The three-dimensional structure in dodecyl phosphocholine micelles of the 26-mer membrane-permeabilizing bacteriocin-like pheromone plantaricin A (PlnA) has been determined by use of nuclear magnetic resonance spectroscopy. The peptide was unstructured in water but became partly structured upon exposure to micelles. An amphipilic α-helix stretching from residue 12 to 21 (possibly also including residues 22 and 23) was then formed in the C-terminal part of the peptide, whereas the N-terminal part remained largely unstructured. PlnA exerted its membrane-permeabilizing antimicrobial activity through a nonchiral interaction with the target cell membrane because the D-enantiomeric form had the same activity as the natural L-form. This nonchiral interaction involved the amphipilic α-helical region in the C-terminal half of PlnA because a 17-mer fragment that contains the amphipilic α-helical part of the peptide had antimicrobial potency that was similar to that of the L- and D-enantiomeric forms of PlnA. Also the pheromone activity of PlnA depended on this nonchiral interaction because both the L- and D-enantiomeric forms of the 17-mer fragment inhibited the pheromone activity. The pheromone activity also involved, however, a chiral interaction between the N-terminal part of PlnA and its receptor because high concentrations of the L-form (but not the D-form) of a 5-mer fragment derived from the N-terminal part of PlnA had pheromone activity. The results thus reveal a novel mechanism whereby peptide pheromones such as PlnA may function. An initial nonchiral interaction with membrane lipids induces α-helical structuring in a segment of the peptide pheromone. The peptide becomes thereby sufficiently structured and properly positioned in the membrane interface, thus enabling it to engage in a chiral interaction with its receptor in or near the membrane water interface. This membrane-interacting mode of action explains why some peptide pheromones/hormones such as PlnA sometimes display antimicrobial activity in addition to their pheromone activity.

Many lactic acid bacteria secrete ribosomally synthesized antimicrobial peptides, termed bacteriocins. These peptides are usually membrane-permeabilizing and cationic and contain between 25 and 60 residues (1, 2). Their production is in some bacteria controlled by a three-component regulatory system consisting of a membrane-associated histidine protein kinase, response regulators, and a peptide pheromone with bacteriocin-like properties (3, 4). Plantaricin A (PlnA), produced by Lactobacillus plantarum C11, is such a peptide pheromone (5, 6). This cationic pheromone has antimicrobial membrane-permeabilizing activity (7), and it is exported out of the cell by a bacteriocin-secretion machinery that specifically recognizes the bacteriocin-like leader sequence on the pheromone precursor peptide (5, 6). PlnA acts as a pheromone by interacting with the membrane-associated histidine protein kinase of a three-component regulatory system, thereby triggering the kinase to phosphorylate two response regulators, which then in turn activate the genes encoding the two two-peptide bacteriocins, plantaricin E/F and J/K (9).

Three PlnA variants are produced by L. plantarum C11: a 26-residue full-length peptide (PlnA-26) and two N-terminally truncated forms containing 23 (PlnA-23) and 22 (PlnA-22) residues. The three variants are all derived from a 48-residue precursor encoded by the plnA gene (5), and they display identical antimicrobial (i.e. bacteriocin) and pheromone activities (6, 10). The D-enantiomeric form (contains only D-amino acids) of PlnA-22, whose structure is the mirror image of the L-form, has the same antimicrobial activity as the L-form (7), indicating that the antimicrobial activity of PlnA does not depend on chiral interactions and thus appears to involve only interactions with the lipids of the target cell membrane. The D-enantiomeric form, however, has no pheromone activity, but it inhibits the pheromone activity of the L-form, indicating that the pheromone activity is dependent on both chiral and nonchiral interactions.

To gain insight into how PlnA functions as a pheromone and antimicrobial peptide, the three-dimensional structure of PlnA-26 in the presence of lipid micelles was determined by use of NMR spectroscopy. Moreover, fragments of PlnA (both L- and D-enantiomeric forms) were used as probes to determine what parts of the peptide were involved in the antimicrobe-dependent nonchiral and the pheromone-dependent chiral and nonchiral interactions. The results of this structure-function analysis indicate that some peptide pheromones/hormones such as PlnA function through a novel membrane-interacting mechanism whereby they adopt a membrane-induced α-helical structure that subsequently enables the peptides to interact with their receptors.

This paper is available on line at http://www.jbc.org
EXPERIMENTAL PROCEDURES

Synthesis, Purification, and Analysis of Peptides—The natural \(\alpha\)-enantiomeric forms of PlnA-26 and -22 (PlnA-26-L and PlnA-22-L), the \(\beta\)-enantiomeric form of PlnA-22 (PlnA-22-D), the 5-mer N-terminal fragment of PlnA-22-L (N-5-L), and the 17-mer C-terminal fragment of PlnA-22-L (PlnA-17-L) were all synthesized according to the sequence reported previously (1). The \(\alpha\)-enantiomeric forms of the 5-mer N-terminal fragment (N-5-D) and the 17-mer C-terminal fragment of PlnA-22 (PlnA-17-D) (Fig. 1) were obtained by cyanogen bromide cleavage of PlnA-22-D as described previously (11). For purification, the peptides were dissolved in 0.1% (v/v) trifluoroacetic acid and applied to a 3-ml RESOURCE RPC reverse phase column (GE Healthcare) equilibrated with 0.1% (v/v) trifluoroacetic acid. The peptides were eluted from the reverse phase column with a linear 0–60% (v/v) 2-propanol gradient containing 0.1% (v/v) trifluoroacetic acid. The fractions containing peptides were collected, diluted five times with water containing 0.1% (v/v) trifluoroacetic acid, and rechromatographed on the reverse phase column. The primary structure and purity of the peptides were confirmed by mass spectrometry using a Voyager-DE RF matrix-assisted laser desorption ionization time-of-flight mass spectrometer (PerSeptive Biosystems) and analytical reverse phase chromatography. Peptide concentrations were determined by measuring the absorbance at 280 nm and using the molar extinction coefficients deduced from the amino acid composition.

Assay for Antimicrobial/Bacteriocin Activity—Antimicrobial (bacteriocin) activity was determined essentially as described earlier (7), using the microtiter plate assay and Lactobacillus sake NCDO 2714 and L. plantarum 965 as indicator cells. Each well of the plate contained 200 \(\mu\)l of MRS broth (Oxoid), peptides at 2-fold dilutions, and the indicator cells at an \(A_{600}\) of about 0.01. The microtiter plate cultures were incubated for 12–15 h at 30 °C, after which growth inhibition of the indicator cells was measured spectrophotometrically at 600 nm by use of a microplate reader. Determination of antimicrobial activity was reproducible within a 2-fold dilution. Activities are presented as minimal inhibitory concentrations, the peptide concentrations that inhibited the growth of indicator cells by 50% under the described assay conditions.

Assay for Pheromone Activity—The pheromone activity was determined essentially as described earlier (7), using L. plantarum C11 cells with a bacteriocin-negative phenotype (6). These cells will convert to a bacteriocin-positive phenotype (i.e. start producing the bacteriocins, plantaricin E/F and J/K) upon adding PlnA (6, 10). An overnight stationary culture of L. plantarum C11 cells with a bacteriocin-negative phenotype was diluted 1/100 with MRS broth and grown for 1 h prior to the assay to be assessed for pheromone activity. The amount of peptide is indicated in Tables IV and V. The transformation of cells from the bacteriocin-negative to the bacteriocin-producing phenotype upon adding peptides was then determined by measuring the amount of bacteriocin produced after 6 h, using the assay for antimicrobial activity described above and the indicator strain L. plantarum 965. The amount of bacteriocin produced is presented as bacteriocin units/ml (BU/ml), where one BU is defined as the amount of bacteriocin that inhibited the growth of the indicator organism by 50%.

Circular Dichroism (CD) Spectroscopy—CD spectra were recorded by using a Jasco J-810 spectropolarimeter (Jasco International Co., Ltd., Tokyo, Japan) calibrated with ammonium d-camphor-10-sulfonate (Icatayama Chemicals, Tokyo). All measurements were performed with a peptide concentration of 0.15 mg/ml in 10 mM potassium phosphate (pH 7.4) and in the presence of 1.4 mM liposomes or various concentrations of TFE (0–50% (v/v); Sigma), SDS, or DPC (0–4 mM; CDN isotopes, Quebec Canada). Measurements were performed at 23 °C using a quartz cuvette (Starna, Essex England) with a path length of 0.1 cm. Samples were scanned eight times at 20 nm/min with a bandwidth of 2 nm and a response time of 2 s, over the wavelength range 190–260 nm. The data were averaged, and the spectrum of a sample-free control was subtracted. The \(\alpha\)-helical content of the various peptides was calculated both by the use of full spectrum methods using CDPro (12, 13) and by a single point method using the mean residual ellipticity at 222 nm (\(\theta_{222}\)) and Equation 1

\[
f_H = \theta_{222}(-40,000(1-2.5n))
\]  

where \(f_H\) and \(n\) represent the \(\alpha\)-helical content and the number of peptide bonds, respectively (14). All measurements were conducted at least twice.

Liposome Preparation—Single bilayer phospholipid vesicles were prepared essentially according to the procedure of Batzri and Korn (15). 24 \(\mu\)mol of dioleoyl-l-cysteine (DOPC, Sigma) or dioleoyl-l-cysteine (DOPG, Sigma) dissolved in chloroform were carefully dried under a stream of ultra pure nitrogen. The dried lipids were redissolved in 1 volume of absolute ethanol and dried again. Subsequently, the lipids were redissovled in 200 \(\mu\)l of absolute ethanol and slowly (about 100 \(\mu\)l/min), and at constant speed, injected into 4 ml of 10 mM potassium phosphate (pH 7.4) at room temperature. The ethanol was removed by dialysis against 10 mM potassium phosphate (pH 7.4).

NMR Sample Preparation—For NMR structure elucidation, 6.1 mg of PlnA-26 was dissolved in 700 \(\mu\)l of 100 mM deuterated DPC (CDN Isotopes) and a water solution with 10% D2O (Cambridge Isotope Laboratories). The final concentration of the sample was 2.9 mM.

NMR Spectroscopy—The NMR spectra of PlnA-26 were obtained at 25 °C on an 800 MHz Varian INOVA 800 NMR spectrometer with four channels, 5-mm 1H (13C,15N) pulsed field gradients probe. TOCSY (16) and NOESY (17, 18) experiments included in the biopack were performed to assign the molecule. Spectra with mixing times of 40 and 80 ms were acquired for the TOCSY pulse sequence, and 150 and 200 ms were acquired for the NOEY pulse sequence. Watergate water decoupling was applied in the TOCSY and NOEY experiments (19).

The natural abundance HSQC (19) spectrum was obtained with 128 increments in the 1 dimension and 1024 complex data points in the 2 dimension, and 128 scans. The data were multiplied with a sine bell window function and zero filled to 256Kx2 data points prior to the Fourier transformation.

All postprocessing was obtained with the application of NMR Pipe (20), and spectral assignments and integration were done with the application of SPARKY (T. D. Goddard and D. G. Kneller, University of California, San Francisco). NOE restrictions were obtained from the structure assignment of the molecule.

Restrains and Structure Calculation—Dihedral angle restraints were obtained by using the TALOS program (21) and the chemical shifts assigned by the application of the HSQC (19) and TOCSY. From this program we obtained restrictions for the torsion angles of the molecule from residue 12 to residue 21. CYANA (22) and the CANDID (23) routine were applied in the NOE assignment and structure calculation. The NOEY assignments obtained by the CANDID (22) routine were then checked by manual assignment and correction of the NOEY restriction prior to the final calculation of the structure. A total of 244 NOE distance constraints were obtained after removal of those that could be violated or found several times, of which 92 were interresidue and 152 were intraresidue NOE restraints. No long range NOE restraint distances larger than 4 residues were observed in the spectrum.

In the final structure calculated, hydrogen bond upper distance limitations for residue 12–21, between the O(H) and H(n+3) and O(n) and N(n+3) of 2 and 3 Å, respectively, were added to the list of restraints. A total of 100 structures were calculated of which the 20 best structures were selected for further evaluation. The target function gave an average of 0.081 ± 0.023. The structures were visualized with the MOLMOL program (24).

RESULTS AND DISCUSSION

Structural Analysis by CD Spectroscopy: \(\alpha\)-Helical Structuring of PlnA upon Exposure to Membrane-minicking Environments—The amino acid sequences of the PlnA peptides and fragments used in this study are shown in Fig. 1. To determine helical contents from CD spectra, we applied three different full spectrum methods (CONTIN/LL, CDSSTR, and SELCON3) belonging to the CDpro package (12, 13). The three methods gave similar values for the percent perfect (\(\alpha_p\)) and distorted (\(\alpha_d\)) helix in the peptides, although CONTIN/LL gave generally the smallest standard deviations. The helical content obtained

**FIG. 1.** Aligned amino acid sequences and residue numbering of the different peptides investigated in this paper.
with the CONTIN/LL full spectrum method is given in Table I along with the helical content obtained with the single point method using the mean residual ellipticity at 222 nm. The helical content obtained with the single point method is, with some exceptions, in fair agreement with the percent perfect helical content calculated from the spectra. One reason for this might be that the Ser residue was in close proximity to the remaining water ridges observed in the spectra. The width of the different lysine residues that partly overlapped in the aliphatic region might explain why the Lys residue was not observed in this region.

The intrar residue NOEs and dihedral angles obtained by the application of the TALOS program for PlnA-26-L in the presence of DPC are shown in Fig. 2. The existence of α-HN (i,i+3), αH-HN (i,i+4) and αHβH (i,i+3) NOEs (Fig. 2) clearly indicates that there is an α-helix stretching from residue 12 to residue 21 (possibly also including residue 22 and 23) in PlnA-26-L (18). The torsion angles obtained from TALOS analysis (21) of the chemical shift values and early structure calculations also clearly indicated that there was an α-helix in this region. H-bonds were consequently introduced in the final annealing of the structure.

The final 20 best structures obtained after the annealing of the structure are shown in Fig. 3; the mean square deviation for the molecule is shown in Table II. Because of the lack of restrictions and chemical shift values for residues 1 and 2, these two residues were not used when averaging the structures in Fig. 3A. The lack of restrictions for this region is also evident from the decrease in the mean square deviation observed when these two residues are left out when averaging the structures (Table II).

The N-terminal helix of PlnA-26-L clearly lacks a well defined structure (Fig. 3B) when exposed to a membrane-like environment. The root mean square deviations and the low number of interresidue NOEs found for this half of the peptide (Table II) are in good agreement with a flexible/unstructured N-terminal region. In Fig. 3A, the helical conformation of PlnA-26-L is only vaguely observable. However, if one superimposes the structures from residue 12 to residue 22 as done in Fig. 3C, the α-helix conformation of PlnA-26-L is well defined. This well defined helix is reflected in the low root mean square deviation of 0.33 (Table II) and the number of restrictions observed for this part of the peptide. The length of the α-helix is about the same as that observed for other antimicrobial peptides, as is also the mean square deviation. Fig. 3D shows the same helix looking down into the helix from the C-terminal end of PlnA-26-L. Fig. 3C indicates that there is a helical structure without the residues closest to the helix.

The helix stretching from Thr to Lys is amphipilic, with

**Table I**

| Membrane-mimicking environment | Percent helicity calculated by | PlnA-26-L | Percent helicity calculated by | PlnA-22-L |
|--------------------------------|-------------------------------|----------|-------------------------------|----------|
|                                | Full spectrum method           | αt, αd   | Single point method            | αt, αd   |
| Water                          | 1                              | 6        | 2                              | 0        |
| 25% TFE                        | 36                             | 22       | 39                             | 10       |
| 50% TFE                        | 40                             | 22       | 46                             | 12       |
| 0.5 mM DPC                     | 1                              | 1        | 5                              | 2        |
| 2.0 mM DPC                     | 43                             | 23       | 44                             | 11–12    |
| 4.0 mM DPC                     | 47                             | 23       | 44                             | 11       |
| 10.0 mM DPC                    | 35                             | 57       | 51                             | 11       |
| 1.4 mM DOPC                    | 6                              | 6        | 7                              | 2        |
| 1.4 mM DOPG                    | 5                              | 21       | 42                             | 11       |
| 4.0 mM DOPG                    | 34                             | 35       | 46                             | 10       |
| 10.0 mM SDS                    | 40                             | 37       | 48                             | 10–11    |

- The number of residues in helix was calculated from the percent helicity determined by the single point method using the mean residual ellipticity at 222 nm.
- The values were obtained by application of the CONTIN/LL full spectrum method using the basis set of soluble and membrane proteins found in the CDpro package (12,13). αt and αd are, respectively, the percent perfect and distorted helix in the peptides.
- The helicity determined by application of the single point method using the mean residual ellipticity at 222 nm.

The helicity determined by application of the single point method using the mean residual ellipticity at 222 nm. The values were obtained by application of the CONTIN/LL full spectrum method using the basis set of soluble and membrane proteins found in the CDpro package (12,13). αt and αd are, respectively, the percent perfect and distorted helix in the peptides.
the polar residues Thr$^{12}$, Lys$^{15}$, Gln$^{16}$, Lys$^{18}$, Lys$^{19}$, Lys$^{22}$, and Lys$^{23}$ along one side of the helix and the nonpolar residues Ala$^{13}$, Ile$^{14}$, Val$^{17}$, Leu$^{20}$, and Phe$^{21}$ along the other side (Fig.

4). The amphiphilic stretch would have extended all the way to residue 7 if the helical struc- turing had continued to this residue. Residues Gly$^{10}$, Met$^{9}$, and Leu$^{7}$ would then be along the hydrophobic side of the helix and Ala$^{11}$ and Gln$^{8}$ along the polar side. The amphiphilic stretch would also have extended to residue Gly$^{25}$ if the helical struc- turing had continued toward the C-terminal end. The last residue (Trp$^{26}$), however, could not have been included because it would have become positioned on the hydrophilic side of the helix near Lys$^{23}$. The large number of lysine residues along the helix going from residue 12 to 22 indicates that electrostatic interactions may play an important role in the binding of this peptide to the membrane surface and/or histidine kinase receptor. The reduced helicity observed with neutral DOPC liposomes compared with anionic

### Table II

| Residue range | Backbone (N,Ca,C) | Heavy atoms |
|---------------|------------------|-------------|
| 1–26          | 4.19 ± 1.38      | 5.51 ± 1.52 |
| 3–26          | 3.64 ± 1.35      | 4.88 ± 1.48 |
| 12–24         | 0.33 ± 0.10      | 1.02 ± 0.26 |
| 1–12          | 3.40 ± 0.78      | 4.90 ± 0.89 |
| 3–12          | 2.83 ± 0.75      | 4.29 ± 0.82 |
Plantaricin A Structure and Mode of Action

Antimicrobial Activity Depends on Nonchiral Interactions between Target Cells and the Amphiphilic α-Helix in the C-terminal Half of PlnA—The naturally produced L-enantiomeric PlnA-26 and -22 peptides and the D-enantiomeric form of PlnA-22 have similar antimicrobial activities (7, 10) (Table III). Moreover, the l- and d-enantiomeric forms permeabilize target cell membranes to the same extent as judged by their ability to dissipate the transmembrane electrical potential and pH gradient of the cell (7). The PlnA peptides thus apparently exert their membrane-permeabilizing antimicrobial activity through nonchiral interactions with the target cell membrane. This interaction involves the amphiphilic α-helical region in the C-terminal half of PlnA-26 because a 17-mer fragment (PlnA-17-L) that contains this amphiphilic α-helical part of the peptide had antimicrobial potency that was similar to that of the l- and d-enantiomeric forms of PlnA-22 (Table III). In contrast, the 5-residue fragment (N-5-L) derived from the nonhelical N-terminal part had little antimicrobial activity (Table III).

Pheromone Activity Depends on Both a Chiral Interaction Involving the Nonstructured N-terminal Half and a Nonchiral Interaction Involving the Membrane-interacting Amphiphilic α-Helical C-terminal Half—Both PlnA-26-L and -22-L display pheromone activity (7). This activity, in contrast to the antimicrobial activity, depends on both chiral and nonchiral interactions because the d-enantiomeric form (PlnA-22-D) has no pheromone activity but nevertheless inhibits the pheromone activity of the l-form (7) (Table IV). The membrane-interacting amphiphilic α-helical C-terminal half of PlnA-26-L appears to be involved in the nonchiral interaction because both the l- and d-enantiomeric forms of the 17-mer fragment (PlnA-17-L and -D) which contains the α-helic part of PlnA-26-L inhibited the pheromone activity of PlnA-22-L (Table IV). The structuring of C-terminal half of PlnA induced by nonchiral interactions with membranes (Figs. 3 and 4) thus appears to be relevant not only for the PlnA membrane-permeabilizing antimicrobial activity, but also for its pheromone activity. As reported earlier (7), the d-enantiomeric form of the PlnA-22 (i.e. PlnA-22-D) also inhibited the pheromone activity of the l-form, in fact somewhat more efficiently than PlnA-17-L and -D (Table IV). In all cases, a large excess of these peptides (compared with the concentration of PlnA-22-L) was necessary to obtain complete inhibition of the pheromone activity. This is as expected if the inhibitory peptides compete with PlnA-22-L for binding to a cellular target site. Moreover, PlnA-22-L induces production of PlnA-26-L, -23-L, and -22-L (in addition to the plantaricin E/F and J/K) (6, 7), and the interaction of PlnA-22-L with its receptor must consequently be inhibited completely to avoid accumulation of large amounts of endogenously produced PlnA pheromones.

In contrast to the C-terminal 17-mer fragments (PlnA-17-L and -D), neither the l- nor d-enantiomeric form of the five-residue fragment (N-5-L) derived from the nonhelical N-terminal part inhibited the pheromone activity of PlnA-22-L (results not shown). High concentrations of the l-form of this fragment (but not the d-form) had, however, pheromone activity, and this activity was inhibited by PlnA-17-L (Table V). This indicates that the unstructured N-terminal residues of PlnA-22-L (see Figs. 3 and 4) are involved in a chiral interaction with the histidine kinase near to or in the membrane interface.

CONCLUDING REMARKS

Taken together, the results indicate that the PlnA action as a pheromone involves an initial nonchiral interaction with membrane lipids, which induces α-helical structuring of the C-terminal part of the peptide (Figs. 3 and 4). Pheromone membrane studies indicate that the amphiphilic α-helix thus formed does not penetrate deeply into the membrane lipids but presumably orients parallel to the membrane with the hydrophobic residues penetrating into the hydrophobic part of the membrane and the hydrophilic residue into and partly through the membrane-water interface (see Fig. 4B). The peptide thereby becomes sufficiently structured and properly positioned in the membrane-water interface, thus enabling its N-terminal residues to engage in a chiral interaction with its histidine kinase receptor in and/or near the water-membrane interface.

It has been postulated that activation of the histidine kinase involves PlnA-triggered dimerization of the kinase (29) and that the kinase contains six or seven transmembrane segments (30). Somewhat surprisingly, six negatively charged aspartate residues are localized near or in the (negatively charged) membrane-water interface on the extracellular side of four of the transmembrane segments of the kinase (30). At least two of these residues (Asp⁷⁴ and Asp⁵⁵) have been shown by mutational analysis to be important for PlnA-induced activation of...
Table IV

**Inhibitory effect of PlnA-fragments on the pheromone activity of PlnA-22-L**

| Concentration of PlnA-22-L | Bacteriocin production induced by PlnA-22-L (BU/ml) | Without fragments | With the following fragments at indicated concentrations |
|---------------------------|--------------------------------------------------|------------------|--------------------------------------------------|
|                           | PlnA-22-D | PlnA-17-L | PlnA-17-D |
|                           | 0.4 µM | 2 µM | 10 µM | 50 µM | 10 µM | 50 µM |
| nM                        | BU/ml | BU/ml | BU/ml | BU/ml | BU/ml | BU/ml |
| 0                         | <100   | 100    | 200   | 150   | 200   | 300   |
| 0.2                       | 1,000  | 1,000  | 1,000 | 1,000 | 1,000 | 1,000 |
| 1.0                       | 4,000  | 4,000  | 4,000 | 4,000 | 4,000 | 4,000 |
| 5                         | 11,000 | 5,000  | 100   | 300   | 300   | 300   |
| 20                        | 18,000 | 18,000 | 1,500 | 18,000| 18,000| 17,000| 300   |

*Pheromone activity is measured as the amount of bacteriocin that was produced as a result of PlnA-22-L-induced bacteriocin production. The amount of the bacteriocin is quantified as bacteriocin units/ml of culture (BU/ml) as described under “Experimental Procedures.”

Table V

**Pheromone activity of N-5-L in the presence and absence of PlnA-17-L**

| Concentration of N-5-L | Bacteriocin production induced by N-5-L |
|-----------------------|---------------------------------------|
| µM                    | N-5-L | N-5-L + 2 PlnA-17-L | N-5-L + 50 µM PlnA-17-L |
| 2                     | ≤40   | –                 | –                     |
| 10                    | ≤150  | –                 | –                     |
| 50                    | ≤20,000| 20000           | ≤200                  |

| Concentration of N-5-L | Bacteriocin production induced by N-5-L |
|-----------------------|---------------------------------------|
| µM                    | N-5-L | N-5-L + 2 PlnA-17-L | N-5-L + 50 µM PlnA-17-L |
| 2                     | ≤40   | –                 | –                     |
| 10                    | ≤150  | –                 | –                     |
| 50                    | ≤20,000| 20000           | ≤200                  |

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