Characterization of the gene cluster involved in the biosynthesis of macedocin, the lantibiotic produced by *Streptococcus macedonicus*

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Abstract

Streptococcus macedonicus ACA-DC 198, a food-grade isolate from naturally fermented Greek Kasseri cheese, produces a lantibiotic named macedocin that has been previously purified and characterized. In the present study, a 15 171 bp region in the *S. macedonicus* ACA-DC 198 chromosome, containing the biosynthetic gene cluster of macedocin, has been sequenced. This region consists of 10 ORFs, which correspond to the genes (*mcd* genes) involved in macedocin biosynthesis, regulation and immunity. The *mcd* genes are organized in two operons and their role is predicted on the basis of similarities to genes of known lantibiotics. Compared with its closest match, the streptococcin A-FF22 gene cluster, the macedocin one contains an additional structural gene and an insertion sequence between the regulatory and the biosynthetic operons.

Introduction

Many lactic acid bacteria produce Class I bacteriocins, also called lantibiotics. Lantibiotics are synthesized as precursor peptides, which subsequently undergo posttranslational modifications (Kupke & Gotz, 1996). These modifications involve dehydration of serine and threonine residues to dehydroalanine and dehydrobutyrine, respectively. Lanthionine or β-methyllanthionine is produced from the reaction of dehydroalanine or dehydrobutyrine, respectively, with sulfydryl groups of cysteine residues. All lantibiotic precursor peptides (prepeptides) contain an N-terminal leader peptide of 29–59 amino acids followed by a C-terminal region that undergoes posttranslational modifications (propeptide).

The successful application of the lantibiotic nisin as a safe food preservative has attracted much attention on lantibiotics study in recent years (Delves-Broughton *et al.*, 1996). So far, streptococcal lantibiotics have been mainly isolated from streptococci found in the oral cavity and the upper respiratory tract of humans and animals, although they may be also isolated from almost any type of clinical specimen (Hardie, 1986). Some examples include salivaricin A produced by *Streptococcus salivarius* (Ross *et al.*, 1993), mutacin 1140 (Hillman *et al.*, 1998), mutacin I (Qi *et al.*, 2000), mutacin II (Novak *et al.*, 1994) and mutacin III (Qi *et al.*, 1999), all produced by *Streptococcus mutans*, bovicin HJ50 produced by *Streptococcus bovis* (Xiao *et al.*, 2004), nisin U produced by *Streptococcus uberis* (Wirawan *et al.*, 2006) and the streptococcins A-FF22 (SA-FF22) (Hynes *et al.*, 1993), A-M49 (SA-M49) (Hynes *et al.*, 1994) and streptin (Wescombe & Tagg, 2003) produced by *Streptococcus pyogenes*. Because lantibiotic-producing oral streptococci cross the borders of pathogenicity, it is obvious that they have no chance to be used in food applications.

Streptococcus macedonicus was first proposed as a new *Streptococcus* species for isolates deriving from naturally fermented Greek Kasseri cheese (Tsakalidou *et al.*, 1998). Schlegel *et al.* (2003) proposed to split *Streptococcus gallolyticus* into three subspecies: *S. gallolyticus* ssp.

gallolyticus, *S. gallolyticus* ssp. *pasteurianus*, *S. gallolyticus* ssp. *macedonicus*. Among the Kasseri isolates, *S. macedonicus* ACA-DC 198 produces a lantibiotic named macedocin that has been purified and characterized (Georgalaki *et al.*, 2002). Macedocin can be considered as a promising biopreservative due to its activity against several lactic acid bacteria, various food spoilage and pathogenic bacteria, among them *Clostridium tyrobutyricum*, which is one of the principle causative agents of cheese late blowing (Georgalaki *et al.*, 2002).

The molecule of macedocin was previously found to be identical with SA-FF22 and SA-M49 produced by the human pathogen *S. pyogenes* (Georgalaki *et al.*, 2002). However, the use of purified bacteriocins in food requires costly purification schemes and toxicology tests and it is generally accepted that using bacteriocin-producing cultures in food, instead of purified bacteriocins, has considerable advantages. From this point of view, only a food grade microorganism, like *S. macedonicus* ACA-DC 198 producing a bacteriocin, could be used as a biopreservative, while with the pathogenic *S. pyogenes* this cannot be the case.

In the present study, a model for the production of macedocin is proposed, based on the determination of the complete nucleotide sequence of the *lan* gene cluster (named as *mcd* genes) and on the similarities to the respective proteins of other lantibiotic clusters.

Materials and methods

Bacterial strains and growth conditions

The macedocin producing strain *S. macedonicus* ACA-DC 198 was grown at 37 °C in M17 medium or in skim milk (10%, w/v) supplemented with 0.3% (w/v) yeast extract, while *Escherichia coli* TOP10F′, used for the cloning experiments, was grown in Luria–Bertani medium supplemented with the appropriate antibiotics.

Amplification, cloning and sequencing of the genes encoding macedocin

All the basic molecular biology techniques were performed according to Sambrook & Russel (2001). Total DNA of *S. macedonicus* ACA-DC 198 used as target in the polymerase chain reaction (PCR) assays was isolated according to the method of Leenhouts *et al.* (1990). PCR amplification of the macedocin gene cluster was performed using several sets of primers listed in Table 1. The heterologous ones were designed according to the respective SA-FF22 genes from *S. pyogenes* (McLaughlin *et al.*, 1999), while the homologous ones were designed according to the resulting nucleotide sequence of the macedocin gene cluster.

Each 50-μL PCR-reaction mixture contained 1 × PCR buffer, 200 μM of each dNTPs, 3 mM MgCl2, 0.2 mM of each primer, 1 U of Dynazyme polymerase (Finnzymes) and 1–2 μL of the sample. PCR conditions were optimized on the basis of theoretical calculations of the melting temperatures of the primer pairs used and on the results of several amplification experiments. Amplified PCR products were cloned to the PCR 2.1. vector, included in the TA-Cloning Ligation Kit (Invitrogen), and sequenced by the Lark Technologies Inc. (UK). To amplify the two opposite ends of the gene cluster, the ligation-mediated PCR technique was applied according to Dufour *et al.* (2000).

Reverse transcriptase-PCR (RT-PCR) experiments

The RT-PCR approach was used to define the operons existing in the macedocin gene cluster. RNA was isolated from *S. macedonicus* ACA-DC 198 grown in skim milk supplemented with yeast extract, using the RNeasy Mini Kit

Table 1. Description of the oligonucleotide primers used in PCR amplifications

Set of primers	Name of primers	Source organism and gene	Sequence (5′ → 3′)
1	ForApyo	*S. pyogenes, scnA*	GGAAAAAATGGTGTGTTTAAAAC
	RevM1pyo	*S. pyogenes, scnM*	TGCCTCCTAGAACACCACTAG
2	ForM1mcd	*S. macedonicus, mcdM*	GGAATTCAGTTCTTTCTACGGG
	RevM2pyo	*S. pyogenes, scnM*	CACCTGTCATTAACCCATAAAC
3	ForM2mcd	*S. macedonicus, mcdM*	GTTTAGAGAATGGATGGATGTGC
	RevT1pyo	*S. pyogenes, scnT*	GACAATATCTAAATGATGACTAAC
4	ForT1mcd	*S. macedonicus, mcdT*	GGGATGAAGCTTTCAGTAATC
	RevFpyo	*S. pyogenes, scnF*	AAGTCCATTAGTAGGTTCATC
5	ForKpyo	*S. pyogenes, scnK*	GTTTAGAAATTTTCAATCCCATACC
	RevAmcd	*S. macedonicus, downstream of mcdA′*	CATATTAGACACTTAGTCATCC
6	ForFmcd	*S. macedonicus,mcdF*	GTTAAGGCCTATTCTCTAGG
	RevEpyo	*S. pyogenes, scnE*	CCCTTTCTTCTTTTTCAGTAAATA
7	ForEmcd	*S. macedonicus, mcdE*	GATGTTTGTAGCAGTAACTTGG
	T7 promoter	Universal primer	TAATACGACTCACTATAGGG
8	RevKmcd	*S. macedonicus, mcdK*	CCATAACTTAATCTATAACTCAGTACTTC
	T7 promoter	Universal primer	TAATACGACTCACTATAGGG

Table 2. Primers used in RT-PCR amplification of the *mcd* gene cluster

Primer (gene target)	Sequence (5′ → 3′)	Pairs of primers in RT-PCR	RT-PCR product (size, bp)
pF1 (*mcdK*)	CTTGAGTCTATCAGGAGAAACC	pF1/pR1	+(1442)
pR1 (*mcdR*)	GGAAATCAACTTTCACTAACTCC		
pF2 (*mcdR*)	GATAGATTGACTAAGTGAGACATCG	pF2/pRtnp	–
pRtnp (*tnp*)	CATGAGCGAAGCTTAGAAGCC		
pR2 (*mcdA′*)	CATATTAGACACTTAGTCATCC	pF2/pR2	–
pF3a (*mcdA*)	ATTTCTTGCTACATGTTGCTCATAA	pF3a/pR3	+(690)
PF3b (*mcdA′*)	GTTTCTTGCCACATGTTGTTCTTGA		
pR3 (*mcdA1*)	GGATTCCAAAGCATCTTGAC	pF3b/pR3	+(530)
pF4 (*mcdA1*)	GTTGGAATAGTTTACAAGC		
pR4 (*mcdM*)	GCTTCCATTGTGAATATCTC	pF4/pR4	+(520)
pF5 (*mcdM*)	GTTTAGAGAATGGATGGATGTGC		
pR5 (*mcdT*)	CAATAGCGGGATCTACAATCG	pF5/pR5	+(473)
pF6 (*mcdT*)	CTTCAGAACAGTCAATACTCTG		
pR6 (*mcdF*)	CTCACCTGAAGTCGGATCTAC	pF6/pR6	+(470)
pF7 (*mcdF*)	GTTAAGGCCTATTCTCTAGG		
pR7 (*mcdG*)	CCAATAAAACTGGACAAAGC	pF7/pR7	+(1446)
pF8 (*mcdE*)	GATGTTTGTAGCAGTAACTTGG		
pR8 (ORF2)	CTGCCGTTAAGGCAACCTCTCC	pF8/pR8	–

pF, forward primer; pR, reverse primer.

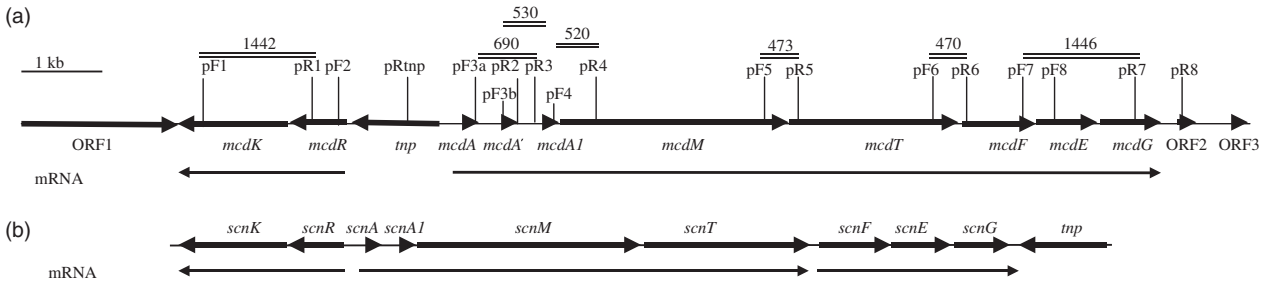

Fig. 1. (a) Organization of the macedocin gene cluster. Bold arrows at the bottom of the genes show transcriptional units determined by RT-PCR. Positions of the primers used for the RT-PCR experiments as well as the size of the RT-PCR products obtained (double lines) are indicated above *mcd* gene cluster. (b) Organization of the SA-FF22 gene cluster, as described by McLaughlin *et al*. (1999).

(Qiagen). RT-PCR was performed with the Titan One Tube RT-PCR System (Roche), according to the instructions of the manufacturer, using the primers listed in Table 2 and shown in Fig. 1.

Nucleotide sequence accession number

The complete sequence of the macedocin gene cluster has been deposited in the GenBank database under accession number DQ835394.

Results and discussion

Previous Edman's degradation analysis revealed the amino acid sequence of macedocin to be comprised of 22 amino acid residues (Georgalaki *et al*., 2002). Eight of them could not be identified, while the rest were identical to the respective ones of the SA-FF22 and SA-M49, both isolated from pathogenic *S. pyogenes* strains (Hynes *et al*., 1993, 1994). On the basis of this identity, all the heterologous primers used for the amplification of the macedocin gene cluster (Table 1) were designed according to the respective SA-FF22 genes (McLaughlin *et al*., 1999).

In total, the inserts of eight overlapping clones, raised from the eight sets of primers of Table 1, were sequenced, representing a region of 15 171 bp, with a G+C content of 30.9%. Computer analysis revealed 14 probable ORFs, 10 of which were named *mcd* (macedocin) genes (Fig. 1). Nucleotide and amino acid similarities to other products of lantibiotic gene clusters were considered, in order to predict the functions of the different *mcd* genes. All 10 *mcd* gene products revealed the highest percentage of amino acid identity (65–92%) to the respective genes of SA-FF22 (Table 3) and lower similarities to other genes of type AII lantibiotics.

Macedocin is putatively encoded by two adjacent structural genes, *mcdA* and *mcdA′*, 156 nucleotides long each, exhibiting 94.9% identity at the nucleotide level. Six

Table 3. Putative ORFs on *mcd* gene cluster and their functions

ORF	Size of putative protein (aa)	Putative function	Closest match (GenBank accession no.)	Alignment region of *mcd* ORF	Alignment region of closest match (size of homolog)	% amino acid identity
ORF1	621	Unknown	*S. suis*, relaxase/mobilization nuclease domain (ZP00874662)	1–621	1–619 (619)	86
mcdK	453	Histidine kinase type sensor protein	SA-FF22*, ScnK (AAB92598)	1–452	1–449 (453)	74
mcdR	232	Response regulator	SA-FF22, ScnR (AAB92599)	1–232	1–232 (232)	83
tnp	391	Transposase	*S. thermophilus* putative transposase, IS*256* family (CAC67525)	1–391	1–391 (391)	99
mcdA	51	Premacedocin	SA-FF22 precursor, ScnA (AAB92600)	1–51	1–51 (51)	92
mcdA′	51	Premacedocin	SA-FF22 precursor, ScnA (AAB92600)	1–51	1–51 (51)	92
mcdA1	53	Unknown	SA-FF22, ScnA1 (AAB92601)	6–53	4–51 (51)	75
mcdM	928	Posttranslational modification of pre-macedocin	SA-FF22, ScnM (AAB92602)	1–925	1–926 (927)	65
mcdT	690	Processing and secretion ABC transporter	SA-FF22, ScnT (AAB92603)	1–689	1–688 (689)	75
mcdF	304	Subunit of ABC transporter involved in immunity.	SA-FF22, ScnF (AAB92604)	3–297	1–295 (299)	83
mcdE	254	Subunit of ABC transporter involved in immunity.	SA-FF22, ScnE (AAB92605)	1–252	1–252 (254)	75
mcdG	245	Subunit of ABC transporter involved in immunity.	SA-FF22, ScnE (AAB92606)	11–244	3–236 (237)	78
ORF2	74	Unknown	*S. agalactiae*, transcriptional regulator (helix turn–helix motif) (NP735764)	1–72	1–72 (73)	84
ORF3 (5′partial)	67	Unknown	*S. suis*, recombinase (resolvase) (ZP00875209)	1–66	1–66 (584)	90

*Streptococcin A-FF22 from *S. pyogenes*.

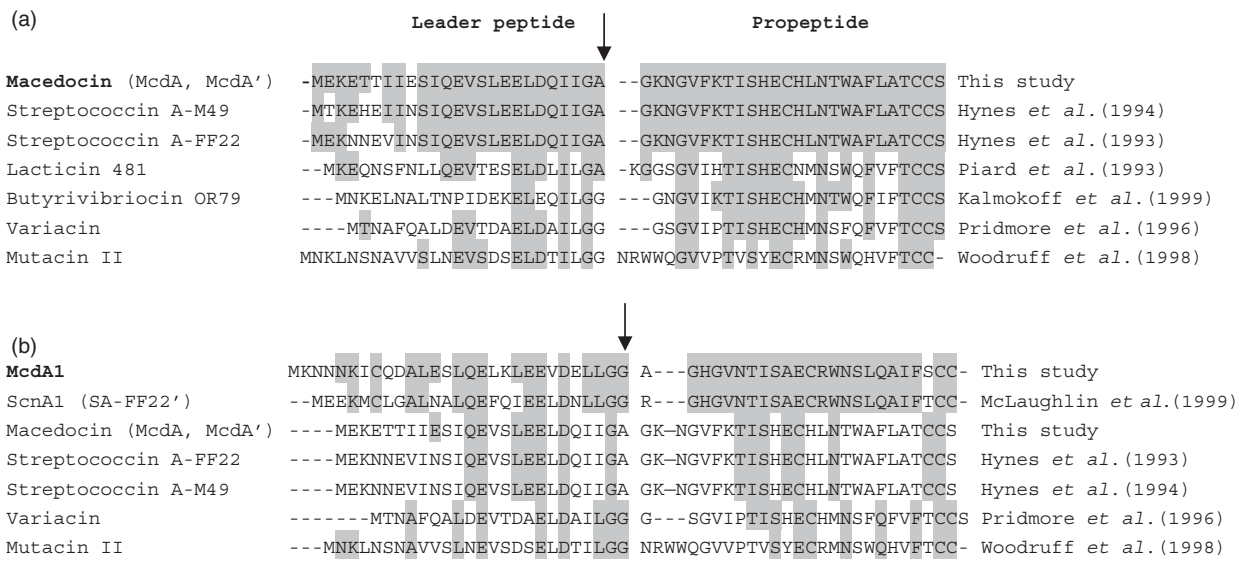

Fig. 2. Comparison of the leader peptides and propeptides of macedocin (a) and McdA1 (b) with lantibiotics of type AII. The arrow indicates the probable cleavage site. Amino acids identical to macedocin's or McdA1's ones are shaded.

basepairs upstream the putative AUG codon of each gene a putative ribosome-binding site (5′-AAAGGA-3′ and 5′-AAAGAGG-3′, respectively) is located. The deduced amino acid sequences of the two genes are 100% identical, and correspond to the macedocin prepeptide. Both *mcdA* and *mcdA′* genes code for the same 51 aa prepeptide, comprised of 25 N-terminal residues corresponding to the leader peptide and the 26 remaining residues corresponding to the macedocin propeptide (Fig. 2a). BLAST search of the macedocin prepeptide revealed homology with six other

prelantibiotics of the lacticin 481 group (type AII group) (Dufour *et al.*, 2007) as presented in Fig. 2a. Macedocin prepeptide contains the characteristic 'GG' or 'GA' peptidase cleavage site, which is the cleavage site for the removal of the leader peptide. The prepeptide terminates in the conserved sequence 'CCS' (Fig. 2a). The deduced amino acid sequence of the macedocin propeptide is identical to SA-FF22 and SA-M49, but their leader peptides differ by five and four residues, respectively.

These results confirmed the previous ones obtained by the amino acid sequence analysis of macedocin, and allowed the determination of the residues not determined by the Edman's degradation analysis (Georgalaki *et al.*, 2002). By applying the RT-PCR, it was shown that the two putative macedocin structural genes, *mcdA* and *mcdA′*, are cotranscribed and they are both part of the same operon (*mcdAA′A1MTFEG*) (Fig. 1). It remains to be clarified whether macedocin's production is associated with both or only one of the two structural genes. Similarly, the SA-M49 is putatively encoded by two adjacent structural genes (*scnA′* and *scnA″*), which are cotranscribed as part of the *scnA′Á″MT* biosynthetic operon, but it is still unknown if the lantibiotic is produced from *scnA′* and/or *scnA″* gene products (Hynes *et al.*, 1994). Structural gene duplication has also been reported for mutacin I (Qi *et al.*, 2000), mutacin III (Qi *et al.*, 1999) and mutacin B-Ny266 (Becal-Si *et al.*, 2002). In the case of mutacin B-Ny266 there is only 57.4% amino acid identity between the two putative structural gene products and there is no evidence for the transcription of the second structural gene. In mutacin I and mutacin III, insertional inactivation of *mutA* structural gene abolished mutacin production, while inactivation of the second structural gene *mutA′* did not, and thus the role of the latter gene remains unknown. Interestingly, the gene cluster of ruminococcin A, a lantibiotic produced by *Ruminococcus gnavus*, includes three almost identical structural genes, all of them encoding the same prepeptide (Gomez *et al.*, 2002).

Another ORF located 463 bp downstream the *mcdA′* gene was named *mcdA1* because it encodes a putative protein with high amino acid sequence identity (75%) with the ScnA1 peptide of *S. pyogenes* (McLaughlin *et al.*, 1999). *scnA1* is a putative structural gene located downstream the *scnA* structural gene of SA-FF22 and its deduced peptide (ScnA1) exhibits high identity with other lantibiotics; however, it is still unknown if this peptide corresponds to a functional lantibiotic (McLaughlin *et al.*, 1999). Moreover, McdA1 gene product exhibits lower identity with the lantibiotics SA-FF22, SA-M49, variacin produced by *Micrococcus varians* (Pridmore *et al.*, 1996) and mutacin II (Woodruff *et al.*, 1998), and it also shares 38.2% amino acid identity with the macedocin prepeptide (*mcdA* and *mcdÁ′* gene products) (Fig. 2b). RT-PCR experiments showed that the *mcdA1* gene is transcribed as part of the *mcdAA′A1MTFEG* transcript unit (Fig. 1, Table 2), but, like its close relative *scnA1* gene (McLaughlin *et al.*, 1999), it remains unknown if it produces a second active lantibiotic. The production of two different lantibiotics by the same bacterium has been reported for some *S. salivarius* and *S. pyogenes* strains (Wescombe *et al.*, 2006a).

The enzyme responsible for the modification of the macedocin propeptide and the protein involved in the removal of the leader peptide and the secretion of the mature macedocin are proposed to be encoded by the *mcdM* and *mcdT* genes, respectively. McdM-deduced peptide exhibits the highest amino acid sequence identity (65%) to the respective ScnM product of SA-FF22 and significantly lower identity (30%) to the respective LanM products of lacticin 481 produced by *Lactococcus lactis* (Rincé *et al.*, 1994) and nukacin ISK-1 produced by *Staphylococcus warneri* (Aso *et al.*, 2005). On the basis of the role proposed for LanM proteins, it is reasonable to suggest that the role of McdM is the catalysis of the prelantibiotic posttranslational modification reactions resulting in the formation of dehydroalanine/dehydrobutyrine and lanthionine/β-methyllanthionine residues (Xie & van der Donk, 2004; Chatterjee *et al.*, 2005). The *mcdT* gene encodes a 690 peptide with 75% identity to the ScnT product of SA-FF22, 39% to the LanT products of nukacin ISK-1 and lacticin 481, and less identity to other ABC-transporters. On the basis of what is known about LctT, it can be proposed that the role of McdT is dual: the cleavage of the leader peptide and the transport of the mature macedocin outside the cell (Chen *et al.*, 1999; Uguen *et al.*, 2005).

The next three ORFs were named *mcdF*, *mcdE* and *mcdG* as they showed nucleotide and amino acid (of the deduced peptides) sequence similarity with the corresponding genes found in other lantibiotics. The putative McdF product exhibits similarity with the ATP-binding domain of ABC transporters assumed to participate in the lantibiotic immunity of the producer strain (Rincé *et al.*, 1997; McLaughlin *et al.*, 1999). The predicted proteins encoded by the next two ORFs, *mcdE* and *mcdG*, were similar to the membrane-spanning domain of this kind of transporters. These results suggest that McdF, McdE and McdG are associated in an ABC transporter-like complex, which is involved in the immunity of *S. macedonicus* ACA-DC 198 against macedocin. It has been proposed that the LanFEG complex prevents the accumulation of the lantibiotic in the area outside the cell membrane and keeps the lantibiotic in a low concentration, which is not sufficient to form pores into the membrane (Peschel & Gotz, 1996).

Upstream the *mcdA*, and in the opposite transcriptional direction relative to the rest *mcd* genes, there is a regulatory operon consisting of two ORFs, *mcdR* and *mcdK*, encoding a typical two-component signal transduction system with

high similarity with that of SA-FF22. It has been proposed that in SA-FF22, this system consisting of the response regulator ScnR and a histidine kinase type sensor protein (ScnK) controls the transcription of all three *scn* operons responding to a signal not yet characterized (McLaughlin *et al.*, 1999). This signal could be SA-FF22 itself, as this lantibiotic autoinduces its production (Wescombe *et al.*, 2006b). The similarity of the McdR and McdK products with ScnR and ScnK, respectively, as well as the same organization of the genes in the two genomes, suggests that they may have the same function. In the case of nisin, there is a two-component regulator system composed of NisR and NisK, and the expression of the gene cluster is autoregulated by nisin itself (Kuipers *et al.*, 1995). Subtilin and Salivaricin A have also been shown to serve as the sensing molecules that trigger the transcription of their prepeptides (Upton *et al.*, 2001; Kleerebezem, 2004). It is worthy to mention that macedocin is only produced when *S. macedonicus* is grown in milk (Georgalaki *et al.*, 2002), and macedocin's production is not autoinduced (Georgalaki *et al.*, 2006). Preliminary induction activity studies indicated that it is induced by a highly hydrophobic peptide that could be eventually produced by milk protein degradation (Georgalaki *et al.*, 2006).

Noteworthy, a putative transposase gene, named *tnp*, is located 114 bp upstream of the *mcdR* gene (Fig. 1). The deduced peptide is 99% identical with a transposase from *Streptococcus thermophilus*, which is part of the mobile element IS1191 (GenBank accession number AAN63693) that belongs to the IS*256* family. The *tnp* gene found upstream *mcdR* is framed by two inverted-repeat sequences of 28 bp each that are identical to the respective ones of the IS1191 mobile element from *S. thermophilus*. Because of the insertion, a short (8 bp) direct target repeat has been generated (5′-AGTAAAAT-3′), flanking the insertion sequence (IS).

Additionally, another putative mobilization element (ORF1) is located 79 bp downstream the *mcdK* gene in the opposite transcriptional direction (Fig. 1). The deduced peptide of ORF1 is 86% identical with a relaxase from *Streptococcus suis* and exhibits lower identity with other relaxases. Relaxases are conjugative plasmid-encoded proteins essential for the horizontal transfer of genetic information contained on plasmids that occurs during bacterial conjugation (Carter & Porter, 1991). Finally, the deduced amino acid sequence of the partially sequenced ORF3 (964 bp downstream the macedocin gene cluster) exhibits 90% identity with a resolvase (N-terminal recombinase) from *S. suis* and lower identity with other resolvases (Fig. 1, Table 3).

The presence of mobilization elements in lantibiotics gene clusters has been previously reported. The lacticin 481 genes are part of a potentially mobile element and so far it is the sole transposon-encoded lantibiotic of its group (Dufour *et al.*, 2000, 2007). A transposase gene was also found 130 bp upstream the mutacin II genes as well as 100 bp downstream the SA-FF22 genes (Chen *et al.*, 1999; McLaughlin *et al.*, 1999). However, the presence of an IS inside the lantibiotic gene cluster is described for the first time in this study. Dufour *et al.* (2007) claim that the transposase gene located next to the SA-FF22 gene cluster in *S. pyogenes* might explain plasmid–chromosome and/or chromosome–plasmid exchanges. Similarly, the presence of the mobile element inside the macedocin gene cluster, as well as the fact that a relaxase and a resolvase gene frame the macedocin gene cluster, could indicate that this gene cluster has been raised after genetic rearrangement.

The G+C content of the area between ORF1 (relaxase) and *tnp*, where the *mcdKR* genes are located, is 28.6% and that of the respective one of the area between *tnp* and ORF3 (resolvase), where the *mcdAA′A1MTFEG* genes are located, is 29.6%. Both the above areas, enclosed by the three putative mobilization elements (relaxase, transposase and resolvase), exhibit lower G+C content compared with the average genome G+C content (38%) of *S. macedonicus* species (Tsakalidou *et al.*, 1998). This fact could indicate that *S. macedonicus* gained the macedocin genes by horizontal transfer of DNA from another organism with different G+C content. This has been suggested for the ruminococcin A genes (Gomez *et al.*, 2002). Moreover, the presence of the IS in the region upstream of the regulation operon *mcdRK* could have affected its initial promoter, as many IS elements have been shown to activate the expression of the neighboring genes (Mahillon & Chandler, 1998).

Finally, the transcription units of the macedocin gene cluster were determined by RT-PCR amplification of the regions between all the vicinal ORFs, using RNA isolated from *S. macedonicus* ACA-DC 198 grown in skim milk supplemented with yeast extract. The primers used as well as the products obtained from each primer pair are listed in Table 2 and shown on *mcd* gene cluster in Fig. 1. The products obtained showed that the *mcd* genes are organized in two operons: the regulatory one consisting of *mcdRK* genes, and the biosynthesis-immunity one consisting of the remaining *mcd* genes, *mcdAA′A1MTFEG* (Fig. 1). These two operons have opposite transcriptional direction. On the contrary, in the case of SA-FF22, which is the closest match of all macedocin genes, the respective *scn* genes are organized in three operons: *scnRK*, *scnAA1MT* and *scnFEG* (McLaughlin *et al.*, 1999). On the basis of the RT-PCR product amplified by pF6/pR6 and pF7/pR7 primer pairs (Fig. 1, Table 2), it can be concluded that in the macedocin cluster, the *mcdFEG* genes are cotranscribed with the *mcdAA′A1MT* genes.

In summary, in this study the entire macedocin biosynthetic gene cluster was identified. On the basis of these results and on the similarity exhibited to other lantibiotics, especially with SA-FF22, and on previous results obtained by the purification of the peptide, it can be concluded that macedocin belongs to an AII linear-type lantibiotic and particularly to the lacticin 481 group. A possible model for the synthesis of macedocin from *S. macedonicus* ACA-DC 198 is also proposed. According to this model, a nonidentified yet environmental signal activates the autophosphorylation of McdK kinase, which in turn phosporylates the McdR response regulator protein. The activated McdR initiates the transcription of the biosynthetic-immunity operon and the McdA precursor peptide is produced. The C-terminal propeptide is modified by the enzymatic action of McdM. McdT transports the modified propeptide out of the cell and cleaves the leader peptide. The ABC-transport complex McdFEG provides immunity to the producer against macedocin, possibly by keeping the macedocin density low outside the cell.

Current experiments by the gene inactivation approach focus on the investigation of the role of *mcdA′* and *mcdA1* in macedocin production. The possibility of synthesis of a second lantibiotic from the *mcdA1* gene will be also examined.

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