harboring an additional disulfide bridge (more like pediocin PA-1), displays lower activity against *Listeria* spp. than natural sakacin P. According to Fimland (23), this clearly indicates that sakacin P, the more flexible molecule, is capable of forming more favorable interactions with some *Listeria* sp. strains. The significance of disulfide bridges for the potency of class IIa bacteriocins may be genus or species dependent. Based on the MICs of different class IIa bacteriocins for one single *L. ivanovii* strain, Guyonnet et al. (32) concluded that the peptides with two disulfide bridges are significantly more effective than those harboring a single disulfide bridge. In a study of LAB and *Listeria* strains, the increased activities of pediocin PA-1 and enterocin A (two disulfide bridges) compared to that of sakacin P seemed to be more pronounced for LAB strains than for *Listeria* spp. (18). Eijsink et al. (18) suggested that the high levels of activity in pediocin PA-1 and enterocin A are at least partly due to the extra disulfide bridge. In the present study only *L. monocytogenes* is analyzed, and the results indicate that for this species, factors other than the number of disulfide bridges are important for the potency of the bacteriocins. Based on susceptibility testing of 200 different strains, it is clear that sakacin P is more potent than pediocin PA-1 and sakacin A against *L. monocytogenes*, under the experimental conditions used here.

If bacteriocins are to be used as biopreservatives in foods, development of resistance to these antimicrobial compounds has to be considered. Recently the mechanism in *L. monocytogenes* for high-level resistance to class IIa bacteriocins has been reported (29). None of the 200 strains examined in this study showed high-level resistance, and none of the strains could be classified as resistant. It has been reported that high-level resistance to one class IIa bacteriocin confers cross-resistance to other class IIa bacteriocins (29, 46). This study shows

that spontaneous high-level resistant mutants, cross-resistant to other class IIa bacteriocins, develop also when sakacin P is the selective agent used in the agar. However, the IC_{50} of nisin for the high-level-resistant mutants was the same as that for the wild type strains, in agreement with other reports (17, 46). Cross-resistance between class IIa bacteriocins has been reported frequently (7, 17, 46–48), but reports concerning cross-resistance between bacteriocins of different classes contain contradictory results (7, 11, 41, 46, 47, 52, 54).

Resistance is a relative term, and there is a need for guidelines for the classification of different species according to their susceptibilities to various bacteriocins. Such guidelines should reflect the concentrations of bacteriocins used or produced in food products when bacteriocins or bacteriocinogenic strains are added as biopreservatives. Intrinsic factors affect the activity of a bacteriocin in a food product (2, 5, 27), but studies have shown that susceptibility differences measured in microtiter plate assays are comparable to differences seen for the same strains of *L. monocytogenes* in food model systems (37). Based on this study, sakacin P appears to be a candidate for inhibition of *L. monocytogenes* in food. It has been shown previously that sakacin P inhibits *L. monocytogenes* without development of resistant strains in food model experiments with cold smoked salmon and chicken cold cuts (36, 37). Bacteriocins can be applied as biopreservatives in many food systems. However, they should not be the only preservative principle used; rather, they should be part of a system with multiple preservative principles, so-called "hurdles" (10, 21).

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