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Mode of action of lactocin 705, a two-component bacteriocin from *Lactobacillus casei* CRL705

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Abstract

Lactocin 705 is a bacteriocin whose activity depends on the complementary action of two peptides (705α and 705β) of 33-amino-acid residues each and is produced by *Lactobacillus casei* CRL705. Biologically active, synthetic lactocin 705 was used to study the mode of action on sensitive cells of *Lactobacillus plantarum* CRL691. The addition of 90 nmol l^{-1} of lactocin 705 to cells of *L. plantarum* dissipated both, the membrane potential ($\Delta\Psi$) and the pH gradient (ΔpH). Energized membrane, obtained after the addition of glucose, were more susceptible to lactocin 705 action leading to the immediate release of intracellular K^+ and inorganic phosphate. When the role of various ions on sensitive cells were analyzed, only Ca^{2+} ion exhibited a protective effect against lactocin 705. These data suggest that the presence of a proton motive force (PMF) promotes the interaction of the bacteriocin with the cytoplasmic membrane of energized cells, leading to pore formation which allows for the efflux of ions, thereby ensuring efficient killing of target bacteria.

Keywords: Bacteriocin; *Lactobacillus*; Mode of action

1. Introduction

Lactic acid bacteria (LAB) produce three main classes of ribosomally synthesized antimicrobial peptides named bacteriocins (Moll et al., 1999a): (i) Class I: lantibiotics, small peptides (<5 kDa) that contain lanthionine and/or β-methyl-lanthionine residues. Some lantibiotics rely on the complementary action of two components, i.e., lactacin 3157 (McAuliffe et al., 1998); (ii) Class II: nonlantibiotic, low-molecular-weight (<10 kDa), heat-stable peptides; and (iii) Class III: nonlantibiotic, large heat-labile peptides (>30 kDa). Subclasses IIa and IIb bacteriocins constitute the majority of lactic acid bacteriocins, these being also the most thoroughly studied (Jack et al., 1995; Nes and Holo, 2000; Ennahar et al., 2000) and are produced by a number of strains usually associated with meat (Aymerich et al., 1998). Subclass IIa bacteriocins, the largest subgroup, are highly effective in killing *Listeria* (Muriana, 1996). On the other hand, subclass IIb involves bacteriocin whose activities depend on the complementary action of two components, i.e., lactococcins G and M from *Lactococcus lactis* (Nissen-Meyer et al., 1992; Moll et al., 1998), lactacin F from *Lactobacillus johnsonii* (Allison et al., 1994), plantaricin A from *Lactobacillus plantarum* (Nissen-Meyer et al., 1993) and acidocin J1132 from *Lactobacillus acidophilus* (Tahara et al., 1996).

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Bacteriocins from GRAS lactic acid bacteria have aroused a great deal of interest to the food fermentation industry as a novel approach to control foodborne pathogens and spoilage microorganisms (Muriana, 1996; Stiles, 1996; Aymerich et al., 1998). The potential application of LAB bacteriocins as food preservatives requires an in-depth knowledge of how they exert their bactericidal effect. In general, most bacteriocins from LAB appear to share a common mechanism of action which is depleting proton motive force (PMF) in target cells through the formation of pores in the cell membrane releasing intracellular ions (Abee, 1995; Montville and Bruno, 1994; Moll et al., 1997, 1999a; Nes and Holo, 2000). However, the mechanisms through which they achieve this appear to differ among the different bacteriocins; ultrastructural studies of treated sensitive cells indicate different mechanisms of membrane destabilization and cell death (Jack et al., 1995).

The meat isolate *Lactobacillus casei* CRL705 produce lactocin 705, a small, hydrophobic and positively charged antimicrobial peptide that belongs to the class IIb bacteriocin whose activity depends upon the complementation of two peptides, termed 705α and 705β. These two peptides, consisting of 33-amino-acid residues each, showed to have different primary structure (Cuozzo et al., 2000). Among the target organisms to this bacteriocin are food spoilage bacteria closely related to the producer (*L. casei*, *L. plantarum*, *Leuconostc* spp.) and food-borne pathogens such as *Streptococcus pyogenes* and *Staphylococcus aureus* (Vignolo et al., 1993). However, lactocin 705 was not active against *Listeria monocytogenes*, but evidence about the production of a second inhibitory substance, an anti-*Listeria* bacteriocin, is currently in progress. In fact, previous work showed the effectiveness of *L. casei* CRL705 in reducing growth of *L. monocytogenes* in broth and meat slurry when a concentrated overnight supernatant was used as a source of bacteriocin (Vignolo et al., 1996).

In this report, the mechanism of action for lactocin 705 has been studied with *L. plantarum* CRL691, one of the most susceptible bacteria to the bacteriocin produced by *L. casei* CRL705. The primary target of lactocin 705 was the plasma membrane, in which it forms pores that allowed free led of solutes (K^+ and P_i). The role and importance of energized membranes in this process was examined as well as the effect of different ions on bactericidal action of lactocin 705.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The lactocin 705 producer *L. casei* CRL705 and the sensitive strain *L. plantarum* CRL691 were isolated from dry-cured sausages (Vignolo et al., 1993) and maintained at −20 °C in MRS (De Man et al., 1960) (Merck) containing 10% glycerol. Cultures were grown aerobically in MRS broth at 30 °C.

2.2. Peptides purification and synthesis

The lactocin 705α and 705β peptides were purified from the supernatant of *L. casei* CRL705 using the adsorption–desorption pH-dependent method and the active extract was further subjected to RP-HPLC and SDS-PAGE as previously described (Palacios et al., 1999). The active antimicrobial band was electroeluted and its amino acid sequence determined by Bio-Synthesis (Lewisville, TX, USA).

2.3. Effect of $CaCl_2$ on the minimum inhibitory concentration (MIC) of lactocin 705

Bacteriocin activity was assayed by the agar-well diffusion method. Thirty microliters of serial twofold dilutions of lactocin 705 (705α + 705β peptides) + 50 mmol l^{-1} $CaCl_2$ after incubation for 15 min at 30 °C were placed into each well of freshly prepared lawns of the indicator strain *L. plantarum* CRL691. In addition, 705α and 705β peptides were separately treated with 50 mmol l^{-1} $CaCl_2$, incubated for 15 min at 30 °C and seeded together (705α + 705β) as stated before. The MIC was recorded as the lowest concentration of the bacteriocin that prevented visible growth after incubation 18–20 h at 30 °C.

2.4. Effect of lactocin 705 on energized membrane of sensitive cells

L. plantarum CRL691 was grown to an OD_{560} of 0.6, harvested by centrifugation, washed twice and resuspended to approximately 10^7 cells ml^{-1} in 5

mmol l^{-1} sodium phosphate buffer (pH 6.5) and MRS broth, both supplemented with 10 and 100 mmol l^{-1} glucose. The bacteriocin, 705α + 705β peptides, was added at a concentration of 90 nmol l^{-1}, incubated for 1 h at 30 °C, and samples were taken at appropriate times to determine the viable cell counts after aerobic incubation at 30 °C in MRS agar.

2.5. Effect of different concentrations of various ions on survival of bacteriocin-treated cells

Cells of *L. plantarum* CRL691 were resuspended to approximately 10^6 cells ml^{-1} in fresh MRS broth containing 90 nmol l^{-1} of lactocin 705 (705α + 705β peptides) and 10, 20, 50 and 100 mmol l^{-1} of $MgCl_2$, $CaCl_2$, NaCl and KCl for studying the effect of the bacteriocin on sensitive cells. Viable cells were determined after incubation for 3 h at 30 °C by plating them onto an MRS agar, while cell growth was measured by optical density at 560 nm. Cell suspensions with lactocin 705 without salts and without lactocin 705 with salts were used as controls.

2.6. Measurement of proton motive force

Transmembrane electrical potential ($\Delta\Psi$) was monitored with the fluorescent probe 3,3-dipropylthia-dicarbocyanine iodide [$DISC_3(5)$] (Molecular Probes, Eugene, OR, USA) according to Wu et al. (1999). This probe measures the electrical potential gradient disruption across the cytoplasmic membrane of intact cells. Fluorescence value measurements were determined with a spectrofluorimeter (Shimadzu RF-5301 PC, Japan) at 30 °C. The excitation and emission wavelengths were set at 623 and 690 nm, respectively. Cells were resuspended in 5 mmol l^{-1} potassium HEPES buffer (pH 7.0) with 100 and 10 mmol l^{-1} glucose. Experiments were performed in the presence of nigericin (1.0 μmol l^{-1}) (Sigma, St. Louis, MO) to prevent the generation of a transmembrane pH gradient (ΔpH) and valinomycin (1.0 μmol l^{-1}) (Sigma). The transmembrane pH gradient (ΔpH), inside alcaline, was measured by loading the cell suspension containing 10 mmol l^{-1} glucose with the fluorescent pH indicator 2′,7′-bis-(2-carboxyethyl)5(and-6)-carboxyfluorescein (BCECF) (Sigma) as previously described by Molenaar et al. (1991). The acid shock used to obtain the optimal loading was 2.5 μl of 0.5 mmol l^{-1} HCl (pH 2.0). In this experiment, glucose was only used in a concentration of 10 mmol l^{-1}, addition of 100 mmol l^{-1} of glucose caused a spontaneous BCECF efflux preventing the probe retention by cells.

2.7. Determination of K^+ and inorganic phosphate contents of cells

Intracellular and extracellular K^+ contents were determined as follows. Cells were resuspended to approximately 10^6 cells ml^{-1} in 2.5 mmol l^{-1} sodium HEPES buffer (pH 7.0) supplemented with 10 and 100 mmol l^{-1} glucose. Lactocin 705 (705α + 705β peptides) was added to a concentration of 90 nmol l^{-1}. At various time intervals, samples (1 ml) were taken and immediately chilled on ice. Cells were removed by centrifugation at 10,000 × *g* for 7 min at 0 °C. Supernatant was removed and stored for the determination of extracellular K^+. The cell pellet was resuspended in 1 ml of 5% (w/v) trichloroacetic acid and frozen overnight at −20 °C. The samples were thawed and incubated at 95 °C for 10 min. Demineralized water (4 ml) was added to each sample, which was then centrifuged at 10,000 × *g* for 15 min. The supernatant was retained for intracellular K^+ determination. The K^+ concentration in the samples was determined by flame photometry (Jenway PFP7, USA). Inorganic phosphate was determined in the supernatants by reduction with ascorbic acid of the formed phosphomolybdate complex (Ames, 1966).

2.8. Statistical analysis

Experiments were carried out in duplicate. One-way analysis of variance (ANOVA) was used (Minitab Statistic Program, release 8.21).

3. Results

3.1. Effect of $CaCl_2$ on the MIC of lactocin 705

The minimum inhibitory concentration of lactocin 705 (705α + 705β peptides) against *L. plantarum* CRL691 was 90 nmol l^{-1}. When lactocin 705 was incubated in presence of $CaCl_2$, a twofold increased MIC value (180 nmol l^{-1}) was found, while a

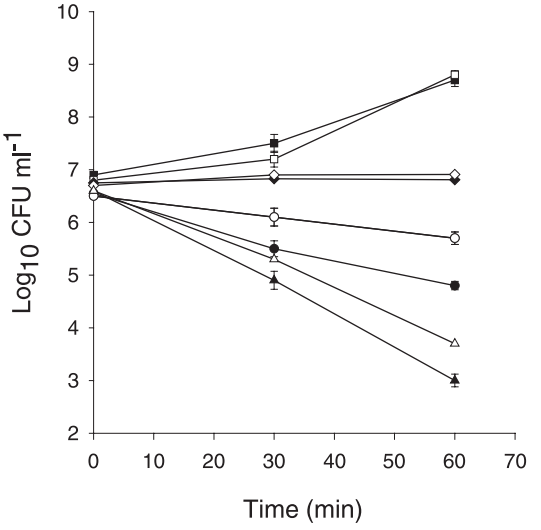

Fig. 1. Effect of lactocin 705 addition on the viability of *L. plantarum* CRL691 cells incubated at 30 °C. MRS broth (●,○); phosphate buffer (▲,△). Controls: MRS broth (■,□); phosphate buffer (♦,◇). Solid symbols: 100 mmol l^{-1} glucose; empty symbols: 10 mmol l^{-1} glucose. Values are mean ± standard deviation of two replicate samples.

dramatic increase in the MIC was obtained when each peptide was separately incubated with Ca^{2+}, the bacteriocin concentration being 1448 nmol l^{-1}.

3.2. Effect of lactocin 705 on viability of energized sensitive cells

The effect of different glucose concentrations on the bactericidal activity of 90 nmol l^{-1} lactocin 705 (705α and 705β peptides) on sensitive cells of *L. plantarum* CRL691 was studied. When 100 mmol l^{-1} of glucose was added to MRS broth, a decline in the viability from 6.85 to 4.80 log cfu ml^{-1} was observed (Fig. 1), while in presence of 10 mmol l^{-1} of glucose, a decrease of only 1 log cycle was obtained after 1 h of incubation. Due to a possible influence of MRS constituents on bacteriocin action, a similar experiment was performed with cells incubated with the same bacteriocin concentration but in sodium phosphate buffer. Under these conditions, the killing efficiency of lactocin 705 was increased, even at the lowest glucose concentration, the final viable counts after 1 h being 3.00 and 3.70 log cfu ml^{-1} for 100 and 10 mmol l^{-1} of glucose, respectively. No difference in viable counts after 1 h was observed in absence of lactocin 705 when MRS was supplemented with 10 and 100 mmol l^{-1} of glucose, both reaching a final population of 8.70 log cfu l^{-1}. Similar results were obtained using phosphate buffer, the final cell counts being 6.88 log cfu l^{-1} in presence of glucose (10 and 100 mmol l^{-1}). Apparently, lactocin 705 may require an energized membrane for insertion.

3.3. Effect of various ions on lactocin 705 treated cells

To investigate the effect of various ions on *L. plantarum* cells treated with the bacteriocin, optical density and the viable count were determined over time. No changes in the bactericidal efficiency of lactocin 705 were observed in the presence of Mg^{2+}, Na^{+} and K^{+} in the concentrations studied (data not shown). However, when the effect of different Ca^{2+} concentrations was assayed on the bactericidal action of 90 nmol l^{-1} of lactocin 705, less *L. plantarum* inhibition was observed (Fig. 2). In the absence of the bacteriocin, *L. plantarum* CRL691 increased its population from an OD_{560} of 0.06 to

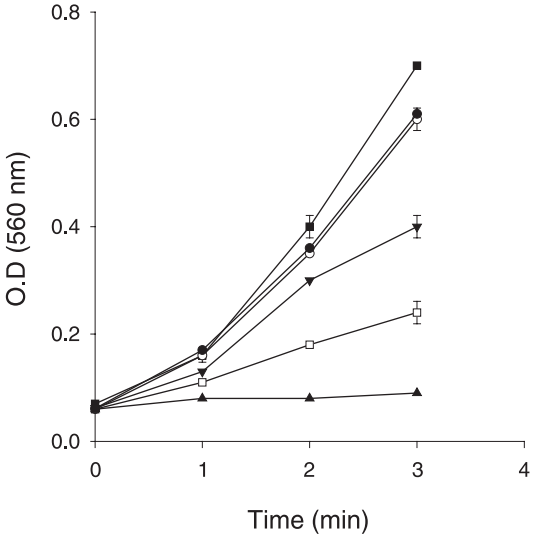

Fig. 2. Effect of different concentrations of Ca^{2+} on the growth of *L. plantarum* CRL691 after the addition of lactocin 705. Control without lactocin 705 with Ca^{2+} (■); control with lactocin 705 without Ca^{2+} (▲); 100 (●), 50 (○), 20 (▼) and 10 (□) mmol l^{-1} of Ca^{2+}. Values are mean ± standard deviation of two replicate samples.

Table 1
Effect of various $CaCl_2$ concentrations on the viability of *L. plantarum* CRL691 cells after the addition of lactocin 705

Treatments	Colony counts (log CFU ml^{-1})	Cell viability (%)
Cells + $CaCl_2$ 10 mmol l^{-1}	6.59 ± 0.01^b	14
Cells + $CaCl_2$ 20 mmol l^{-1}	6.94 ± 0.07^b	32
Cells + $CaCl_2$ 50 mmol l^{-1}	7.22 ± 0.03^a	63
Cells + $CaCl_2$ 100 mmol $^{-1}$	7.38 ± 0.02^c	88
Control 1	7.43 ± 0.06	100
Control 2	5.27 ± 0.03	0.7

Control 1: *L. plantarum* CRL691 + Ca^{2+} without lactocin 705. Control 2: *L. plantarum* CRL705 + lactocin 705 without Ca^{2+}. Cells viability (%): expressed vs. controls 1 and 2.
Values are the means of two replicated measurements ± S.D. Difference between means: $^aP<0.05$; $^bP<0.001$; ^{c}not significant.

0.70 after 3 h of incubation at 30 °C. However, when compared with lactocin 705 alone (without the addition of Ca^{2+}), the final OD_{560} was 0.09. Increasing concentrations of Ca^{2+} added together with the bacteriocin produced a reduction of lactocin 705 effectiveness against *L. plantarum* inhibition. In the presence of 10, 20 and 50 mmol l^{-1} of Ca^{2+}, OD_{560} values for sensitive cells after 3 h of incubation were 0.24, 0.40 and 0.60, respectively. Similar results were obtained when the viable counts of *L. plantarum* were determined showing a viability increase from 14% to 63% corresponding to 10–50 mmol l^{-1} of Ca^{2+} treatment (Table 1). No difference was detected in presence of 50 and 100 mmol l^{-1} of Ca^{2+} when optical density was measured while higher percentage of viables were observed when cell viability was determined.

3.4. Lactocin 705 dissipates the PMF

To determine if lactocin 705 acts on the cytoplasmic membrane of target organisms, the effect of the bacteriocin on the components of the proton motive force (PMF), i.e., $\Delta\Psi$ and ΔpH, was examined. The $\Delta\Psi$ was measured qualitatively with the fluorescent probe 3,3-dipropylthia-dicarbocyanine iodide [$DISC_3(5)$]. Generation of a $\Delta\Psi$ (inside negative) on addition of glucose to the cells resulted in quenching of the fluorescent signal. In the presence of the K^+/H^+ exchanger nigericin (1.0 μmol l^{-1}), the cells were able to maintain a maximum $\Delta\Psi$, which could be completely dissipated by the addition of the K^+ ionophore valinomycin (1.0 μM) (data not shown). The addition of lactocin 705 to cells of *L. plantarum* CRL691 maintaining a maximum $\Delta\Psi$ led to immediate depolarization of the cytoplasmic membrane as shown by the increase in fluorescence values (Fig. 3). No further dissipation was observed when valinomycin was added to cells treated with the bacteriocin, indicating that complete dissipation had occurred as a result of bacteriocin action. In fact, lactocin 705 was as effective at dissipating the $\Delta\Psi$ as valinomycin. Moreover, 10 mmol l^{-1} glucose added to the cells was also capable to dissipate, even incompletely, the transmembrane electrical potential (Fig. 3).

Intracellular BCECF can be used to monitor the transmembrane pH gradient, provided that a correction is made for the fluorescence changes due to extrusion or loss of BCECF from the *L. plantarum* cells. The addition of nigericin (1.0 μmol l^{-1}) caused a rapid and complete collapse of the ΔpH, leading to an equilibration of the internal pH with the outside environment. To ensure that the ΔpH was the sole component of the PMF analyzed, 1.0 μmol l^{-1}

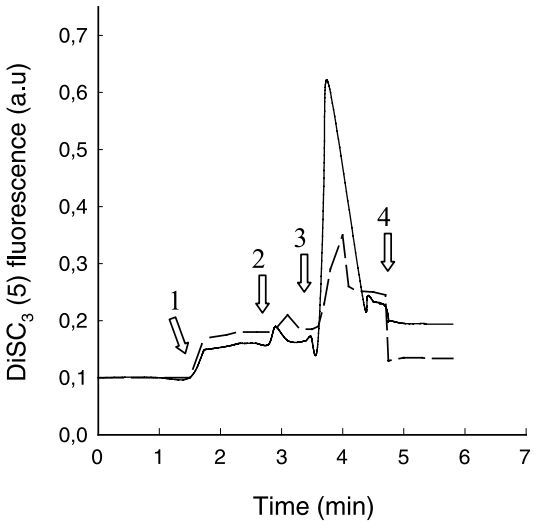

Fig. 3. Lactocin 705 dissipate transmembrane electrical potential ($\Delta\Psi$) in *L. plantarum* CRL691. Cells loaded with 3,3-dipropylthia-dicarbocyanine iodide [$DISC_3(5)$] were suspended in 5 mmol l^{-1} K-HEPES buffer (pH 7.0) and energized with 100 mmol l^{-1}(continuous line) and 10 mmol l^{-1} glucose (dashed line) (arrow 1). Subsequently, nigericin (1.0 μmol l^{-1}) (arrow 2), lactocin 705 (90 nmol l^{-1}) (arrow 3) and valimomycin (1.0 μmol l^{-1}) (arrow 4) were added.

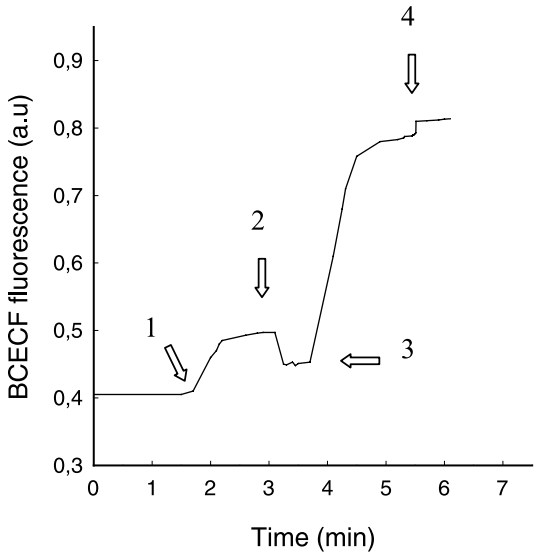

Fig. 4. Lactocin 705 causes the efflux of the fluorescence dye BCECF. Cells loaded with 2′,7′-bis-(2-carboxyethyl)5(and-6)-carboxyfluorescein (BCECF) were diluted into 50 mmol l^{-1} potassium phosphate (pH 7.0) (arrow 1). Subsequently, valimomycin (1.0 μmol l^{-1}) (arrow 2), lactocin 705 (90 nmol l^{-1}) (arrow 3) and nigericin (1.0 μmol l^{-1}) (arrow 4) were added.

valinomycin was added. As shown in Fig. 4, the addition of valinomycin resulted in a decrease in fluorescence, indicating the interconversion of the membrane potential to pH gradient. The fluorescence of the probe was rapidly increased on addition of lactocin 705, indicating an increase in the internal pH due to the extrusion of H^+ ions. The addition of nigericin did not show any further effect, demonstrating that lactocin 705 was responsible of the complete collapse of the ΔpH. The above results indicate that lactocin 705 was able to dissipate both components of PMF.

3.5. Efflux of K^+ ions and inorganic phosphate is the result of bacteriocin action

To determine whether the bacteriocin has an impact on the internal K^+ pool of *L. plantarum* CRL691, control cells and cells treated with the bacteriocin were permeabilized with trichloroacetic acid, and the intracellular and extracellular K^+ concentrations were measured by flame photometry. In the absence of lactocin 705, cells of *L. plantarum* maintained an intracellular concentration of K^+ of approximately 110 μmol l^{-1}. The subsequent addition of lactocin 705 to glucose-energized cells (100 mmol l^{-1}) caused a dramatic loss of cellular K^+ (Fig 5). Measurement of the extracellular K^+ content indicated that the bacteriocin had induced massive leakage of K^+ from the cells, as the concentration outside had increased. The release of K^+ was immediate, and after 5 min of treatment with 90 nmol l^{-1} of lactocin 705, the cells retained a low level of K^+ intracellular (11 μmol l^{-1}). The same experiment was carried out with cells energized with 10 mmol l^{-1} of glucose, the K^+ efflux being lower (51 μmol l^{-1}) after 5 min. On the other hand, the extracellular K^+ concentration increased after lactocin 705 addition, reaching 107 and 83 μmol mol l^{-1} after 25 min in presence of 100 and 10 mmol l^{-1} glucose, respectively.

Lactocin 705 was shown to make cells permeable to inorganic phosphate as well as K^+ ions (Fig. 6). Therefore, the samples were assayed for internal and external inorganic phosphate contents. Lactocin 705 induced the loss of intracellular phosphate from *L. plantarum* at a concentration of 90 nmol l^{-1}. In cells energized with 100 mmol l^{-1} of glucose, the release was very rapid, as was found with K^+. After lactocin 705 addition, intracellular phosphate decreased from 96 to 10 μmol l^{-1} and from 96 to 35 μmol l^{-1} for

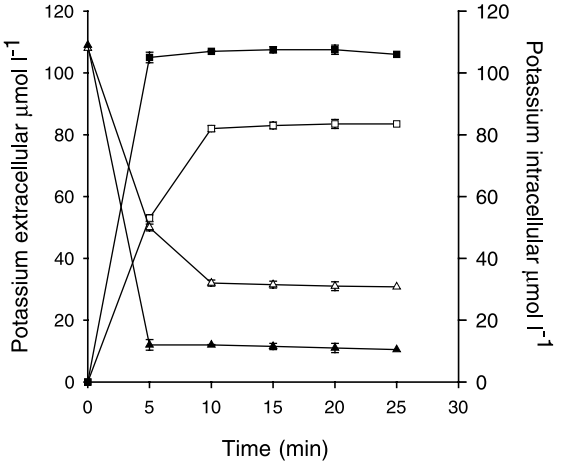

Fig. 5. Lactocin 705 induced K^+ efflux from energized *L. plantarum* CRL691 cells. Solid symbols: 100 mmol l^{-1} glucose; empty symbols: 10 mmol l^{-1} glucose. Extracellular K^+ concentration (■,□); intracellular K^+ concentration (▲,□). Values are mean ± standard deviation of two replicate samples.

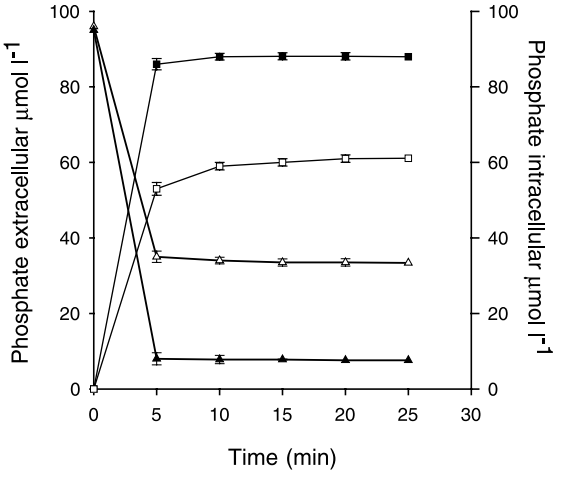

Fig. 6. Lactocin 705 induced phosphate inorganic efflux from energized *L. plantarum* CRL691 cells. Solid symbols: 100 mmol l^{-1} glucose; empty symbols: 10 mmol l^{-1} glucose. Extracellular P_i concentration (■,□); intracellular P_i concentration (▲,□). Values are mean ± standard deviation of two replicate samples.

cells energized with 100 and 10 mmol l^{-1}, respectively. When extracellular phosphate was analyzed, results were similar as with extracellular K^+, the efflux being 84 and 53 µmol l^{-1}, respectively, after 5 min for both glucose concentrations.

4. Discussion

The present work provides evidence of the mechanism of action of the two-component bacteriocin lactocin 705, which activity relies upon the complementation of 705α and 705β peptides of 33-amino-acid residues each and belong to subclass IIb bacteriocin (Cuozzo et al., 2000). Its secondary structure was predicted to form an amphiphilic α-helix (Palacios et al., 1999), this conformation being essential to the antimicrobial action of the bacteriocin. Our data suggest that the bacteriocin induces cell death by making sensitive cell membrane permeable, allowing for the efflux of K^+ ions and inorganic phosphate. This action results in the dissipation of the $\Delta\Psi$ and ΔpH components of the PMF. In this regard, Nes and Holo (2000) reported that class IIb bacteriocins seem to form relatively specific pores that were all found to dissipate the transmembrane potential. Montville and Bruno (1994) reported that bacteriocins from lactic acid bacteria acted by the common mechanism of depleting proton motive force; however, some differences have been observed between the various antimicrobial systems. The two-peptide bacteriocin lacticin 3147 and lactococcin G were found to selectively dissipate the membrane potential and hydrolyse internal ATP leading to an eventual collapse of the pH gradient (McAuliffe et al., 1998; Moll et al., 1997). On the other hand and similarly to lactocin 705, plantaricins EF and JK and acidocin J1132 (Moll et al., 1999b; Tahara et al., 1996) permeabilized the target cells dissipating the transmembrane electrical potential and pH gradient in a nonselective manner.

As with other lactic acid bacteriocins, the primary site of action of lactocin 705 appears to be the cytoplasmic membrane (Abee et al., 1994; Barrena-Gonzalez et al., 1996; Nes and Holo, 2000). Results from killing assays performed with lactocin 705 suggest that the action of the bacteriocin was enhanced when target cells were energized, leading to the conclusion that the changes generated in the PMF may favour insertion of the bacteriocin into the membrane as well as pore formation (Moll et al., 1999a). Lactocin 705 can also induce cell death in less energized cells (10 mmol l^{-1} glucose), although somewhat less effectively. McAuliffe et al. (1998) reported similar results when the action of lacticin 3147 on energized and unenergized cells was compared. These energy requirements for bacteriocin activity have direct implications on the potential use of these antimicrobial peptides as food preservatives. Nevertheless, complex and nutritionally rich environments are available, inducing the maintenance of fully energized food spoilage and pathogen cell membranes.

Treatment of *L. plantarum* CRL691 cells with lactocin 705 also resulted in the release of almost all detectable internal phosphate; as with K^+, this release occurred almost immediately through the nonselective pores formation. The level of extracellular phosphate in unenergized cells of *L. plantarum* was much lower than in energized cells. The loss of these essential ions may be a consequence of the ATP hydrolysis that occurs as a result of lactocin 705 action, presumably due to the consume of the available ATP in a futile attempt to reaccumulate K^+ and phosphate by ATP-dependent uptake systems, resulting in an collapse of the ΔpH.

The effect of different ions was explored because they play a role in cellular physiology such as the

preservation of intracellular pH (Otto et al., 1983) and the stimulation of ATPase bound to cytoplasmic membrane (Maloney, 1997). It was demonstrated that treatment of susceptible cells with Ca^{2+} ions resulted in a protective effect against the bacteriocin showing a markedly lower bactericidal effect. The reduced efficiency of some bacteriocins against Gram-positive target cells in presence of di- and trivalent cations could be explained by the interaction with the negatively charged phospholipid head groups present in the cytoplasmic membrane (Abee et al., 1994). Moreover, the presence of ions in the environment might make it possible to compensate, at least in part, for the cations lost from cells after the addition of lactocin 705 action as reported for plantaricin SA6 by Rekhif et al. (1995). Regarding the NaCl effect on lactocin 705 efficiency, no changes were observed, this result being partially in agreement with Gänzle et al. (1999), who reported that curvacin A and especially sakacin P activity were increased by NaCl addition, but nisin activity against *L. curvatus* and *L. innocua* remained unaffected. The results of the MIC with peptides previously treated with Ca^{2+} suggest that this ion affected the appropriate complementation of 705α and 705β peptides to exert its bactericidal activity, leading to the observed protective effect. This can result in the potential reduction of the efficiency of bacteriocins action against Gram-positive spoilage bacteria and pathogens in food systems containing calcium.

On the basis of the data presented here, our results suggest that the bactericidal action of this two-component bacteriocin occurs through complementation of the two peptides which form poration complexes in the cytoplasmic membrane, thereby dissipating ion gradients and resulting in inhibition of the growth of target microorganims. These events were observed to be highly stimulated in presence of glucose and adversely affected by Ca^{2+} ions, thus these parameters together with others food components can have significant influence on the activity of the bacteriocin.

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