Lysine-Oriented Charges Trigger the Membrane Binding and Activity of Nukacin ISK-1

Sikder M. Asaduzzaman,1 Jun-ichi Nagao,1 Yuji Aso,1 Jiro Nakayama,1 and Kenji Sonomoto1,2*

Laboratory of Microbial Technology, Division of Microbial Science and Technology, Department of Bioscience and Biotechnology, Faculty of Agriculture, Graduate School, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan,1 and Laboratory of Functional Food Design, Department of Functional Metabolic Design, Bio-Architecture Center, Kyushu University, 6-10-1 Hakozaki, Higashi-ku, Fukuoka 812-8581, Japan2

Received 24 March 2006/Accepted 6 July 2006

The antibacterial activities and membrane binding of nukacin ISK-1 and its fragments and mutants were evaluated to delineate the determinants governing structure-function relationships. The tail region (nukacin1–7) and ring region (nukacin7–27) were shown to have no antibacterial activity and also had no synergistic effect on each other or even on nukacin ISK-1. Both a fragment with three lysines in the N terminus deleted (nukacin4–27) and a mutant with three lysines in the N terminus replaced with alanine (K1-3A nukacin ISK-1) imparted very low activity (32-fold lower than nukacin ISK-1) and also exhibited a similar antagonistic effect on nukacin ISK-1. Addition of two lysine residues at the N terminus (+2K nukacin ISK-1) provided no further increased antibacterial activity. Surface plasmon resonance sensograms and kinetic rate constants determined by a BIAcore biosensor revealed that nukacin ISK-1 has remarkably higher binding affinity to anionic model membrane than to zwitterionic model membrane. Similar trends of strong binding responses and kinetics were indicated by the high affinities of nukacin ISK-1 and +2K nukacin ISK-1, but there was no binding of tail region, ring region, nukacin1–27, and K1-3A nukacin ISK-1 to the anionic model membrane. Our findings therefore suggest that the complete structure of nukacin ISK-1 is necessary for its full activity, in which the N-terminus three lysine residues play a crucial role in electrostatic binding to the target membrane and therefore nukacin ISK-1’s ability to exert its potent antibacterial activity.

The emergence of resistance to traditional antibiotics among microorganisms has drawn the keen attention of researchers to ameliorate the antibiotic repertoires. In search of alternative antibacterial agents, lantibiotics seem to be one of the most promising candidates. Lantibiotics are synthesized on the ribosome as prepeptides that undergo several posttranslational modification events associated with several enzymes to form biologically active peptides (9, 25). Engineering of the naturally occurring novel lantibiotics might provide significant information for rational design of lantibiotics with improved activity and/or spectra that would be potent antimicrobials to augment, supplement, or replace the currently used antibiotics.

The antibacterial mode of actions of lantibiotics has been found to be diverse, and their activities pertain mostly to attacking the bacterial membrane, which eventually is induced to release ions, small molecules, and ATP from sensitive cells (16, 25, 27, 31). In addition, lantibiotics are reported to inhibit outgrowth of bacterial spores (22) and hinder cell wall biosynthesis (31). Among the structures/functions of lantibiotics, the type A(I) lantibiotic nisin has been explicitly studied to know the structures responsible for its work as a potential antibiotic. The cationic nature of nisin allows it to bind to the phospholipid membrane by electrostatic interactions (4, 12, 33), and it exhibits higher affinity to anionic than to zwitter-ionic model membrane (8). N-terminal backbone amides of nisin interact with the pyrophosphate moiety of lipid II (14), and the transmembrane orientation of the molecule involves the insertion of its C-terminal part for pore formation (30). However, type A(II) lantibiotics that have two distinct regions (N-terminus tail and C-terminus ring) have not yet been studied well, and little is known about the structures of these peptides that are important for different steps necessary to function as antibiotics.

Nukacin ISK-1 is a novel type A(II) lantibiotic produced by Staphylococcus warneri ISK-1, isolated in our laboratory from well-aged Nukadoko, a bed of fermented rice bran (19, 20). It consists of 27 amino acids, including two molecules of lanthionine, one molecule of 3-methylthionionine and one residue of dehydrobutyryne (Fig. 1) (1, 26). In this study, we generated and evaluated the fragments and mutants of nukacin ISK-1 to determine the roles of the structures involved in membrane binding and antibacterial activity. We used surface plasmon resonance (SPR) determined by BIAcore biosensor to investigate the binding behavior of nukacin ISK-1 and its fragments and mutants to model membrane. SPR is a fast and powerful tool for real-time monitoring of binding of the membrane-active peptides that supersedes the other relevant techniques (24). Our results suggested that the complete structure of nukacin ISK-1 is necessary for its full activity, and the first three lysine residues in the tail region play the vital role in its antibacterial activity, in which the positive charges are the key determinant for membrane binding of nukacin ISK-1. Since there is still little information on the mode of action of type A(II) lantibiotics, this study was designed to provide new in-
plasmids, pNZAK1-3A and pNZ9000 were done according to the method developed by Holo and Nes (13). The resultant buffer, pH 8.0, containing 0.5 mM CoCl$_2$ to make a 1-ml reaction volume, and N was purified in accordance with the protocol described by Aso et al. (2). 

Sight into how type A(II) lantibiotics function in terms of the primary mode of action.

MATERIALS AND METHODS

Synthesis and purification of nukacin ISK-1 and its fragments. Nukacin ISK-1 was purified in accordance with the protocol described by Aso et al. (2).

The tail region (nukacin$_{2-27}$) of nukacin ISK-1 was synthesized chemically by a solid-phase method using a 9-fluorenlymethoxy carbonyl (Fmoc) strategy with p-alkoxybenzyl alcohol resin (0.1 mmol; Kokusai Chemical, Tokyo, Japan) as the solid support. After chain assembly, the peptide was cleaved off from the support and the solid chain-protecting groups were also deprotected by treatment with a trifluoroacetic acid (TFA)-trisopropylsilane-water mixture (95:2.5:2.5, vol/vol/vol) for 90 min. The reaction mixture was passed over an HILIC-DISK 13 (Kanto Chemical, Tokyo, Japan), and cold diethyl ether was added. The mixture was kept standing overnight at ~20°C. The precipitant was collected on a polytetrafluoroethylene membrane filter (ADVANTEC, Ehime, Japan). The crude peptide was dissolved in 20% acetonitrile–0.1% TFA and then purified by reverse-phase (RP) column (PepRPC HR 5/5; Amersham Bioscience, Uppsala, Sweden) integrated in an LC-10A high-performance liquid chromatography (HPLC) system (Shimadzu, Kyoto, Japan). Peptides were eluted with a linear gradient of 15 to 90% acetonitrile–0.1% TFA at a flow rate of 1 ml/min and were analyzed by electro spray ionization-mass spectrometry (ESI-MS) (Shimadzu, Tokyo, Japan).

The ring region (nukacin$_{7-27}$) was obtained by digestion of nukacin ISK-1 (180 µg) with Pfu N-acetyl deacetylating aminopeptidase (Takara, Shiga, Japan) (30 µg) in the buffer supplied by the manufacturer (250 mM N-ethylmorpholine-acOH buffer, pH 8.0, containing 0.5 mM CoCl$_2$) to make a 1-ml reaction volume, and the final reaction mixture was incubated at 50°C for 72 h. The nukacin$_{4-27}$ fragment was generated by deletion of three lysine residues from the N terminus of nukacin ISK-1 (42 µg) by digestion with endoproteinase Lys-C (Sigma-Aldrich, St. Louis, MO) dissolved in 100 mM NH$_4$HCO$_3$ buffer (pH 8.5) to make a 1-ml reaction mixture and was incubated at 25°C for 24 h. After purification of the fragments by RP-HPLC, masses of the peptides were determined by ESI-MS.

Generation of nukacin ISK-1 mutants. K1-3A nukacin ISK-1 and +2K nukacin ISK-1 were generated by amplification of the nukacin ISK-1 structural gene (nukA) by PCR using pNZAK1-3A and pNZ9000 (13). The resultant plasmids, pNZAK1-3A and pNZ900A +2K, were extracted by the method of O’Sullivan and Claenhammer (23) and were then introduced into L. lactis NZ9000 harboring plasmid plnukdA, which contains all of the nukacin ISK-1 biosynthetic genes except for the nukA gene (1). Nukacin ISK-1 mutants were expressed by a nisin-controlled expression system basically in accordance with the method of Aso et al. (1). The recombinant strains were grown in chemically defined medium (18) with 5 µg/ml each chloramphenicol and erythromycin at 30°C. Nisin solution (crude nisin) (Sigma-Aldrich) was added to 10-µg/ml final concentrations to the culture at an optical density at 600 nm (OD$_{600}$) of 0.6, and incubation continued for another 5 h.

For purification of expressed K1-3A nukacin ISK-1 and +2K nukacin ISK-1, culture supernatant was collected by centrifugation of the culture at 6,000 × g at 4°C for 15 min. Ten milliliters of culture supernatant was loaded onto a Sep-Pak C$_{18}$ cartridge column (100 mg; Waters, Milford, MA), washed with 2 ml of 0.1% TFA, and eluted with 3 ml of 50% acetonitrile–0.1% TFA. Concentrated eluates were injected directly into the RP-HPLC column, and the mass of the peptides was determined by ESI-MS. The N-terminal amino acid sequences of the mutants were obtained by Edman degradation performed on a PPSQ-21 gas-phase automatic protein sequence analyzer (Shimadzu).

Antibacterial activity. Antibacterial activities of purified nukacin ISK-1 and its fragments and mutants dissolved in water (pH 7.0) were determined by the spot-on-lawn method (20). Lactobacillus agar (Becton Dickinson, Sparks, MD) was overlaid on MRS agar medium with the specific indicator strains, and serial twofold-diluted peptides with water (pH 7.0) in 96-well plates (96 microwell plates, Nunc, Roskilde, Denmark) were spotted onto the surface of the medium. By observation of the clear zone of inhibition, antibacterial activities were expressed as the MIC. Lactobacillus sakei subsp. sakei JCM 11577 and Lactococcus mesenteroides subsp. mesenteroides subsp. niger JCM 6124T, grown in MRS broth at 30°C, and Pediococcus pentosaceus JCM 5885, grown in MRS broth medium at 37°C, were used as indicators to determine antibacterial activity. Inhibitory activities of the fragments/mutants were determined with equimolar concentrations of nukacin ISK-1 and the fragment/mutation.

Preparation of model membranes. The phospholipids (Sigma-Aldrich) used were 1,2-dimyristoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (anionic) and 1,2-dioleoyl-sn-glycero-3-phosphocholine (zwitterionic). Small unilamellar vesicles were prepared separately by dissolving 3 mg of each of the phospholipids in 10 ml chloroform. The suspension was evaporated under N$_2$ and then dissolved in 10 mM HEPES buffer (final liposome concentration, 0.5 mM). After sonication, the liposome was dispensed into a glass tube and stored at ~30°C.

SPR biosensor. SPR spectroscopy using a BIACore biosensor (BIACore AB, Uppsala, Sweden) was used to determine the interaction of nukacin ISK-1 and its fragments and mutants with the previously described model membrane by an HPA sensor chip (BIACore AB). All of the experiments were performed at 25°C in HBS buffer (10 mM HEPES, 150 mM NaCl, pH 7.4). Anionic or zwitterionic vesicle (0.5 mM, 80 µl each) was applied to the flow cell of the HPA sensor chip surface at a flow rate of 2 µl/min. To remove the multilamellar lipid vesicles and also to regenerate the model membrane, 10 mM NaOH (25 µl) was injected at a flow rate of 5 µl/min to result in a stable baseline corresponding to the lipid monolayer. Nukacin ISK-1 and its fragments and mutants were injected (7 µl, 15 µl) onto the lipid surface at a flow rate of 5 µl/min. The bulk effect of buffer composition was subtracted from each set of experimental sensograms. The kinetic values, $k_a$ (association rate constant) and $k_d$ (dissociation rate constant) of the resultant sensograms were determined by the BIACalculator 2.1 software (BIACore AB). The dissociation constant ($K_d$) was calculated as $k_d/k_a$. SPR spectroscopy was done at least twice for each experiment.

RESULTS

Comparison of antibacterial activities of nukacin ISK-1 and its fragments and mutants. Chemically synthesized tail region (nukacin$_{2-27}$), Pfu N-acetyl deacetylating aminopeptidase-digested ring region (nukacin$_{2-27}$), endoproteinase Lys-C-digested nukacin$_{2-27}$ fragment, and genetically engineered K1-3A nukacin ISK-1 and +2K nukacin ISK-1 (Fig. 1) were successfully obtained, and the masses of each were determined exactly to their calculated values. RP-HPLC-purified fragments and mutants were used to determine antibacterial activities along with their synergistic effects on each other and also on nukacin ISK-1 against L. sakei subsp. sakei JCM 11577, L. mesenteroides subsp. mesenteroides JCM 6124T, and P. pentosaceus JCM 5885 as indicators. No activity of the ring region and tail region.
TABLE 1. Antibacterial activities of nukacin ISK-1 and its fragments and mutants along with their inhibitory effects on nukacin ISK-1 and each other

<table>
<thead>
<tr>
<th>Nukacin ISK-1 or derivative</th>
<th>MIC (µM)</th>
<th>L. sakei</th>
<th>P. pentosaceus</th>
<th>L. mesenteroides</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nukacin ISK-1 (control)</td>
<td>0.26</td>
<td>0.52</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>+2K nukacin ISK-1</td>
<td>0.26</td>
<td>0.52</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Nukacin ISK-1 + tail region</td>
<td>0.26</td>
<td>0.52</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Nukacin ISK-1 + ring region</td>
<td>0.26</td>
<td>0.52</td>
<td>0.26</td>
<td></td>
</tr>
<tr>
<td>Nukacin ISK-1 + K1-3A</td>
<td>1.04</td>
<td>2.08</td>
<td>1.04</td>
<td></td>
</tr>
<tr>
<td>Nukacin ISK-1 + nukacin4–27</td>
<td>1.04</td>
<td>2.08</td>
<td>1.04</td>
<td></td>
</tr>
<tr>
<td>K1-3A nukacin ISK-1</td>
<td>8.32</td>
<td>16.64</td>
<td>8.32</td>
<td></td>
</tr>
<tr>
<td>Nukacin4–27</td>
<td>8.32</td>
<td>16.64</td>
<td>8.32</td>
<td></td>
</tr>
<tr>
<td>Tail region (nukacin4–27)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Ring region (nukacin7–27)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Tail region + ring region</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
</tbody>
</table>

*Antibacterial activity was determined at least twice for each derivative. Results are shown for L. sakei subsp. sakei JCM 11577T, P. pentosaceus JCM 5885, and L. mesenteroides subsp. mesenteroides JCM 6124T. ND, not detected.

Binding affinity of nukacin ISK-1 to the model membranes.

SPR determined by the BIAcore biosensor suggested strong binding responses of nukacin ISK-1 to anionic model membrane in contrast to the zwitterionic model membrane (Fig. 2). The integrity of the sensor chip HPA was also checked by allowing nukacin ISK-1 to bind to the free surface of its flow cells, and no affinity to the sensor chip was found (data not shown). Differences between the kinetics of each of the sensorgrams suggested different binding affinities towards the model membrane and provided further evidence for stronger binding of nukacin ISK-1 to anionic (K_D, 5.15 µM) than to zwitterionic (K_D, 32.5 µM) model membrane (Table 2). The variations in dissociation constant (K_D) of nukacin ISK-1 to the anionic and zwitterionic model membranes were due to variations of both the association rate constant (k_a) and dissociation rate constant (k_d). These differences can be explained quantitatively to emphasize the binding behavior of nukacin ISK-1. The rate constants (k_a and k_d) distinctly indicate strong association and high stability of nukacin ISK-1 to anionic model membrane in contrast to zwitterionic model membrane, which were reflected similarly by the entire sensorgram showing high association (high number of resonance units [RU]) and low dissociation with anionic membrane and vice versa for zwitterionic membrane (Table 2). Kinetics data therefore strongly supported the sensorgrams and indicated high binding affinity of nukacin ISK-1 to anionic membrane in comparison with the zwitterionic model membrane. Variation of nukacin ISK-1 concentrations (5, 15, and 20 µM) allowed us to obtain high binding responses to anionic model membrane at higher concentrations (Fig. 3). Binding of nukacin ISK-1 to the model membrane thus correlated well with the amount of nukacin ISK-1.

Comparison of the binding affinities of nukacin ISK-1 and its fragments and mutants to the model membranes.

The SPR data detected as RU for the signal intensity obtained by the BIAcore biosensor were the functions of nukacin ISK-1 and its fragments and mutants for binding to the anionic model membrane (Fig. 4). The concentrations of nukacin ISK-1 and its fragments and mutants were 7 µM throughout this study. Strong binding responses of nukacin ISK-1 and +2K nukacin

![FIG. 2. Binding affinity of nukacin ISK-1 to anionic [1,2-dimyristoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt] and zwitterionic [1,2-dioleoyl-sn-glycero-3-phosphocholine] model membranes determined by SPR biosensor. Sensorgrams for 7 µM nukacin ISK-1 bound to each of the anionic (a) and zwitterionic (b) model membranes are indicated.](image)

![FIG. 3. Dose response of nukacin ISK-1 towards the anionic model membrane. Nukacin ISK-1 concentrations were 20 (a), 15 (b), and 5 (c) µM.](image)
nukacin ISK-1 were detected by the entire sensorgrams that were further supported by the affinity rate constants for recognition and stability. Comparable but somewhat stronger (~1.5-fold) affinity ($K_D$, 5.15 μM and 2.97 μM for nukacin ISK-1 and +2K nukacin ISK-1, respectively) was observed for +2K nukacin ISK-1 (Table 2). No affinity of nukacin$_{4-27}$, K1-3A nukacin ISK-1, tail region, and ring region to the anionic model membrane was detected by the SPR response under the specified conditions. The hypothesis that nukacin ISK-1 would have affinity towards negatively charged membrane was strongly supported by the binding of nukacin ISK-1 and +2K nukacin ISK-1 to anionic phospholipid membrane. The results therefore suggested that the association of nukacin ISK-1 with the model membrane is primarily by the electrostatic interactions for the presence of positively charged residues that dramatically increased the number of resonance units of SPR. The apparent argument for the possibility of the tail region to bind to the membrane was clarified by determining its secondary structure. Except for the tail region, nukacin ISK-1 and other fragments were shown to form a similar secondary structure (data not shown). The SPR results were thus quite robust for proving that the complete structure of nukacin ISK-1, especially the presence of the positively charged lysine residues in the tail region, was crucial for strong binding of nukacin ISK-1 to the anionic model membrane.

**DISCUSSION**

We tried to depict our understanding of which structure of nukacin ISK-1 is responsible for membrane binding and exerts its antibacterial activity. In this study, we used purified nukacin ISK-1, its tail region (nukacin$_{1-7}$), ring region (nukacin$_{7-27}$), nukacin$_{4-27}$ fragment, K1-3A nukacin ISK-1, and +2K nukacin ISK-1 (Fig. 1) to determine the antibacterial activity and their synergistic effects on nukacin ISK-1 as well as each other (Table 1). No activity of the ring or tail region was observed, these regions showed no synergistic effect in combination, and neither of them had any effect on the activity of nukacin ISK-1. Activities of nukacin$_{4-27}$ and K1-3A nukacin ISK-1 were 32-fold lower than that of nukacin ISK-1, and both nukacin$_{4-27}$ and K1-3A nukacin ISK-1 exhibited an inhibitory effect (four-fold lower) on the antibacterial activity of nukacin ISK-1. Our results are in good agreement with the findings that for the nisin$_{1-12}$ fragment, deletion of five residues and deletion of a further nine residues from the C terminus of nisin resulted in no activity and 10-fold- and 100-fold-lower activities, respectively, along with the antagonistic effect of nisin$_{1-12}$ on nisin activity (7). Truncation of five residues from the N terminus of lacticin 481 has also been reported to show 10-fold-lower activity (28).

Peptide antibiotics are thought primarily to be effective by their cationic and amphipathic nature. Nukacin ISK-1 has three net positive charges (determined by GENETYX-WIN; Software Development, Tokyo, Japan), so the anionic bacterial membrane would be the target for binding of nukacin ISK-1. However, we also tested and compared the zwitterionic vesicle to prove the fidelity of anionic vesicle as a model membrane. Stronger binding to anionic than to zwitterionic membrane (Table 2 and Fig. 2) and dose-dependent binding proved the electrostatic interactions of nukacin ISK-1 with anionic model membrane (Fig. 3). Though binding evaluation of lantibiotics by SPR has not yet been reported by others, our work was concurred with the binding responses of other antibiotic peptides to the model membrane (21, 24).

We used the affinity-related structure-function relationship as the parameter for biological activity. The binding of nukacin ISK-1 and its fragments and mutants to the model membrane was evaluated to determine the relationship between binding ability (Fig. 4) and antibacterial activity (Table 1). Binding affinities shown by SPR sensorgrams were significantly correlated with their observed antibacterial activities. High binding of nukacin ISK-1 and +2K nukacin ISK-1 to the anionic membrane reflected their high antibacterial activities. Increase of the cationic property of nukacin ISK-1 by two additional lysine residues at the N terminus (+2K nukacin ISK-1) provided a relatively stronger (~1.5-fold) ability to bind to the model membrane (Fig. 2). However, this extra binding ability did not contribute further to enhancement of the antibacterial activity of +2K nukacin ISK-1. This was probably because the charges required for the electrostatic interactions to bind to the membrane would have already been satisfied by the three lysine residues to act as a novel antibacterial. Therefore, the increase in charges did not increase nukacin ISK-1's effectiveness as a potential antibiotic. Binding of nukacin ISK-1 to the membrane was thus proved to be primarily by the N terminus, and its antibacterial action might be dependent on the C terminus. No observable antibacterial activity of the tail and ring region was reflected, as expected by their inability to bind to the anionic model membrane. Many membrane-active peptides have been shown to form a secondary structure to undergo necessary alteration for various conformational changes needed to become an antibacterial (10, 11, 16, 17, 29). Our result also suggests that lack of secondary structure formation was the possible reason for the tail region not to bind to the anionic model membrane and not to show antibacterial activity, though the three lysine residues are present in the tail region. Low antibacterial activities of nukacin$_{4-27}$ and K1-3A nukacin ISK-1 were detected, but none of them showed electrostatic interaction to bind to the anionic model membrane. Since the degrees of binding were found to be directly related to endowing nukacin ISK-1 and +2K nukacin ISK-1 with antibacterial activity, the low activities of nukacin$_{4-27}$ and K1-3A nukacin ISK-1 might result from their binding by other weak
associations (e.g., hydrophobic interaction) with the cytoplasmic membrane of an indicator strain. Nukacin4–27 and K1-3A nukacin ISK-1 showed inhibitory activities against nukacin ISK-1, though they did not bind to the model membrane. It has become well established that binding to the membrane is the primary step of a membrane-active antibacterial peptide, and in vivo activity depends on more than one factor. Existing structure-based antibacterial activity of nukacin ISK-1 might also be possible, as was discussed later for other lantibiotics. Therefore, the competition of nukacin4–27 and K1-3A nukacin ISK-1 against nukacin ISK-1 would probably be at the next steps of its activity (e.g., competition for docking molecule or a binding motif) or might even be against enzyme function inhibition.

The mode of actions of type A(I) (nisin type) lantibiotics has unambiguously been clarified. The overlapping killing activities of nisin have been elicited to be complex. Nisin and nisin-like (e.g., subtilin) lantibiotics use lipid II as a docking molecule for high-affinity binding that combines pore formation and inhibition of cell wall biosynthesis (3, 6, 31). The nisin-lipid II complex has a novel lipid II-binding motif in which the N-terminal backbone amides of nisin coordinate the pyrophosphate moiety of lipid II (14). Besides, some other lantibiotics have already been shown to target the peptidoglycan precursor lipid II for their potent mode of actions. Wiedemann et al. (32) recently found that the overall inhibitory features of plantaricin C are more similar to those of nisin, where it strongly inhibits for their potent mode of actions. Wiedemann et al. (32) recently found that the overall inhibitory features of plantaricin C are more similar to those of nisin, where it strongly inhibits

ACKNOWLEDGMENTS

We are grateful to M. Kimura of Kyushu University, Fukuoka, Japan, for his kind permission to get easy access to use the BAcore biosensor. We are thankful to Yoshiko Morinaga for her sincere help with part of this study.

S.M.A. acknowledges a Monbukagakusho (MEXT, Japan) fellowship. This work was partially supported by grants from the Japan Society for the Promotion of Science (JSPS), the Japan Science Society, the Novartis Foundation (Japan) for the Promotion of Science, the Novozymes Japan Research Fund, and the Nagase Science and Technology Foundation.

REFERENCES


