Mesentericin Y105 gene clusters in *Leuconostoc mesenteroides* Y105

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Because of their potential usefulness as natural food preservatives, increased interest has focused on bacteriocins from lactic acid bacteria. Mesentericin Y105 is a small non-lantibiotic bacteriocin (class II) encoded within a 35 kb plasmid from *Leuconostoc mesenteroides* Y105 and it is active against *Listeria monocytogenes*. Using reverse genetic methodologies, an 8 kb *DraIII* fragment has been cloned that contains the mesentericin Y105 structural gene, *mesY*, which encodes a precursor of the bacteriocin with a 24 amino acid N-terminal extension ending with a Gly–Gly motif upstream of the cleavage site, which is typical of class II bacteriocins. Four other putative genes are associated with *mesY* within two divergent putative operons. In addition to *mesY*, the first putative operon is predicted to encode a protein, similar to that encoded by ORF2 in the leucocin A operon, whose function remains to be elucidated. The second putative operon contains three ORFs, two of which, *mesD* and *mesE*, encode proteins that resemble ATP-dependent transporters and accessory factors, respectively. For three other class II bacteriocin systems (lactococcin A, pediocin PA-1, colicin V), these proteins have been shown to be involved in bacteriocin secretion independently of the general sec-dependent secretion pathway. The last putative gene (*mesC*) does not resemble any previously characterized gene. Results concerning the heterologous expression of the cloned *mesY* in *Lactobacillus johnsonii* NCK64 suggest that the maturation and secretion functions dedicated to lactacin F (another class II bacteriocin) are efficient for mesentericin Y105 as well. This characteristic may be of great interest in the development of industrial fermentation starters producing multiple bactericidal activities.

Keywords: bacteriocin, mesentericin Y105, lactic acid bacteria, ABC transporter, *Listeria*

INTRODUCTION

Because of the trend to reduce the use of chemical additives in food protection against pathogens and other undesirable organisms, increased interest has been focused on bactericidal compounds produced naturally by organisms that are 'generally recognized as safe'. Antimicrobial activities of lactic acid bacteria have been widely investigated over the past decade (De Vuyst & Vandamme, 1994). Among such natural inhibitors, proteinaceous compounds, called bacteriocins, inhibit closely related species (Tagg *et al.*, 1976) and, therefore, appear to be good candidates for use as biological food preservatives.

The lactic acid bacterium *Leuconostoc mesenteroides* Y105 isolated from goat's milk produces a bacteriocin (mesentericin Y105) that is bactericidal against *Listeria* species (Héchard *et al.*, 1992a, b). This peculiar characteristic may be of great interest in the development of strains that are active against pathogens without affecting the natural microflora of dairy products and other fermented foods. Mesentericin Y105 is a small heat-stable peptide, devoid of lanthionine residues, and contains the N-terminal YGNGVXC consensus motif (Héchard *et al.*, 1992a) commonly found in all anti-*Listeria* bacteriocins and typical of the pediocin-like bacteriocins, as defined by Nieto Lozano *et al.* (1992). Therefore, it can be classified as a class IIa bacteriocin, according to the classification of Klaenhammer (1993). Mesentericin Y105 as well as other class II bacteriocins provoke a depolarization of the cytoplasmic membrane of sensitive bacteria (van Belkum

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et al., 1991b; Chikindas *et al.*, 1993; Maftah *et al.*, 1993; Venema *et al.*, 1993; Abee *et al.*, 1994).

Over the past five years, there has been a burst of research activity at the molecular level on the class II bacteriocins produced by lactic acid bacteria. To date, the primary structures for a few of these bacteriocins have been elucidated and their structural genes characterized. These include lactacin F from *Lactobacillus johnsonii* (Muriana & Klaenhammer, 1991; Fremaux *et al.*, 1993; Allison *et al.*, 1994), lactococcin A, B and M from *Lactococcus lactis* subsp. *cremoris* (van Belkum *et al.*, 1991a, 1992; Holo *et al.*, 1991), leucocin A from *Ln. gelidum* (Hasting *et al.*, 1991), pediocin PA-1 from *Pediococcus acidilactici* (Marugg *et al.*, 1992), sakacin A from *Lb. sake* (Holk *et al.*, 1992), and carnobacteriocin A from *Carnobacterium piscicola* (Worobo *et al.*, 1994). Colicin V is another bacteriocin that can be classified within class II, even though it is produced by *Escherichia coli*, a Gram-negative organism (for a review see Fath *et al.*, 1992). Colicin V export is independent of the general secretion pathway and involves two genes genetically linked and associated with the bacteriocin structural gene. These two genes, encoding an ATP-dependent transporter and an accessory factor, have been well characterized for colicin V export. Of the class II bacteriocins from lactic acid bacteria, ABC transporter genes have only been cloned for lactococcins and pediocin PA-1 (Marugg *et al.*, 1992; Stoddard *et al.*, 1992). However, there is no biological evidence for their effective involvement in bacteriocin export.

In this study, the mesentericin Y105 structural gene (*mesY*) has been cloned from *Ln. mesenteroides* plasmid pHY30 using reverse genetics, and transferred to a strain of *Lb. johnsonii*. Heterologous expression was obtained and the bacteriocin was efficiently produced. The nucleotide sequence of *mesY* and its flanking regions have been determined. It appears to be encoded within an operon together with another ORF that encodes the putative immunity peptide against mesentericin Y105. A second operon, divergent from the *mesY* operon, encodes three proteins. Two of them, MesD and MesE, resemble the ABC transporters and their accessory factors, respectively. The third protein encoded within the operon does not display any similarity with proteins from databases.

METHODS

Bacterial strains, cultures and plasmids. Bacterial strains and plasmids used in this study are detailed in Table 1. *E. coli* strains were propagated at 37 °C in LB broth or on agar (1.5%, w/v) (Sambrook *et al.*, 1989). Ampicillin, erythromycin, IPTG and X-Gal were used at concentrations of 75, 150, 50 and 50 µg ml⁻¹, respectively. Erythromycin-resistant transformants of *E. coli* were selected on brain heart infusion agar plates (Difco). Lactic acid bacteria were propagated in MRS (Difco) broth or on agar (1.2%, w/v) at 30 °C for *Ln. mesenteroides* strains and at 37 °C for *Lb. johnsonii*. When appropriate, erythromycin was added to 3 µg ml⁻¹. *L. monocytogenes* E20 was grown at 30 °C in tryptic soy broth (Difco). To obtain plasmid-cured derivatives of *Ln. mesenteroides* Y105, cultures were performed in MRS broth containing a sublethal concentration of novobiocin (5 µg ml⁻¹).

After about 15 generations, colonies were isolated, assayed for bactericidal activity and their plasmid content was analysed as described below.

Bacteriocin assays. The critical dilution assay (Mayr-Harting *et al.*, 1972; Barefoot & Klaenhammer, 1983) was used for bacteriocin titration. Mesentericin Y105 was assayed in MRS broth cultures that were propagated overnight. Twofold serial dilutions of cell-free supernatant were spotted on MRS agar and then overlaid with 5 ml tryptic soy agar (0.6%, w/v) inoculated to 1% with the indicator strain. After overnight incubation at 30 °C, bacteriocin titres were determined and expressed in activity units per ml (AU ml⁻¹).

DNA manipulation, cloning and transformation. Plasmids from *E. coli* and lactic acid bacteria were extracted and purified as previously described by Sambrook *et al.* (1989) and Muriana & Klaenhammer (1987), respectively. Plasmid DNA was digested with restriction enzymes (Gibco-BRL) according to the supplier's recommendations. Analytical and preparative agarose gel electrophoresis in Tris-borate/EDTA (pH 8.3) was done according to the methods of Sambrook *et al.* (1989). DNA restriction fragments were purified from agarose gels using the Prep-a-Gene kit (Bio-Rad). Recombinant DNA was obtained using the T4 DNA ligase (Gibco-BRL) following the manufacturer's recommendations. *E. coli* competent cells were prepared and transformed according to the methods of Hanahan (1983). Lactic acid bacteria were transformed by electroporation in 0.2 cm cuvettes in a Gene Pulser apparatus (Bio-Rad; settings 25 µF, 2.5 kV, 200 Ω). Electro-competent cells were prepared according to the method of Raya *et al.* (1992).

Hybridization. Plasmids and DNA fragments cleaved with the appropriate restriction enzyme were transferred from agarose gels to nylon membranes (Hybond-N+; Amersham) as described by Southern (1975), then treated according to the supplier's instructions. Probes were labelled by random priming as described by Sambrook *et al.* (1989).

PCR. *mesY* gene amplification by PCR was done using primers YN1, 5'-GGATCCAAGTATTATGGTAACGGAGTTCAT-TGCACA, and YN2, 5'-ATGAATTCCCAGAAACCATTT-CCACCAT, deduced from the mesentericin Y105 amino acid sequence (Hécharde *et al.*, 1992a). Amplifications were carried out with *Taq* DNA polymerase (Perkin Elmer Cetus) according to the supplier's recommendations using the Gene Aaq thermocycler (Pharmacia) under the following conditions: 30 × (denaturation, 94 °C for 30 s; annealing, 53 °C for 30 s; elongation, 72 °C for 1 min).

DNA sequencing and computer analysis. Part of the 8 kb *Dra*II (5235 bp) insert from pCFYC2(+) and -(−) has been sequenced. The insert was first subcloned in parts (see below, Fig. 2, pCFYC3 and pCFYC4), then random nested deletions were created using the Erase-a-Base system (Promega) to generate plasmid for sequencing. Sequencing, based on the chain-termination method (Sanger *et al.*, 1977), was done with the Auto-read sequencing kit (Pharmacia) using either standard or specific primers with an automated DNA sequencer (ALF, Pharmacia). Sequence analysis was performed with the programs of the GCG sequence analysis software package.

RESULTS

Location of the *mesY*

To obtain a DNA probe specific to *mesY*, two oligonucleotide primers (YN1 and YN2) deduced from the amino acid sequence of the secreted mesentericin Y105 (MesY) were synthesized. YN1 fits the N-terminus of the

Table 1. Bacterial strains and plasmids

Strain/plasmid	Relevant characteristics	Reference/source
Strain		
<i>E. coli</i>		
DH5 α	<i>recA EndA1 gyrA96 thi-1 hsdR17 supE44</i> $\Delta lacU169(\Phi 80 \Delta lacZ \Delta M15) deoR F^- \lambda^-$	BRL
CFYC2	DH5 α (pCFYC2 + / - *)	This study
CFYC3	DH5 α (pCFYC3)	This study
CFYC4	DH5 α (pCFYC4)	This study
CFYC5	DH5 α (pCFYC5)	This study
<i>Ln. mesenteroides</i>		
Y105	MesY ⁺ , pHY30, pHY15, pHY3, pHY2	Hécharde <i>et al.</i> (1992)
Y30	MesY ⁻ , pHY15, pHY3, pHY2	This study
Y31	MesY ⁻ , pHY15, pHY3, pHY2	This study
Y32	MesY ⁻ , pHY15, pHY3, pHY2	This study
<i>Lb. johnsonii</i>		
NCK64	NCK88 derivative, <i>lafA729</i> , Laf ⁻ , MesY ^R	Allison <i>et al.</i> (1994)
NCK65	NCK88 derivative, Δlaf , Laf ⁻ , MesY ^R	Allison <i>et al.</i> (1994)
CFYC6	NCK64(pGKV210)	This study
CFYC7	NCK65(pGKV210)	This study
CFYC8	NCK64(pCFYC5), MesY ⁺	This study
CFTC9	NCK65(pCFYC5), MesY ⁺	This study
<i>L. monocytogenes</i>		
E20	MesY-sensitive indicator	IUT la Rochelle; Hécharde <i>et al.</i> (1992)
Plasmid		
pBluescript II SK +	Amp ^R , <i>lacZ</i> , cloning vector, 2.9 kb	Stratagene
pUC18	Amp ^R , <i>lacZ</i> , cloning vector, 2.9 kb	Pharmacia
pGKV210	Erm ^R , <i>E. coli</i> -lactic acid bacteria shuttle vector, 4.4 kb	van der Vossen <i>et al.</i> (1987)
pYHYC1	pUC18::127 bp <i>EcoRI</i> - <i>Bam</i> HI PCR fragment	This study
pCFYC2 + / - *	pBluescript II SK(+):8.0 kb <i>Dra</i> II fragment from pHY30	This study
pCFYC3	pBluescript II SK(+):4.0 kb <i>Sac</i> I- <i>Dra</i> II fragment from pHY30	This study
pCFYC4	pBluescript II SK(+):4.0 kb <i>Dra</i> II- <i>Sac</i> I fragment from pHY30	This study
pCFYC5	pGKV210::1.7 kb <i>Nsi</i> I- <i>Pst</i> I fragment from pHY30	This study

* + / - Depending on the insert orientation.

mature MesY with a *Bam*HI restriction site 5'-extension, and YN2 fits the C-terminus of MesY with an *Eco*RI restriction site 5'-extension. Using these primers, PCR amplification of a plasmid DNA preparation from *Ln. mesenteroides* Y105 generated a unique DNA fragment of 127 bp. This fragment was cloned within the *Bam*HI and *Eco*RI sites of pUC19, generating pYHYC1, and its sequence was determined. The DNA sequence of the cloned fragment encoded a deduced peptide that is identical to the mature MesY.

The *Bam*HI-*Eco*RI insert from pYHYC1 was purified from an agarose gel and used as a probe in Southern blot

hybridization with plasmid DNA extracted from *Ln. mesenteroides* Y105. The strain contains four plasmids, pHY30, pHY15, pHY3 and pHY2, of about 35, 15, 3.5 and 2.5 kb, respectively (Fig. 1a); the probe hybridized to pHY30 (Fig. 1b). Similar analysis with total DNA extracted from *Ln. mesenteroides* Y105 displayed no hybridization to chromosomal DNA (not shown). These data located *mesY* within pHY30. In addition, *Ln. mesenteroides* Y105 derivatives (*Ln. mesenteroides* Y30, Y31 and Y32) that did not produce the bacteriocin were obtained by novobiocin treatment (0.5 μ g ml⁻¹). Plasmid profiling revealed the absence of pHY30 from these strains (Fig. 1a), thus providing correlative evidence for the involve-

Fig. 1. Agarose gel electrophoresis (a) and Southern blot hybridization (b) of plasmid extracts with a *mesY*-specific DNA probe isolated from pYHYC1. Plasmids were extracted from *Ln. mesenteroides* Y32 (lane 1), Y31 (lane 2), Y30 (lane 3), Y105 (lane 4). Arrowheads indicate the plasmids and their size has been estimated (see text) by comparison with a supercoiled DNA standard ladder (GIBCO-BRL). Traces of chromosomal DNA (chr) are arrowed.

ment of pHY30 in bacteriocin production. A partial restriction map of a 20 kb fragment from pHY30 which contains *mesY* was prepared and is shown in Fig. 2.

DNA sequencing of the *mesY* region

An 8 kb *Dra*II fragment, purified from pHY30, which hybridized to the 127 bp *Bam*HI-*Eco*RI *mesY*-specific sequence in pYHYC1 was cloned within the *Dra*II restriction site of pBluescript II SK(+), generating pCFYC2(+) and -(−), depending on the insert orientation (Fig. 2). Plasmids pCFYC3 and pCFYC4 were generated from pCFYC2(+) and -(−) by deletion of a 4 kb fragment using *Sac*I restriction sites (one internal to the *Dra*II insert and the other located within the multicloning site). Using these plasmids and deleted derivatives, and specific synthetic primers, 5235 bp out of the 8 kb *Dra*II fragment have been sequenced (Fig. 3). Computer analysis of the sequence, in all possible reading frames, identified two ORFs named *mesI* and *mesY* on one strand, and three ORFs on the other strand, named *mesC*, *mesD* and *mesE*, thus defining two gene clusters. Each ORF, except *mesD*, is preceded by a putative ribosome-binding site. Within the first gene cluster, *mesY* encoded a protein which consists of 61 amino acid residues. Amino acid residues 25 to 60, predicted from *mesY* (Hécharde *et al.*, 1992), correspond entirely to the amino acid sequence

determined for mesentericin Y105. This indicates that the *mesY* gene encodes a precursor of the bacteriocin with a 24 amino acid N-terminal extension, which is probably cleaved during secretion. Positioned 20 bp downstream of *mesY*, *mesI* is likely to start with the less frequently used TTG initiation codon, and encodes a putative protein of 113 amino acids. In *E. coli*, TTG replaces the ATG initiation codon in some genes (Kozak, 1983), whereas in *Leuconostoc* sp. this phenomenon has been described only once (Hastings *et al.*, 1991). Just downstream of *mesI*, computer analysis predicted a stem-loop structure (the stem and the loop are 27 and 9 bp long, respectively) that might act as a terminator (calculated free energy of formation, ΔG_0 , $-42.2 \text{ kcal mol}^{-1}$; $-176.6 \text{ kJ mol}^{-1}$). The second group of putative genes starts with *mesC* which encodes a 137 amino acid protein. It is followed at 46 bp by two ORFs separated by 9 residues, *mesD* then *mesE*, that encode two large proteins of 722 and 457 amino acid residues, respectively.

These two clusters of putative genes are transcribed in opposite directions, and are separated by a 154 bp non-coding region that should contain both promoter regions (Figs 2 and 3). Within this area, three putative promoters P1, P2 and P3, as described in Fig. 3, present strong identities with characterized constitutive promoters driving the transcription of several genes in lactic acid bacteria (van der Vossen *et al.*, 1987; Koivula *et al.*, 1991). This organization suggests that these five ORFs may be transcribed as two operons. Indeed, messenger RNA analyses need to be performed to confirm this hypothesis. A schematic representation of the mesentericin Y105 region organization is displayed in Fig. 2.

Heterologous expression of the mesentericin Y105

A 1.7 kb *Pst*I-*Nsi*I fragment from pCFYC3 (Fig. 2) was isolated and inserted within the unique *Pst*I restriction site of pGKV210, an *E. coli*-lactic acid bacteria shuttle vector encoding erythromycin resistance and a promoterless *cat* (van der Vossen *et al.*, 1987). The resulting construction (pCFYC5; Fig. 2), contains *mesY*, *mesI*, and their upstream region, including their putative promoter and *mesC* with a truncated C-terminus. No erythromycin-resistant (*Erm*^R) transformants were obtained with *Ln. mesenteroides* Y105 and Y30.

Consequently, *Lb. johnsonii* NCK64 and NCK65, derived from the lactacin F-producing strain *Lb. johnsonii* NCK88 (Muriana & Klaenhammer, 1987), were investigated for their ability to express mesentericin Y105. Both are lactacin F non-producing strains and are insensitive to mesentericin Y105. NCK64 has a frameshift mutation within the lactacin F structural gene and NCK65 has a chromosomal deletion of about 10 kb affecting the lactacin F operon (Allison *et al.*, 1994). Good transformation efficiencies were obtained using pGKV210 and erythromycin selection; however, the efficiency decreased by two orders of magnitude when pCFYC5 was used for the transformations. Moreover, the resulting transformants had poor growth rates and decreased plasmid copy numbers. Their ability to produce mesentericin Y105

Fig. 2. Schematic overview of the genetic organization of the mesentericin Y105 gene clusters. The upper part of the diagram displays a partial restriction map of the 20 kb *Hind*III fragment from pHY30. The restriction fragments cloned within plasmids pBluescript II SK(+) (for pCFYC2, pCFYC3 and pCFYC4) and pGKV210 (for pCFYC5) are indicated by shaded bars in the bottom part of the figure. The diagram locates the ORFs and their respective orientation (arrowed boxes). The putative function of the proteins deduced from ORFs is indicated. P and T indicate putative promoter and terminator regions, respectively. The solid bar represents the portion of the insert whose sequence has been determined on both strands.

was analysed (Table 2). NCK64(pGKV210) and NCK65(pGKV210) were not bactericidal against *L. monocytogenes* E20. NCK64 and NCK65 transformed with pCFYC5 stably produced variable amounts of mesentericin Y105 ranging from 100 to 3200 AU ml⁻¹. To analyse the efficiency of the putative promoter located downstream of *mesI*, chloramphenicol MICs were determined for CFYC8 and -9 and compared to those of CFYC6 and -7 (containing pGKV210). No difference was observed between these strains. Thus, because of the absence of obvious coding sequences downstream of *mesI* (sequence not shown), the inverted repeats located behind *mesI* are likely to act as a transcriptional terminator.

Similarity of the mesentericin Y105 to the leucocin A-UAL 187

Hastings *et al.* (1991) purified leucocin A, an anti-*Listeria* bacteriocin from *Leuconostoc gelidum* UAL 187. In addition, they analysed a 1088 bp plasmid-derived fragment from *Ln. gelidum* UAL 187 that contains the precursor leucocin A gene (ORF1), the putative immunity gene (ORF2), and their upstream and downstream sequences. Hécharde *et al.* (1992a) pointed out some striking identities between this bacteriocin and the purified mesentericin Y105. Differences involved replacements of residues 22 and 26 of the mature peptide and an additional tryptophan at the C-terminus of the leucocin A. Analysis of the peptide sequence deduced from *mesY* confirmed the differences in position 22 and 26. However, similarly to leucocin A, MesY ended with a tryptophan residue. Analysis of the native MesY and leucocin A, deduced from their gene sequence, displayed a less conserved N-terminal extension (7 differences over 24 amino acids, data not shown).

Amino acid sequence alignment showed important similarities (74.5% identity, mostly located within the C-terminus) between the protein deduced from *mesI* (MesI) and the putative leucocin A immunity protein (deduced from ORF2, data not shown). However, no sequence similarity between *mesI* and ORF2 was found downstream. In addition, upstream of the leucocin A structural gene a truncated ORF has been found that is 72% identical to the N-terminus of MesC, and the resulting intergenic region is 77% identical to the promoter region between *mesY* and *mesC* (data not shown).

Similarity of the *mesC*–*mesD*–*mesE* gene cluster to ATP-dependent transport systems

Searches for protein homologies revealed that the proteins deduced from *mesD* (MesD) and *mesE* (MesE) share significant similarity with proteins known as ABC transporters and accessory factors, respectively. ABC transporters and accessory factors are two proteins that form secretion systems independent from the signal peptide-dependent export pathway in various prokaryotic organisms (Fath & Kolter, 1993). The predicted MesD consists of a large, mostly hydrophobic, N-terminus (with seven potential membrane-spanning segments), and a C-terminus containing the conserved 200 amino acid domain unique to ATP-dependent transporters (Fig. 3) (Fath & Kolter, 1993). The best similarity score was obtained with LcnC (75.8% similarity), the putative ATP-dependent transporter dedicated to lactococcins A, B and M (Stoddard *et al.*, 1992; van Belkum, 1994). Similarly, MesD is homologous to ComA (75%) and PedD (66.2%), the putative ATP-dependent transporters for the competence factor of *Streptococcus pneumoniae* (Hui & Morrison, 1991) and the pediocin PA-1 (Marugg *et al.*,

AAAATGTCCAATAATTAACCTAATACCTGTCAACATTCAAGTAAATATATCCAAAATCC	60	CTGGCTTTTATGCGGCTTTTCCAAAGTAAATATGACCAATGCAAGCAGGTTCGAT	2400
AGTTAACAAGTAATATATTGATTATCTATTCGAAATTCATCATGTAGTTTCATCCATA	120	<u>W L F M R P F S K M N N D Q M Q A G S M</u>	347
TTTCCCTAGAGTTTAATCATATTTAAAGCCTGTACAATTGAAAAATTAATGTAC	180	GTTGAGCTCTTCATTATGAAGATATTAATGGCGTTGAGACGATTAAAGCGCTGAATAG	2460
(end mesI)		<u>L S S S I I E D I N G V E T I K A L N S</u>	367
AAGGCTTTAAATATGATTACTGATCGAAACGCTGTAACCTGAGACTGTCTGAAA	240	TGAAGCAACGCGCTATCAAAATGATCATGAATTTGACATTAATTAGAGAAATCATT	2520
- Q D F V S Y F Q S K D S F	101	<u>E A T A Y H K I D H E F V T Y L E K S F</u>	387
ATCAGCCATATACTGACCATTAAGTCAGCTTTTGTCCAATACCAAGTTTATAAT	300	TGTTTACGCTAAACAGAGCTGTTCAAAATGCGATTAAAGTCTCTTACAGCTCTCTT	2580
D A M Y Q G N L G A K Q G I V G L K I I	81	<u>V Y A K T E A V Q N A I K S L Q L S L</u>	407
CAATTCCTCTCTTTTGTAAAAAATAATTTGTATGTTGAAGCAGTAATACGAATATA	360	AAATGCTGATTTTATGGGTTGGCGCAACCTGGTCATGACGAATAAATAGTGTGGG	2640
L E E E K K S F I I K Y T S A T I R I Y	61	<u>N V V I L W V G A Q L V M T N K I S V G</u>	427
ATTAACCAATCGATTAAATAGCGCTTCAACACTTGTAGTATTATCCAACTCTTGAAGAC	420	CCAATGATCACTTACATGCTTTATAGGATTTTACAGATCCCTTGCAAAATATTAT	2700
N V L R N I L A E V S Q T N D L K K S V	41	<u>Q L I T Y N A L L G F F T D P L Q N I I</u>	447
TTGAAGCAAACTCGCAATATCTGAGTATCCAGGTTTGTCAACATTATCAATACAG	480	TAATTTCAAACTAAGCTCCAACAGGCTCAGTCGTAATAACCGTTTGAAACGAAGTTA	2760
Q L L V D C I D S Y G P K D V N D V L L	21	<u>N L Q T K L Q Q A S V A N N R L N E V Y</u>	467
(start mesI)		TTTGGTAGATTGAGAATTTAAAGATAGTCATCAATGACAGAAAAATACGCCCAATAG	2820
AGAATAGAGTGTACTAGTGAATTTTGTCTATCTTAAATACCGATCTTTTTCAA	540	<u>L V D S E F K D S H Q M T E K I T P N S</u>	487
S Y L T S T Y N K D E L Y R Y K K K L	1	CTCACTAGTAGCCGATCATATCACTATAAATACGGTTTGGCGCCCGAGCGATTGATGA	2880
(end mesI)		<u>S L V A D H I T Y K Y G F G A P A I D D</u>	507
ACTAATCTCTCTACTGATTTTACCAAAATCCATTTCCACCATTTGCCAAACGATGTATG	600	TGTTTCACTAAGCATTACAGCGGTTGAAAAATCGCTTTGGTGGGATTTGGATCGAGG	2940
- W F G N G G N A L R H I	50	<u>V S L T I T A G E K I A L V G I S G S G</u>	527
CCAGCTGAGGAGCTTCCCGACTTAACAGCATCCACTTTTGTACAGTGAACACCA	660	GAAGTCAACTGATTAATTTGCTGGTAAATTTCTTCAACAGAACTAGGAGCTATCTC	3000
G A S A A E G W N V S C G S K T C H V G	30	<u>K S T L V K L L V N F F Q P E S G T I S</u>	547
TTCCATAATACTTTCCACCAACACTTTCTTGAGATTCTGGTATCTAATTTGCTGATAT	720	ACTGGGACCAACCACTCGTAACTCTGATAACATGAGCTAAGAGGACGATAATTA	3060
N G Y Y K T G G V V K K L N Q N D L Q Q Y	10	<u>L G P T P L A N L D K H E L R G H I N Y</u>	567
(start mesI)		TTTACCACAAGAACCTTTATCTTTCCGGTTCATCATGGAACCTGTTATAGGTGC	3120
GCTTCCACAGACTTCATATTCGTATAAATTTTCTCTTTTCTTACTATTTAAT	780	<u>L P Q E P F I F S G S I M E N L L L G A</u>	587
A E V S K M N T M	1	TAAACAGGACACCAAGAAGATATTAACAGGCGGTAGTAATGCTGAATTAAGA	3180
<-10 <.....P1.....<-35 <		<u>K F G T T Q E D I I R A V E I A E I K D</u>	607
CACATTGATTATATCTAAATTTTAAATCAATATTTCTTGTATATAAATATTTT	840	TGATTATGAAAAATGTCAAGGATTGGCCTGAATCGCCGAAGCGGTAAATATATC	3240
>-35 >.....P2.....>-10 >		<u>D I E K M S Q G F G T E L A E S G N I S</u>	627
>-35 >.....P3...		GGTGGTCAAAAACACGCTTTAGCTAGAGCCATTTAGTTGATTCTCGGTACT	3300
(end mesI)		<u>G G Q K Q R I A L A R A I L V D S P V L</u>	647
TTTATTTTATGTTAGAATAATGAGTGTATGTTTCTCTTTTAAATGAGATAAGGT	900	----->	
...>-10 >		GATTTAGTAGTCAACCAATCTTGTATGTTTAAACAGAAAAAGATTATTGATAA	3360
(start mesC)		<u>I L D E S T S N L D V L T E K K I I D N</u>	667
ATGCTGATTAAACATAAATGAAGATTGATTAATACTACCAAACTAACGAATGAT	960	TCTCATGAAGTTAACCAGAAAACTTATCTTTGAGCGACCGCTTAACACTTTTACA	3420
M P D L N I N E D L I N N Y Q K L T N D	20	<u>L M K L T E K T I I F V A H R L T I S Q</u>	687
ATAGAAATATTTCAAGAAATATCTACATCGATTTTTATAAATGAATATGAAAA	1020	CGAGTGGATCGTATTCTAACCATGCAAAAGTGGCAAGTATCGAAGATGGCAGCATGA	3480
I E I F H E I S Y I D F Y N K M N N G K	40	<u>R V D R I L T M Q S G K I I E D G T H D</u>	707
AATAGTTAATTTACCTTGGTAAACCAAGTGTCTATTTGTGCTCAATTTGTCCTATG	1080	(end mesD) (end mesI)	
N S L I Y L G K P I C T C I C V K F V M	60	CACCTACTTAAAGTGGTCTTCTATCGCTCATATTTAACTATTAAGGAGCTGAT	3540
CTGCATGACATTTTGGCTGCTAAAAATATGATATTTGATTACTTTAATGTTGATCTTTT	1140	<u>T L L K A G G F Y A S L F N H -</u>	722
L H D I L A A K N M H I D Y F N V D T F	80	1	
TTTGAAATAATCTCTGATAAATTAATTAATTTTTCAGACTTTAATATTT	1200	mesI	
F E N N S S D K I N Y I N F F Q T L N I	100	GTTTATCCAAAATACTTAGAAAGTGGCGAATTTATCAACGCTGTTACCGCAATTTCC	3600
TCCCACTTCCATCTTTGATTTTACTCATGAGATATGAATCAACGTTTACCACT	1260	<u>F D P K Y L E S G E F Y Q R R Y R N F P</u>	21
S Q L P S L I F T H G D M N Y Q R L P I	120	AACCTGATTATTTGCGCCATTTTGTGTTGTTTATGTTCTCTTGTAGCTATT	3660
(end mesC)		<u>T L I I V P I F L L V V F I V L F S L F</u>	41
TATACATAAAACCAATAAATGCTGGATACAGCAATTAACGACAAATGACAAAT	1320	TGTAAGCGTGAATTTGTTGCAAGCGAGTGGCGAAATTTCCAGCTAAAGTGTATC	3520
Y C T I K T P I N A I T A I N D K -	137	<u>A K R E I V V K A S G E I P A K V L S</u>	61
(start mesD)		AGATATCCAATCAACGAGTAATAATGCCATGATAGTAACTAGCCGAAATTAAT	3580
TATGTCAAAACTCTAAACATCATCAACGAATTAATAATGTTAAATCCCAATGTT	1380	<u>D I Q S T S N N A I D S N Q L A E N K M</u>	81
M V K T P M F	7	GGTAAAAAGGTGATACATTAGTGACATTTACTAGTGGTAATGAAAAATATCGTCTCA	3840
TCATAAAAAATAGATTATTTCAAGATGATGACGCTGATTGTGGTGGTGGCTGACT	1440	<u>V K K G D T L V T F T S G N E K I S S Q</u>	101
H K K I D Q L G K I A D P D N T C G V A A L	27	ATTATTGACGCAACAAATTAATCACTTAACCAATCGGATACAAAGTCTTGATCTTATA	3900
CGCTATGATTTAGCTCATTACAAAACGCGCTATCTTTAGCCAGCTACGTGATTAGC	1500	<u>L L T Q I N N L N N R I A Q L D Y K</u>	121
A M I L A H Y K T R L S L A K L R D L A	47	GCATGATTATTGATGGACGAAGTGAATTTGGTGGCAGATCAATTTGTTATGATA	3960
CAAAACGGATATGAAGAACGAGCTGCTTTAGGCAATGTTAAAGCTGCTAATGCGCTAGA	1560	<u>H S I I D G R S E F G G T D Q F G Y D N</u>	141
K T D M E G T T A L G I V K A N A L D	67	TCTATTCAACGGCTATATGGCGCAAGTTGATACGTTGACGAGTGAATTTAATCAAAAA	4020
CTTTGAACCTATGCGCTTACGCTGACTTGAATTTATCGATAAAAAGATTTACCGTA	1620	<u>L F N G Y M A Q V D T L T S E F N Q Q N</u>	161
F E T M P I Q A A D L S L F D K K D L P Y	87	TAGTGATAAAACCAAGCTGATCAACAGCTAATCATCAATTTGACGTTTAAAAACAGG	4080
TCCTTTTATGCTATGTGATTAAAGAAAGCAATACCGCAATTAACGCTTGTCTATGG	1680	<u>S D K Q T A D Q Q A N H Q I D V L K G</u>	181
P F I A H V I K E G K Y P H Y Y V V Y G	107	CCAATCTAAAAAATCAACAAATAGGCAATTTCAAGCTATTTTAAACGATTATAATAG	4140
CATGAAAGGTGATCAGCTATTAATCGCTGATCCAGATTAATCGTTGGGAAGCAATAAT	1740	<u>Q S K N N Q Q L A N Y Q A I L T S I N S</u>	201
M K G D Q L L I A D P D N T G K T K M	127	CAACACTAAACCACTAATAATCCCTATCAGTCCATTTATGATAATTAGCAGCCAGTT	4200
GACGAAAGCGCATTTAATGAGGAGTGGACGGTGTGCTCAATTTTATGCGCAATCC	1800	<u>N T K P T N N P Y Q S I Y D N Y A A Q L</u>	221
T K A H F N E E W T G V S I F I A P N F	147	AAAATCAGCAACAACTGATGATAAGAGCAAGTCAAGCAACTGCCTTAAGTATGT	4260
AACCTACAGCAACCAAGAAAAAGCGCTTCCCTGACTTTTATTCCTCGGTGATCAC	1860	<u>K S A Q T T D D K E Q V Q I D V L S</u>	241
T Y K P T K E K K R S L T S F I P V I T	167	GCAACAAATTTGATCAATTACAAACACGAGTATGTTGATGATGATCAATTTGCTGG	4320
ACGACAAAACTATAGTTATCAATATTTGTGATGCTGCTTATTTAGTCAACCTGCTAG	1920	<u>Q Q Q I D Q L Q T T S S S Y D S Q I A G</u>	261
R Q K L L V I N I V I A A L L V T L V S	187	TATTACAAAAAGTGGCTTTATCTCAAGCAGTACCTTAGATAAATCGCTGACTTGA	4380
CATTTTAGGATCTTTATTTTACAAGGTATCATTGATCTATATTTCCCAATAACATGAA	1980	<u>I T K S G P L S Q S S T L D K I A C T L K</u>	281
I L G S Y Y L Q G I I D T Y I P N N M K	207	GCACCAACCACTAGCAAGTCTCAAAAAGAAATCAATGATCAGCAACATTCCTAGATGA	4440
AAACACCTAGGAATTTGTGCTAGGCGTATTTTGGGTATGTTTACCAATTAAT	2040	<u>Q Q Q L A S A Q K E I N D Q Q Q S L D G</u>	301
N T L G I V S L G L I F A Y V I Q Q L L	227	ATTAAAGCCAAAGCATCATCTGCTAATGAGGACTCAAGATACGGTTATTAAAGCAC	4500
ATCTATGCTAGTGATTTATTTAATTTGTCAATGGGCAACGCTCTCAATGATATTAT	2100	<u>L K A K Q S S A N E D Y Q D T V I K A P</u>	321
S Y A R L L I V M G Q R L S I D I I	247	AGAAAGCGGTATTCTGCATTTAACCAGTGATAAAGCACTATAAAGTATTTCCCAAGG	4560
CTTGCTTTATCAACACATTTTGAACGCCAATGTCTTTTTCGCAACGCTGCTAG	2160	<u>E S G I L H L T S D K A T I E A L A S</u>	341
L S Y I K H I F E L P M S F F A T R R T	267	TACTACAGTTACCAAAATTTATCTATGCTAAACAAAGCAAAATTTAGTGTGAGTA	4620
AGGTGAAATTTGAGCGCTTTTACGAGCGCTAATGCCATTATTTGAAGCTCTGGCAAGC	2220	<u>T V T Q I Y P M L N K R T K L S V E Y</u>	361
G E I V S R F E W T D A N A I E A L A S	287	TTATGTTCTACAGTAATAGTGTGGTTTAAACGAGGACCAAACTAAGATTTGTCGC	4680
AATGTTATGATCTTTTATGACTTGGCATTTTGGTCAITTTGGCAGAGTGTAGTGGT	2280	<u>Y V P T S N S V G L K R G Q N I R F V A</u>	381
M L S V F L D L G I L V I V G T V L V V	307		
GCAAAATCTACCTGTTTCTTCTCTCTGATTTCCGGCTTATCGCCTAGTGTG	2340		
Q N S T L F L I S L I A I P A Y A L V V	327		

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TAATCAAAATGTTACTAAACCAATTAAATTAACCGGAACAATTAAACGATTAGTCTGC 4740
N Q N V T K P L I L T G T I K T I S S A 401
ACCCATAATAATTAAAGAGGAATCTTTTATAAGTGATAGCCAGTATAACGTTAACGT 4800
P I I I K E E S F Y K C I A S I N V N V 421
AAGAACACGAAACAATCAATATGGCCTTGCTGGCAAGTAACAACCACTAAAGGAAC 4860
R E H E Q I K Y G L A G K V T T T K G T 441

      (end mesE) ----->
AAAAACTGGTTTAAATTAATTAAGACATTCGCTAGGAACACTAATTGAGTACAAAA 4920
K T W F N Y Y K D I L L G N T N - 457

<-----
TATAAGATTTTGTACTCAATTGTACAGTAAAAATATTATATACTTAATGGGTTAGA 4980
AAATATTTTATTTACATACACTGTACAATAAAATGGTTTGTAAATTTTATATCGAACTAA 5040
TTATCTATGGTCATATAATAATTTCAATATTACTTGCTTTAGTTTCTAAAACACAATTGC 5100
TCAACCAAAATAAAGGTTCTCTGTATGATATATAGACAAAGGACTTTTAAAAAAAAT 5160
TAAGTTATTCTGCGTTGTGAGTAAAGGACTTTTATATGATAGAATTTCTAAATTACCA 5220
TACTAAAAACAAAA 5235

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Fig. 3. Partial nucleotide sequence of the *DraII* fragment cloned within pCFYC2, containing the mesentericin Y105 region. ORF names are given in parentheses according to Fig. 2. Putative -10 and -35 promoter regions and putative ribosome-binding sites (RBS) are displayed. Stem-loop structures are located by facing horizontal arrows. The amino acid sequences derived from the ORFs are shown below the nucleotide sequence. The mesentericin Y105 N-terminal processing site is indicated by a vertical arrow (†) within the protein sequence. The regions of the sequence noted 'Site A' and 'Site B' correspond to the A and B motifs conserved within the ATP-dependent transporter family (Fath & Kolter, 1993). Potential membrane-spanning segments in MesD and MesE are double-underlined.

Table 2. Levels of mesentericin Y105 production exhibited by derivatives of *Lb. johnsonii* NCK64 and NCK65

Strain	Relevant plasmid characteristics	Mesentericin Y105 activity (AU ml ⁻¹)
<i>Ln. mesenteroides</i>		
Y105	pHY30	12800
<i>Lb. johnsonii</i>		
CFYC6	Erm ^R	< 100*
CFYC7	Erm ^R	< 100*
CFYC8	Erm ^R , <i>mesY</i> , <i>mesI</i>	100–3200†
CFYC9	Erm ^R , <i>mesY</i> , <i>mesI</i>	100–400†

* Bactericidal activity, if any, was below the threshold of detection.

† Level of mesentericin Y105 production was variable depending on the transformant analysed.

1992), respectively. Hydrophobicity analysis of the deduced MesE predicted a mostly hydrophilic protein with the exception of the region from residues 20 to 42. Within this region, computer analysis (Klein *et al.*, 1985) indicates a putative α -helix (residues 22 to 42, Fig. 3). It contains only hydrophobic residues surrounded by positively charged amino acids, and therefore may be a trans-membrane anchor. Sequence alignments (not shown) displayed similarities (35.2% identity and 17.5% similarity) between MesE and the putative accessory factor LcnD which is required for lactococcin secretion (Stoddard *et al.*, 1992), thus, suggesting a possible role for

MesE in the mesentericin Y105 secretion process. Comparison of the predicted protein sequence MesC with protein sequences of data bases did not show any significant similarity.

DISCUSSION

As reported previously, *Ln. mesenteroides* Y105 produces a small heat-labile bacteriocin (class II, according to Klaenhammer, 1993), designated mesentericin Y105, which is active against various *Listeria* species (Hécharde *et al.*, 1992a). We have located the mesentericin Y105 genetic determinants on plasmid pHY30 and described a DNA region from this plasmid that is involved in the production of the mesentericin Y105. A 8 kb *DraII* fragment from pHY30 has been cloned that contains the mesentericin Y105 structural gene, *mesY*. Sequence analysis of 5235 bp of the cloned fragment revealed, in addition to *mesY*, the presence of four complete ORFs organized in two gene clusters. One cluster includes *mesY* and *mesI*, the putative gene encoding immunity to mesentericin Y105, and is preceded by a putative promoter and followed by a stem-loop structure that may act as a terminator. Thus, *mesY* and *mesI* are likely to form an operon. Likewise, *mesC*, *mesD* and *mesE* may form a second operon-like structure that is transcribed in the opposite direction with a promoter region overlapping the promoter region of the *mesY*–*mesI* operon. Messenger RNA analyses are underway to verify this hypothesis. To date, this genetic organization seems unique for bacteriocin operons from lactic acid bacteria; indeed, equivalent genes in other bacteriocin systems such as lactococcin A and M (Stoddard *et al.*, 1992; van Belkum, 1994) and pediocin PA-1 (Marugg *et al.*, 1992) are all located onto the same DNA strand.

Comparison between the amino acid sequence deduced from the gene with that of the purified bacteriocin (Hécharde *et al.*, 1992a) indicates that mesentericin Y105 is first translated as a 61 amino acid precursor, then post-translationally processed by cleavage of a 24 residue N-terminal extension at a specific site (val–val–gly–gly↓Lys). This specific cleavage site is believed to be common to all class II bacteriocins (Håvarstein *et al.*, 1994), and indeed, analysis of the mesentericin Y105 propeptide points out some of the striking similarities common to all the class II bacteriocin propeptides (Fremaux *et al.*, 1993). Gene sequence determination confirmed the amino acid sequence of mesentericin Y105 obtained from the purified peptide except for an additional tryptophan in the terminal position. Therefore, in spite of minor differences in the amino acid sequence, this bacteriocin is believed to be truly different from the leucocin A, because of their different activity spectra and their mode of action (bactericidal versus bacteriostatic). In addition, comparison of the DNA sequence over 1088 bp between leucocin A and mesentericin Y105 genetic loci showed numerous differences, mostly located at the wobble position in the coding regions (not shown). This probably reflects a divergent genetic evolution of a common ancestor

plasmid that underwent mutagenesis and rearrangement, the latter explaining the low similarity level observed within the regions downstream of the operon.

For many bacteriocin systems in lactic acid bacteria, an immunity gene has been characterized and located downstream of the bacteriocin structural gene (van Belkum *et al.*, 1991a, 1992; Marugg *et al.*, 1992). Located directly downstream of the bacteriocin structural gene, *mesI* encodes a protein of 113 amino acids. Therefore, *mesI* represents a good candidate for the immunity determinant.

A protein homology search revealed important similarities (from 66 to 76%) between LcnC, PedD and ComA and the protein deduced from *mesD*. LcnC and PedD proved to be required for the production of lactococcin A (Stoddard *et al.*, 1992) and pediocin PA-1 (Marugg *et al.*, 1992), respectively. ComA is one of the proteins required to facilitate genetic transformation of *S. pneumoniae* (Hui & Morrison, 1991). Phylogenetic analysis indicates that these four proteins formed one branch of a large phylogenetic tree grouping the ATP-dependent transporters (Fath & Kolter, 1993). Globally, these data suggest that MesD participates in mesentericin Y105 exportation, and indeed, as for all the ATP-dependent transporters, the central region of MesD presents a membrane-spanning domain containing several putative trans-membrane-helices, and the C-terminal region contains the highly conserved ATP-binding domain. Many of the dedicated bacterial exporters require additional proteins beside the transporter itself to form a functional complex. One protein is referred to as the accessory factor, where its structural gene is usually linked to the ATP-dependent transporter gene within an operon (Fath & Kolter, 1993). In particular, LcnD and ComB are required for the production of lactococcin A and the competence factor from *S. pneumoniae*, respectively, and they share sequence similarity with the accessory factor from Gram-negative bacteria. The gene *mesD*, encoding the putative transporter of mesentericin Y105, is genetically associated with two other genes in an operon (*mesC* and *mesE*), from which *mesE* encodes a protein having 52.7% similarity to LcnD, thus suggesting a possible role in bacteriocin secretion.

To analyse the mesentericin Y105 operon, shuttle-plasmid pCFYC5 containing *mesY* and *mesI* was constructed. Unfortunately, none of the attempted electroporation protocols yielded any transformant of *Ln. mesenteroides* Y30. Consequently, trials to express the cloned structural gene of mesentericin Y105 were done in a heterologous host that does not express any anti-*Listeria* activity. *Lb. johnsonii* NCK64, a non-producing derivative of the natural lactacin F producer (see Table 1) with a frameshift mutation in the lactacin F structural gene, was chosen. Bactericidal activity against *Listeria* was obtained with NCK64 clones containing pCFYC5, suggesting that NCK64 contains the whole machinery required for the production of the mesentericin Y105. It is possible that the lactacin F maturation and secretion functions can support heterologous expression of mesentericin Y105.

However, the lower level of bacteriocin, compared to the parental mesentericin Y105 producer, likely reflects decreased affinity of these functions for mesentericin Y105. Another explanation lies in an insufficient expression level of these chromosomally encoded functions for the expression of the bacteriocin gene located on the multi-copy plasmid pCFYC5. Heterologous bacteriocin production has also been obtained for lactacin F in *Ln. gelidum* UAL187, which naturally produces leucocin A (Klaenhammer *et al.*, 1994). Therefore, these overall data, in addition to protein sequence similarities and common processing sites, provide strong evidence for the operation of homologous processing and export systems for the production of the class II bacteriocins from lactic acid bacteria. This peculiar feature may be of great interest for future development of engineered strains for industrial fermentations that produce various bacteriocins that are active against a broad spectrum of pathogens.

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REFERENCES

- Abee, T., Klaenhammer, T. R. & Letellier, L. (1994). Kinetic studies of the action of lactacin F, a bacteriocin produced by *Lactobacillus johnsonii* that forms poration complexes in the cytoplasmic membrane. *Appl Environ Microbiol* **60**, 1006–1013.
- Allison, G. E., Fremaux, C. & Klaenhammer, T. R. (1994). Expansion of bacteriocin activity and host range upon complementation of two peptides encoded within the lactacin F operon. *J Bacteriol* **176**, 2235–2241.
- Barefoot, S. F. & Klaenhammer, T. R. (1983). Detection and activity of lactacin B, a bacteriocin produced by *Lactobacillus acidophilus*. *Appl Environ Microbiol* **45**, 1808–1815.
- van Belkum, M. J. (1994). Lactococcins, bacteriocins from *Lactococcus lactis*. In *Bacteriocins of Lactic Acid Bacteria*, pp. 301–318. Edited by L. De Vuyst & E. J. Vandamme. London: Chapman & Hall.
- van Belkum, M. J., Hayema, B. J., Jeeninga, R. J., Kok, J. & Venema, G. (1991a). Organization and nucleotide sequences of two lactococcal bacteriocin operons. *Appl Environ Microbiol* **57**, 492–498.
- van Belkum, M. J., Kok, J., Venema, G., Holo, H., Nes, I. F., Konings, W. N. & Abee, T. (1991b). The bacteriocin lactococcin A specifically increased the permeability of lactococcal cytoplasmic membrane in a voltage-independent, protein-mediated manner. *J Bacteriol* **173**, 7934–7941.
- van Belkum, M. J., Kok, J. & Venema, G. (1992). Cloning, sequencing, and expression in *Escherichia coli* of *lcnB*, a third bacteriocin determinant from the lactococcal plasmid p9B4-6. *Appl Environ Microbiol* **58**, 572–577.
- Chikindas, M. L., Garcia-Garcera, M. J., Driessen, A. J. M., Ledebouer, A. M., Nissen Meyer, J., Nes, I. F., Abee, T., Konings, W.

- N. & Venema, G. (1993). Pediocin PA-1, a bacteriocin from *Pediococcus acidilactici* PAC1.0, forms hydrophilic pores in the cytoplasmic membranes of target cells. *Appl Environ Microbiol* **59**, 3577–3584.
- De Vuyst, L. & Vandamme, E. J. (1994). Antimicrobial potential of lactic acid bacteria. In *Bacteriocins of Lactic Acid Bacteria*, pp. 91–142. Edited by L. De Vuyst & E. J. Vandamme. London: Chapman & Hall.
- Fath, M. J. & Kolter, R. (1993). ABC transporters: bacterial exporters. *Microbiol Rev* **57**, 995–1017.
- Fath, M. J., Skvirsky, R., Gilson, L., Mahanty, H. K. & Kolter, R. (1992). The secretion of colicin V. In *Bacteriocins, Microcins, and Lantibiotics*, pp. 331–348. Edited by R. James, C. Ladzunsky & F. Pattus. Heidelberg: Springer-Verlag.
- Fremaux, C., Ahn, C. & Klaenhammer, T. R. (1993). Molecular analysis of the lactacin F operon. *Appl Environ Microbiol* **59**, 3906–3915.
- Hanahan, D. (1983). Studies on transformation of *Escherichia coli* with plasmid DNA. *J Mol Biol* **166**, 557–580.
- Hastings, J. W., Sailer, M., Johnson, K., Roy, K. L., Vederas, J. C. & Stiles, M. E. (1991). Characterization of leucocin A-UAL 187 and cloning of the bacteriocin gene from *Leuconostoc gelidum*. *J Bacteriol* **173**, 7491–7500.
- Håvarstein, L. S., Holo, H. & Nes I. F. (1994). The leader peptide of colicin V shares consensus sequences with leader peptides that are common among peptide bacteriocins produced by Gram-positive bacteria. *Microbiology* **140**, 2383–2389.
- Héchar, Y., Dérjard, B., Letellier, F. & Cenatiempo, Y. (1992a). Characterization and purification of mesentericin Y105, an anti-*Listeria* bacteriocin from *Leuconostoc mesenteroides*. *J Gen Microbiol* **138**, 2725–2731.
- Héchar, Y., Jayat, C., Letellier, F., Julien, R., Cenatiempo, Y. & Ratinaud, M. H. (1992b). On-line visualization of the competitive behavior of antagonistic bacteria. *Appl Environ Microbiol* **58**, 3784–3786.
- Holk, A., Axelsson, L., Birkeland, S.-E., Aukrust, T. & Blom, H. (1992). Purification and amino acid sequence of sakacin A, a bacteriocin from *Lactobacillus sake* Lb706. *J Gen Microbiol* **138**, 2715–2720.
- Holo, H., Nilsen, O. & Nes, I. F. (1991). Lactococcin A, a new bacteriocin from *Lactococcus lactis* subsp. *cremoris*: isolation and characterization of the protein and its gene. *J Bacteriol* **173**, 3879–3887.
- Hui, F. M. & Morrison, D. A. (1991). Genetic transformation in *Streptococcus pneumoniae*: nucleotide sequence shows *comA*, a gene required for competence induction, to be a member of the bacterial ATP-dependent transport protein family. *J Bacteriol* **173**, 372–381.
- Klaenhammer, T. R. (1993). Genetics of bacteriocins produced by lactic acid bacteria. *FEMS Microbiol Rev* **12**, 39–85.
- Klaenhammer, T. R., Ahn, C. & Muriana, P. M. (1994). Lactacin F, a small hydrophobic heat-stable bacteriocin from *Lactobacillus johnsonii*. In *Bacteriocins of Lactic Acid Bacteria*, pp. 377–396. Edited by L. De Vuyst & E. J. Vandamme. London: Chapman & Hall.
- Klein, P., Kanehisa, M. & DeLisi, C. (1985). The detection and classification of membrane-spanning proteins. *Biochim Biophys Acta* **815**, 468–476.
- Koivula, T., Sibakov, M. & Palva, I. (1991). Isolation and characterization of *Lactococcus lactis* subsp. *lactis* promoters. *Appl Environ Microbiol* **57**, 333–340.
- Kozak, M. (1983). Comparison of initiation of protein synthesis in procaryotes, eucaryotes and organelles. *Microbiol Rev* **47**, 1–45.
- Maftah, A., Renault, D., Vignoles, C., Héchar, Y., Bressolier, P., Ratinaud, M. H., Cenatiempo, Y. & Julien, R. (1993). Membrane permeabilization of *Listeria monocytogenes* and mitochondria by the bacteriocin mesentericin Y105. *J Bacteriol* **175**, 3232–3235.
- Marugg, J. D., Gonzalez, C. F., Kunka, B. S., Ledebroer, A. M., Pucci, M. J., Toonen, M. Y., Walker, S. A., Zoetmulder, L. C. M. & Vandenberg, P. A. (1992). Cloning, expression, and nucleotide sequence of genes involved in production of pediocin PA-1, a bacteriocin from *Pediococcus acidilactici* PAC1.0. *Appl Environ Microbiol* **58**, 2360–2367.
- Mayr-Harting, A., Hedges, A. J. & Berkeley, R. C. W. (1972). Methods for studying bacteriocins. *Methods Microbiol* **7A**, 315–422.
- Muriana, P. M. & Klaenhammer, T. R. (1987). Conjugal transfer of plasmid-encoded determinants for bacteriocin production and immunity in *Lactobacillus acidophilus* 88. *Appl Environ Microbiol* **53**, 553–560.
- Muriana, P. M. & Klaenhammer, T. R. (1991). Cloning, phenotypic expression, and DNA sequence of the gene for lactacin F, an antimicrobial peptide produced by *Lactobacillus* spp. *J Bacteriol* **173**, 1779–1788.
- Nieto Lozano, J. C., Nissen Meyer, J., Sletten, K., Peláz, C. & Nes, I. F. (1992). Purification and amino acid sequence of a bacteriocin produced by *Pediococcus acidilactici*. *J Gen Microbiol* **138**, 1985–1990.
- Raya, R. R., Fremaux, C., De Antoni, G. L. & Klaenhammer, T. R. (1992). Site-specific integration of the temperate bacteriophage Φ adh into the *Lactobacillus gasseri* chromosome and molecular characterization of the phage (*attP*) and bacterial (*attB*) attachment site. *J Bacteriol* **174**, 5584–5592.
- Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: a Laboratory Manual*, 2nd edn. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.
- Sanger, F., Nicklen, S. & Coulson, A. R. (1977). DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci USA* **74**, 5463–5467.
- Stoddard, G. W., Petzel, J. P., van Belkum, M. J., Kok, J. & McKay, L. L. (1992). Molecular analysis of the lactococcin A gene cluster from *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* WM4. *Appl Environ Microbiol* **58**, 1952–1961.
- Southern, E. M. (1975). Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* **98**, 503–517.
- Tagg, J. R., Dajani, A. S. & Wannamaker L. W. (1976). Bacteriocins of Gram-positive bacteria. *Bacteriol Rev* **40**, 722–756.
- Venema, K. T., Abee, T., Haandrikman, A. J., Leenhouts, K. J., Kok, J., Konings, W. N. & Venema, G. (1993). Mode of action of lactococcin B, a thiol-activated bacteriocin from *Lactococcus lactis*. *Appl Environ Microbiol* **59**, 1041–1048.
- van der Vossen, J. M. B. M., van der Lelie, D. & Venema, G. (1987). Isolation and characterization of *Streptococcus cremoris* Wg2-specific promoters. *Appl Environ Microbiol* **53**, 2452–2457.

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