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Requirement of Autolytic Activity for Bacteriocin-Induced Lysis

M. CARMEN MARTÍNEZ-CUESTA,1 JAN KOK,2 ELISABET HERRANZ,1 CARMEN PELÁEZ,1 TERESA REQUENA,1,4 AND GIRBE BUIST2
Department of Dairy Science and Technology, Instituto del Frío (CSIC), Ciudad Universitaria, 28040 Madrid, Spain,1 and Department of Genetics, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, 9751 NN Haren, The Netherlands2

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The bacteriocin produced by Lactococcus lactis IFPL105 is bactericidal against several Lactococcus and Lactobacillus strains. Addition of the bacteriocin to exponential-growth-phase cells resulted in all cases in bacteriolysis. The bacteriolytic response of the strains was not related to differences in sensitivity to the bacteriocin and was strongly reduced in the presence of autolysin inhibitors (Co2+ and sodium dodecyl sulfate). When L. lactis MG1363 and its derivative deficient in the production of the major autolysin AcmA (MG1363acmAΔ1) were incubated with the bacteriocin, the latter did not lyse and no intracellular proteins were released into the medium. Incubation of cell wall fragments of L. lactis MG1363, or of L. lactis MG1363acmAΔ1 to which extracellular AcmA was added, in the presence or absence of the bacteriocin had no effect on the speed of cell wall degradation. This result indicates that the bacteriocin does not degrade cell walls, nor does it directly activate the autolysin AcmA. The autolysis was also responsible for the observed lysis of L. lactis MG1363 cells during incubation with nisin or the mixture of lactococcins A, B, and M. The results presented here show that lysis of L. lactis after addition of the bacteriocins is caused by the resulting cell damage, which promotes uncontrolled degradation of the cell walls by AcmA.

Bacteriocins are antimicrobial polypeptides synthesized ribosomally by bacteria (34). Most bacteriocins from lactic acid bacteria exert their antibacterial effect by permeabilizing the target cell membrane, whereby the cells lose their viability (1, 5, 29). Apart from damaging cell membranes, some bacteriocins have also been reported to cause bacteriolysis. Bierbaum and Sahl (4) were among the first to show the involvement of autolysins in the bacteriolytic effect of a bacteriocin. Autolysins are peptidoglycan hydrolases that are capable of causing bacterial autolysis (39). The authors showed that the bacteriocins Pep5, produced by Staphylococcus epidermidis, and nisin, produced by Lactococcus lactis, activate an N-acetylmuramoyl-\(\beta\)-alanine amidase and an \(\beta\)-N-acetylglucosaminidase of S. simulans (4). Plantaricin C has been shown to be bacteriolytic for Leuconostoc fermentum LM 13554 and L. delbrueckii subsp. bulgaricus LMG 13551, while no reduction of the optical densities (ODs) of mid-exponential-phase cultures of L. sake CECT 906, L. helveticus LMG 13555, or Leuconostoc mesenteroides was observed (14, 15). Microscopic analysis of L. fermentum cells treated with plantaricin C showed that changes had taken place in the cell wall. The authors suggested that cell lysis could be a secondary effect of the bacteriocin caused by a deregulation of the autolytic system of the sensitive cells resulting in destruction of the peptidoglycan layer. While no lysis of Lc. mesenteroides cells treated with plantaricin C was seen, a clear reduction of the OD was observed when these cells were incubated with pediocin AcH (3). This effect was not observed with Lactobacillus plantarum, although intracellular components were released. Transmission electron microscopic analysis of cells of both bacterial species revealed the presence of lysed ghost cells upon treatment with pediocin AcH (18). The action of nisin against Listeria monocytogenes Scott A cells resulted in the loss of cellular material following lysis, as shown by electron microscopic analysis (10). The antibacterial cyclic peptide AS-48 produced by Enterococcus faecalis S-48 also has bactericidal and bacteriolytic activity against several L. monocytogenes strains (28). These authors show that cells adapted to AS-48 have a changed fatty acid composition of their cytoplasmic membrane and a thicker cell wall and become more resistant to autolysins. For L. monocytogenes and E. faecalis growing cells, it was observed that loss of viability was much more rapid than the observed reduction of the OD. L. monocytogenes growing cells also lysed upon addition of pediocin PA-1, while the amount of bacteriocin activity added did not have a great influence on the degree of reduction of the OD (37).

A bacteriolytic effect of bacteriocins on lacticocci was first reported by Kok et al. (21), who described that treatment of lacticoccal cells with lactococcin A (LcnA) resulted in the release of UV-absorbing material. Using the same bacteriocin, Morgan et al. (30) obtained bacteriolysis and subsequent release of an intracellular enzyme from sensitive lacticoccal cells only when LcnA acted in concert with the lactococcins B and M. Another bacteriocin which has been shown to cause lysis of sensitive L. lactis cells is the bacteriocin produced by L. lactis IFPL105 (previously identified as Lactobacillus curvatus IFPL105 (9). This secreted broad-spectrum bacteriocin has been shown to cause lysis of logarithmically growing L. lactis and Lactobacillus casei (26). The importance of the lytic effect of this bacteriocin in accelerating cheese proteolysis has been demonstrated in cheese curd slurries manufactured with sensitive strains as starter and bacteriocin-producing adjuncts (27). Increase of starter cell lysis and free amino acid concentration in Cheddar cheese have been described by Morgan et al. (31) after using as starter adjunct a lactococcal strain producing lactococcins A, B, and M.

The major autolysin activity described for lacticocci and lactobacilli is that of an N-acetylmuramidase (24, 32). The gene (acmA) encoding the enzyme in L. lactis has been cloned and sequenced (6). The construction of an acmA deletion mutant
by replacement recombination has allowed to demonstrate that AcmA is required for cell separation and autolysis of cells during stationary growth phase (6, 7). Several factors, such as starvation for a carbon source, reagents which cause depletion of either the electrical or pH gradients of cellular membranes or cause disruption of these membranes, as well as proteolytic degradation, have been shown to influence the autolytic behavior of cells (8, 11, 19, 20, 24).

The object of this work was to investigate whether bacteriolysis by the bacteriocin produced by L. lactis IFPL105 on different strains of lactococci and lactobacilli was a direct or indirect effect of the bacteriocin. The results show that bacteriolysis is observed only when active autolysins are present in the sensitive cells. The bacteriocin does not activate the autolysin AcmA of L. lactis. Rather, depletion of cellular energy causes an imbalance in the control of the action of the auto-
lysin, resulting in cell wall degradation and, thus, lysis of cells.

MATERIALS AND METHODS
Bacterial strains and growth conditions. The bacteriocin producer L. lactis IFPL105 and its mutant L. lactis IFPL1053 (Bac AcmA) (9) are from the Culture Collection of the Instituto del Frio, Madrid, Spain. The bacteriocin-sensitive microorganisms used in this study are listed in Table 1. L. casei JCL1227 and L. rhamnosus JCL1227 were kindly provided by Juan Jimeno (FAM Sektion Biochemie, Liebefeld CH-3003, Bern, Switzerland). Other lactococcal strains used were L. lactis subsp. cremoris MG1363 (12), its derivative MG1363acmAΔ (6), and L. lactis subsp. cremoris 9B4 (41), which produces lactococccins A, B, and M. Culture media were M-17 broth (Adsa-Micro, Pharmalaster SA, Barcelona, Spain) containing lactose or glucose (5 g/liter) for lactococci and MRS broth (M. Culture Collection of the Instituto del Frio, Madrid, Spain) containing lactose or glucose (5 g/liter) for lactococci and MRS agar plates.

Effect of metal ions and chemical reagents on bacteriolysis. The effect of the bacteriocin produced by L. lactis IFPL105 on the mixture of lactococci and lactobacilli was also tested by the addition of the autoclaved crude bacteriocin (300 AU/ml) to exponential-phase cultures (OD600 of 0.7) of L. lactis MG1363 or MG1363acmAΔ. Lysis was followed during incubation at 30°C by monitoring the decrease in OD600 and the release of intracellular X-prolyl dipetidyl aminopeptidase (PepX) activity as described before (8). PepX activity was measured in culture supernatants (100 μl) filtered through a 0.22-μm-pore-size filter (Millipore Co.), using as substrate 100 μl of 1 mM Gly-Pro-p-nitroanilide (Sigma) solution in 50 mM phosphate buffer (pH 7.0). The total volume of the reaction mixture was brought to 1 ml with phosphate buffer, and incubation was at 37°C using a Peltier CUPS-240A temperature controller in a model UV-1601 spectrophotometer (Shimadzu Inc., Columbia, Md.). Release of p-nitroaniline was measured as the increase in absorbance at 410 nm (εmax = 8,800), and PepX activity was expressed as units of supernatant per milliliter.

TABLE 1. Cell viability and lysis after 3 h of incubation at 37°C in 20 mM sodium phosphate buffer (pH 6.8) of suspensions of log-phase cells of Lactococcus and Lactobacillus strains with or without bacteriocin

<table>
<thead>
<tr>
<th>Strain</th>
<th>Initial countsa (CFU/ml)</th>
<th>Incubation in buffer</th>
<th>Incubation with bacteriocin (150 AU/ml)</th>
<th>Lysisb (%, mean ± SE)</th>
<th>Counts (CFU/ml)</th>
<th>Lysisb (%, mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactococcus lactis IFPL359</td>
<td>3.3 × 10⁹</td>
<td>1.0 × 10⁹</td>
<td>32.5 ± 2.3</td>
<td>4.8 × 10⁷</td>
<td>100 ± 1.0</td>
<td>51.3 ± 1.2</td>
</tr>
<tr>
<td>L. lactis T1</td>
<td>1.5 × 10⁹</td>
<td>5.0 × 10⁷</td>
<td>22.4 ± 2.8</td>
<td>3.8 × 10⁷</td>
<td>56.4 ± 3.5</td>
<td>28.4 ± 0.9</td>
</tr>
<tr>
<td>L. lactis IFPL22</td>
<td>2.0 × 10⁹</td>
<td>5.0 × 10⁷</td>
<td>15.6 ± 1.3</td>
<td>3.6 × 10⁷</td>
<td>29.8 ± 1.3</td>
<td>28.4 ± 0.9</td>
</tr>
<tr>
<td>L. lactis IFPL1053</td>
<td>ND</td>
<td>4.3 ± 1.1</td>
<td>19.4 ± 1.5</td>
<td>8.7 × 10⁷</td>
<td>26.0 ± 0.8</td>
<td>25.7 ± 0.8</td>
</tr>
<tr>
<td>Lactobacillus casei IFPL731</td>
<td>1.0 × 10⁸</td>
<td>1.0 × 10⁸</td>
<td>14.9 ± 1.5</td>
<td>2.3 × 10⁷</td>
<td>30.2 ± 0.8</td>
<td>74.5 ± 4.4</td>
</tr>
<tr>
<td>L. plantarum IFPL935</td>
<td>9.9 ± 10⁸</td>
<td>1.0 × 10⁸</td>
<td>15.2 ± 4.8</td>
<td>3.0 × 10⁷</td>
<td>74.5 ± 3.7</td>
<td>74.5 ± 3.7</td>
</tr>
<tr>
<td>L. rhamnosus JCL1211</td>
<td>6.8 ± 10⁸</td>
<td>15.6 ± 1.3</td>
<td>15.6 ± 1.3</td>
<td>3.0 × 10⁷</td>
<td>74.5 ± 3.7</td>
<td>74.5 ± 3.7</td>
</tr>
</tbody>
</table>

a Cell viability expressed as the number of CFU after plating two appropriate dilutions of the cell suspensions in M-17 agar (lactococci) and MRS-agar (lactobacilli).

b OD600 at 600 nm (OD600) of 0.7 in M-17 or MRS broth with 0.2% (wt/vol) autoclaved starter broth. AcmA activity. Nisin was purchased from Sigma Chemical Co., St. Louis, Mo. (crude bacteriocin) was autoclaved (121°C, 10 min) to avoid residual autolysin activity. AcmA activity. Nisin was purchased from Sigma Chemical Co., St. Louis, Mo.

The titers of bacteriocin activity (arbitrary units [AU]) were assayed by a serial twofold dilution test as described previously (9), using L. lactis IFPL359 or MG1363 as the indicator strain.

Analysis of the bacteriocin effect by plate counting and by measuring OD600 reduction and the release of pepdase activity. Exponentially growing cultures (OD600 of 0.7) in M-17 or MRS broth were harvested by centrifugation at 10,000 × g for 10 min at 4°C. Pellets were washed and suspended in 20 mM sodium phosphate buffer (pH 6.8) containing 150 AU of bacteriocin per ml. Lysis was monitored during 3 h of incubation at 37°C by recording the decrease in OD600 using a Spectronic 20 D (Milton Roy Co., Rochester, N.Y.). Percentage of lysis was determined as 100 – (A1/A2 × 100), where A1 is the lowest and A2 is the highest value of the OD600 measured during incubation of the cell suspensions.

Controls for components other than the bacteriocin included culture supernatant from L. lactis IFPL1053 (Bac AcmA) precipitated with 40% ammonium sulfate, loaded on C18 cartridges, and eluted with 40% 2-propanol in 0.1% TFA as described for the parental strain.

Counts were performed in triplicate, and results were statistically compared by using one-way analysis of variance to determine significant differences (P < 0.05) in percentage of lysis among incubation conditions and strains.
was incubated at 30 or 37°C (depending on the strain) for 3 h. Activity was determined by the decrease in the OD600 of the cell suspension per minute.

Lytic activities of the strains were also tested by renaturing SDS-polyacrylamide electrophoresis (PAGE) (zymograms) as described by Potvin et al. (36), using SDS–12.5% polyacrylamide gels containing 0.2% (wt/vol) autolysed cells. M. lysodeikticus cells were used as substrate for L. lactis samples, while samples of the Lactobacillus strains were assayed on autolysed cells of the tested strain. Samples (5 ml) were obtained at different intervals during incubation of cell suspensions in buffer or broth cultures, with or without bacteriocin, and centrifuged (10,000 × g, 5 min, 4°C). Before loading, the samples (cell pellets and lyophilised supernatants) were treated with Laemmli buffer (22) as described by Valade and Lortal (40). Electrophoresis was done in a Mini-Protean II cell unit (Bio-Rad Laboratories, Hercules, Calif.) at 180 V for 1 h. Gels were washed with distilled water, and proteins were renatured in 25 mM Tris-HCl (pH 7.0, 7.5, or 8.0, depending on the strain tested) containing 1% Triton X-100. The renatured cell wall hydrolytic activities appeared as clear bands on the opaque background. The contrast was enhanced by staining the gels with 1% methylene blue in 0.01% KOH and destaining in distilled water.

Effect of the bacteriocin produced by L. lactis IFPL105 on autolysin activity was also tested by mixing 300 AU of bacteriocin per ml with a lactococcal cell wall fraction derived from L. lactis MG1363 or MG1363ΔaemAΔl and suspended (to give a final OD600 of 0.7) in the supernatant fraction of overnight cultures of the two strains (6). Native cell walls were obtained at 4°C from exponentially growing cells, harvested by centrifugation (8,000 × g, 15 min), suspended in 50 mM potassium phosphate buffer (pH 7.0), mixed (1:1, vol/wt) with glass beads (150 to 212 μm in diameter; Sigma), and disrupted for 16 min (four intervals of 4 min each) in a Mini Blend (Sunbeam-Oster Co. Inc., Miami, Fla.). Whole cells were removed by centrifugation at 1,000 × g for 15 min, and the cell walls were recovered from the supernatant by centrifugation at 14,000 × g for 15 min at 4°C. The cell wall fragments were suspended in 20 mM sodium phosphate buffer (pH 6.8) containing 0.02% sodium azide. Reduction of the OD600 of the cell wall suspensions during incubation at 37°C was followed over time using a Shimadzu UV-1601 spectrophotometer.

DNA analysis and manipulation. Genomic DNA of lactococci and lactobacilli was extracted by the method of Anderson and McKay (2). Total DNA was restricted with EcoRI (Roche, Mannheim, Germany), separated in a 1.7% agarose gel, and blotted onto positively charged nylon membranes (Roche). A 1.1-kb DNA fragment from the aemA gene of L. lactis MG1363, amplified by PCR as described by Buist et al. (6), was used as the probe. Probe labeling, hybridization, and immunodetection were performed with the DIG High prime labeling and detection kit according to the instructions of the manufacturer (Roche).

RESULTS

Cell lysis and viability of various lactococci and lactobacilli in the presence of the bacteriocin of L. lactis IFPL105. Lysis of cells of lactococci and lactobacilli during incubation in phosphate buffer with 150 AU of a 40% 2-propanol-elicited preparation of bacteriocin produced by L. lactis IFPL105 per ml was followed by measuring the percentage of decrease in OD600 (Table 1). To ensure that the bacteriocin preparation contained no cell wall-degrading enzymes, it was subjected to SDS-PAGE in the presence of M. lysodeikticus autolysed cells. No bands of clearing, indicative of the presence of cell wall hydrolases, were detected (data not shown).

The lytic response of the various strains to the bacteriocin was statistically different (P < 0.05). Addition of the supernatant fraction of L. lactis IFPL105 (Bac+) had no significant effect on the reduction of cell viability and lysis compared to the incubation of cells in phosphate buffer. Autolysis of the four lactococcal strains after 3 h of incubation in phosphate buffer differed considerably, ranging from 4 to 32%. Addition of the bacteriocin and incubation over the same period resulted in 25 to 30% lysis of L. lactis IFPL22 and IFPL1053 and 50 to 55% of lysis of the L. lactis IFPL359 and T1 strains, showing that the bacteriocin-induced lysis differs among different lactococcal strains. The extents of bacteriocin-induced lysis of the two L. casei strains were found to be similar, while the highest percentage of lysis was obtained for the L. plantarum and L. rhamnosus strains.

Cell viability of the different strains 3 h after addition of the bacteriocin (150 AU/ml) to suspensions of cells taken from the logarithmic phase of growth is also shown in Table 1. Interestingly, the two Lactobacillus strains showing the highest lytic response to the bacteriocin, L. plantarum IFPL935 and L. rhamnosus JCL1211, showed the least loss of viability, whereas L. casei IFPL731 and JCL1227 exhibited a loss of viability similar to that of the Lactococcus strains.

In all strains studied, reduction of cell viability and OD600 were not simultaneous. Figure 1 shows the decrease in OD600 of three representative strains after addition of the bacteriocin to exponentially growing cultures. In the case of the lactobacilli, the cell density of the cultures hardly changed within 0.5 to 1 h after addition of the bacteriocin and then decreased rapidly in L. rhamnosus JCL1211 to reach 71.3% of lysis after 2 h of further incubation.

Effect of the bacteriocin of L. lactis IFPL105 on autolysin activity. The observation that loss of viability was not concurrent with cell lysis suggested that the latter phenomenon was not directly caused by the bacteriocin but, likely, was the result of activities of other enzymes such as cell wall-degrading enzymes. The involvement of the autolytic enzymes in cell lysis was studied by adding autolysin activity inhibitors. The results showed that 1 mM Co2+ (Fig. 2) and 0.40 mg of SDS per ml (Fig. 3) strongly reduced the lytic response of L. lactis IFPL359 to the bacteriocin. Results of previous experiments showed...
that autolysis of lactococci is severely reduced upon the addition of Co²⁺ (35). No reduction of lysis was observed when these components were added to a mixture of *L. rhamnosus* JCL1211 cells and the bacteriocin. In this case, a 50% reduction of lysis was observed during the incubation with bacteriocin (500 AU/ml) in the presence of 0.04 mg of cardiolipin per ml.

**Detection of the cell wall hydrolytic activities.** Results of the analysis by renaturing SDS-PAGE of the cell wall hydrolytic activities present in the cell and supernatant fractions of all lactococcal strains showed a banding pattern similar to that of AcmA (6). No other activities could be detected (results not shown). Hybridization experiments using a PCR probe directed against *acmA* showed that the gene was present in all lactococcal strains. The cell wall hydrolytic activity patterns obtained for the three *Lactobacillus* strains were all different. A clearing band of 110 kDa obtained for the *L. plantarum* strain used was of the same size as that obtained for several strains of this species (25). The bands present in the samples of *L. rhamnosus* and *L. casei* were 35 and 70 kDa, respectively (data not shown).

Cell wall hydrolytic activity of the strains in the presence of the bacteriocin from *L. lactis* IFPL105 was analyzed spectrophotometrically and by renaturing SDS-PAGE using autoclaved *M. hysooeleiticus* cells or autoclaved cells of the tested strain as a substrate for *L. lactis* and *Lactobacillus* strains, respectively. Total autolysin activity did not increase when the bacteriocin was present in the assays (results not shown).

**AcmA is responsible for bacteriolysis of lactococci.** The involvement of the autolysin AcmA in cell lysis after loss of viability was investigated by comparing the effects of the bacteriocin on lysis of *L. lactis* MG1363 and its mutant *L. lactis* MG1363*acmanΔI*, which cannot produce autolysins. The addition of a crude extract of autoclaved bacteriocin of *L. lactis* IFPL105 (300 UA/ml) to logarithmic-phase cells of *L. lactis* MG1363 growing in broth resulted in a sharp decrease in OD (56% of lysis after 5 h of incubation with the bacteriocin [Fig. 4]). This lysis was concomitant with the release of intracellular material (0.022 U of PepX activity per ml). No lysis or release of PepX was observed after addition of a crude extract from the supernatant of *L. lactis* IFPL105 (Bac⁻) or after addition of the autoclaved bacteriocin preparation to logarithmic-phase cells of *L. lactis* MG1363*acmanΔI* (Fig. 4). However, cell counts carried out over the same period resulted in a reduction of cell viability to 10³ CFU/ml for both *Lactococcus* strains (MG1363 and MG1363*acmanΔI*) 3 h after addition of the bacteriocin.

**The bacteriocin of *L. lactis* IFPL105 does not activate AcmA activity.** To study whether the effect of the bacteriocin on cell lysis was caused by direct activation of the autolysin AcmA, autoclaved bacteriocin (300 AU/ml) was added to native cell walls of *L. lactis* MG1363 suspended in a supernatant of an overnight culture of *L. lactis* MG1363*acmanΔI*. The results of cell wall degradation, as determined by decrease in OD₆₀₀ of the mixture after 4 h of incubation at 37°C, are shown in Table 2. Hydrolysis of *L. lactis* MG1363 cell walls was not influenced by the presence of the bacteriocin, nor was activation of AcmA found when native cell walls of *L. lactis* MG1363*acmanΔI*, suspended in *L. lactis* MG1363 supernatants containing extracellular AcmA, were incubated with the bacteriocin (Table 2). No significant OD₆₀₀ reduction was observed after incubation

<table>
<thead>
<tr>
<th>Cell walls from:</th>
<th>AcmA (supernatant)</th>
<th>Cell wall degradation (%; mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Bacteriocin (300 AU/ml)</td>
</tr>
<tr>
<td><em>L. lactis</em> MG1363</td>
<td>−</td>
<td>21.9 ± 0.1</td>
</tr>
<tr>
<td><em>L. lactis</em> MG1363<em>acmanΔI</em></td>
<td>+</td>
<td>22.8 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>−</td>
<td>3.8 ± 0.8</td>
</tr>
</tbody>
</table>

**FIG. 4.** Evolution of OD₆₀₀ (lines) and release of PepX (bars) during growth at 30°C of *L. lactis* MG1363 in M-17 broth (•: striped bars) and during incubation with 300 AU of the bacteriocin of *L. lactis* IFPL105 per ml of logarithmic-phase cells of *L. lactis* MG1363 (•: open bars) and *L. lactis* MG1363*acmanΔI* (■: solid bars). The arrow indicates point of bacteriocin addition.
of native cell walls of *L. lactis* MG1363 in a supernatant without AcmA activity.

**AcmA is also responsible for bacteriolysis observed with other lactococcal bacteriocins.** The effect of the bacteriocin of *L. lactis* IFPL105 on lysis of sensitive lactococcal cells was compared with that of nisin and the mixture of lactococccins A, B, and M (Table 3). Cell lysis and the subsequent release of PepX was observed with all three bacteriocin preparations on exponential-phase cells of *L. lactis* MG1363. Lysis was absent when the bacteriocin of *L. lactis* IFPL105 or nisin was added to the *acmA* deletion mutant, while a very small reduction of the OD<sub>600</sub> was detected when the lactococccin mixture had been added. Also, the release of PepX from the AcmA-negative strain was much less than that obtained for the wild-type strain for all bacteriocins tested. These results indicate that the autolysis is also required for lysis of *L. lactis* MG1363 cells sensitive to bacteriocins other than the bacteriocin from *L. lactis* IFPL105.

**DISCUSSION**

Addition of the bacteriocin produced by *L. lactis* IFPL105, nisin, or a mixture of the lactococccins A, B, and M to logarithmic-phase cultures causes effective lysis of *L. lactis* MG1363 cells but not in its autolysin-negative derivative *L. lactis* MG1363<sub>acmAΔ1</sub> (Fig. 4 and Table 3). Apparently, the bacteriocins themselves are not capable of lysing lactococcal cells. The results presented here clearly demonstrate that cell lysis induced by addition of lactococccin bacteriocins to bacteriocin-sensitive strains is, in fact, caused by the autolytic system of these strains.

The fact that cell lysis caused by addition of the bacteriocin of *L. lactis* IFPL105 to *Lactobacillus* and *Lactococcus* strains was not concurrent with loss of viability (Fig. 1 and Table 1) suggests that it involves two steps. First, viability is lost due to insertion of the bacteriocin into the membrane of the sensitive cell and depletion of cellular energy (38, 41, 42). Second, a gross imbalance between cell wall build up and degradation caused in *L. lactis* by AcmA leads to the observed cell lysis. Autolysis as a secondary effect of bacteriocin action has been suggested previously, but the causative agent has never been definitely pinpointed. Some delay between the decrease in cell viability and cell lysis has been observed in the mechanism of action of other bacteriocins (3, 30). Morgan et al. (30) showed that more than 99% of the cells of a lactococcal culture were killed within 10 min upon treatment with a mixture of lactococccins A, B, and M. Ten hours after addition, only 57% of the total amount of the activity of an intracellular marker was released, indicating that bacteriolysis follows loss of viability.

Similarity in the autolytic activities present in the cell and supernatant fraction of the various lactococcal strains investigated was not consistent with their lytic responses to the bacteriocin, which differed considerably. One possible explanation for this observation could be that the cell walls of the different strains have different compositions. The difference in the lytic response of the *Lactobacillus* strains might also be the result of the expression of different cell wall hydrolytic activities. This could also explain the different effects of the autolysin inhibitors used on *L. lactis* and *L. rhamnosus* cells.

Topological regulation of autolytic enzymes by the electro-chemical potential of the cell membrane, by cell wall lipoteichoic acids, or by extracellular proteinases has been shown for several species of gram-positive bacteria (4, 8, 13, 19, 20). Incubation of the bacteriocin of *L. lactis* IFPL105 with native cell wall fragments of *L. lactis* MG1363 and its AcmA-defective mutant had no effect on lysis of these cell walls, indicating that the bacteriocin does not activate AcmA, either bound to the cell wall or in supernatants. In light of these results, direct activation of AcmA, as postulated for the autolysin *N*-acetyl-muramoyl-t-alanine amidase of *S. simulans* by its replacement from the teichoic acids by cationic bacteriocins (4), does not occur.

Bacteriocins are capable of causing lesions in the cytoplasmic membrane of sensitive cells based on their small size, high hydrophobicity, and hydrophobic regions predicted to form amphipathic α helices (16, 17, 33). This originates dissipation of the proton motive force (5, 29, 42), which has a direct effect on autolysis (19, 20). This effect is shared with other substances such as holin protein of bacteriophages (11, 43). The differences in decrease in OD and in release of the intracellular PepX activity upon addition of the different bacteriocins used might be caused by the difference in effectiveness of pore formation.

Apart from providing fundamental insights into bacteriocin action, the result of this work is also of practical interest. Since the bacteriocin producer *L. lactis* IFPL105 can be used as an adjunct in cheese manufacture (27) and the bacteriocin it produces has a broad spectrum of action, this strain and bacteriocin have both technological and preservative potentials.

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