

## ORIGINAL ARTICLE

# Gene cluster for biosynthesis of thermophilin 1277 – a lantibiotic produced by *Streptococcus thermophilus* SBT1277, and heterologous expression of TepI, a novel immunity peptide

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**Abstract****Aims:** To identify genes cluster for thermophilin 1277 produced by *Streptococcus thermophilus* SBT1277.**Methods and Results:** To identify genes for thermophilin 1277 production, the chromosomal DNA region surrounding the structural gene, *tepA*, was sequenced using a primer-walking method. The thermophilin 1277 biosynthesis gene locus (*tep*) is a 9.9-kb region, which consists of at least ten open reading frames (ORFs) in the following order: *tepAMTFEGKRI* and *ORF4*. Homology analysis showed high similarity to genes involved in bovicin HJ50 production by *Streptococcus bovis* HJ50. *tepI* encodes a novel, small, positively charged hydrophobic peptide of 52 amino acids, which contains a putative transmembrane segment. By heterologous expression in *Lactococcus lactis* ssp. *cremoris* MG1363, the TepI-expressing strain exhibited at least 1.3 times higher resistance to thermophilin 1277.**Conclusions:** Thermophilin 1277 biosynthesis genes were encoded by a 9.9-kbp region containing at least ten ORFs. TepI is a novel immunity peptide, which protected *Strep. thermophilus* SBT1277 against thermophilin 1277 in addition to TepFEG, a putative ABC transporter.**Significance and Impact of the Study:** This is the first report regarding a lantibiotic gene cluster produced by *Strep. thermophilus* strain.**Introduction**

Lantibiotics are synthesized as precursor peptides and subsequently undergo post-translational modifications (Kupke and Gotz 1996). Lanthionine and  $\beta$ -methylanthionine in these molecules are produced from the reaction of sulfhydryl groups of cysteine residues with dehydroalanine and dehydrobutyrine, after dehydration of serine and threonine residues, respectively. Jung (1991) divided the lantibiotics into two groups, type A (linear) and type B (globular), on the basis of their secondary structures. Sahl and Bierbaum (1998) further divided each group into three subgroups, the nisin (AI), lactacin 481 (AII) and lactococcin S (AIII)

types, on the basis of the primary structural similarities of the peptides. Major lantibiotic gene clusters have been clarified, which include the structural gene (*lanA*), modification enzyme genes (*lanB* and *lanC*, or *lanM*), the secretion ABC-type transporter gene (*lanT*), the leader peptidase gene (*lanP*), self-protection (immunity) genes (*lanFEG* and *lanI*, or *lanH*) and regulatory genes (*lanR* and *lanK*). The gene cluster for nisin A is arranged in the sequence *nisABTCIPRKFEFG* (McAuliffe *et al.* 2001).

Nisin A, an AI-type lantibiotic, is the most widely investigated, because it is the first found for bacteriocin and it is the only lantibiotic approved as a food preservative (Chatterjee *et al.* 2005). The successful application of

nisin as a safe food preservative has attracted much attention over the 50 countries (Delves-Broughton *et al.* 1996). So far, streptococcal lantibiotics have been reported in the oral cavities and upper respiratory tracts of humans and animals. However, they may also be isolated from almost any type of clinical specimen. Some examples include salivaricin A produced by *Streptococcus salivarius* 20P3 (Ross *et al.* 1993), mutacin 1140 produced by *Streptococcus mutans* JH1000 (Hillman *et al.* 1998) and streptococin SA-M49 produced by *Streptococcus pyrogenes* (Hynes *et al.* 1994). Lantibiotic-producing oral streptococci is pathogenic; therefore, it is obvious that they cannot be used in foods. *Streptococcus macedonicus* ACA-DC 198, which was isolated from the cheese and nonpathogenic streptococcal species, produced a lantibiotic, macedocin (Papadelli *et al.* 2007).

There are numerous reports and reviews on LAB bacteriocins produced by lactococci, lactobacilli, pediococci, leuconstoc and enterococci; however, to date, only three class IIa bacteriocins produced by *Streptococcus thermophilus* strains have been cloned and sequenced – thermophilin 13 (produced by *Strep. thermophilus* Sfi 13) (Marciset *et al.* 1997), thermophilin A (produced by *Strep. thermophilus* ST134) (Ward and Somkuti 1995; Whitford *et al.* 2001) and thermophilin 9 (produced by *Strep. thermophilus* LMD-9) (Fontaine and Hols 2008). Furthermore, lantibiotics produced by *Strep. thermophilus* strains have never been reported. *Streptococcus thermophilus* is one of the most widely used bacteria in the manufacture of dairy products (yoghurt and cheese) and is considered to be a generally recognized as safe (GRAS) organism. Recently, the complete genome sequences of three *Strep. thermophilus* strains were published, and comparative genome analyses were performed (Hols *et al.* 2003; Bolotin *et al.* 2004). Comparative genomics and multilocus sequencing analyses suggest that this species is undergoing a process of regressive evolution, to adapt to growth in milk. While pathogenic streptococci are recognized for their high capacity to expose proteins at the cell surfaces to achieve cell adhesion or to escape host immune systems, *Strep. thermophilus* has lost these features, along with many virulence-related functions. These results are strongly suggestive of the safety status of *Strep. thermophilus* and the bacteriocins it produces.

Thermophilin 1277, produced by *Strep. thermophilus* SBT1277, is an AII-type lantibiotic, which contains two  $\beta$ -methylanthionines and a disulfide bridge. Thermophilin 1277 is identical in terms of primary amino acid sequence to bovicin HJ50 from *Streptococcus bovis* HJ50 (Xiao *et al.* 2004). Furthermore, the structural gene *tepA* has been cloned (Kabuki *et al.* 2009).

Here, we sequenced the biosynthesis gene cluster of thermophilin 1277 and examined the heterologous expression of a small hydrophobic immunity peptide,

TepI, which shows no similarity to other lantibiotic accessory proteins.

## Materials and methods

### Bacterial strains, plasmids and culture conditions

The strains and plasmids used in this study are listed in Table 1. The bacteriocin-producing strain *Strep. thermophilus* SBT1277 was isolated from raw milk (Kabuki *et al.* 2007). All strains were maintained as frozen stocks at  $-80^{\circ}\text{C}$ . M17 medium (Becton, Dickinson & Co., Sparks, MD, USA) with 0.5% (w/v) glucose (GM17) was routinely used for culturing *Strep. thermophilus* SBT1277 at  $37^{\circ}\text{C}$ , and *Lactococcus lactis* ssp. *cremoris* MG1363 at  $30^{\circ}\text{C}$ . To select and maintain transformants, erythromycin (Sigma, Tokyo, Japan) was used at a concentration of  $5\ \mu\text{g ml}^{-1}$  in GM17 for *L. lactis* ssp. *cremoris* MG1363.

### Molecular cloning and nucleotide sequencing

Molecular cloning techniques were performed as described by Sambrook *et al.* (1989). Chromosomal DNA was prepared using a previously reported method (Pospiech and Neumann 1995). PuReTaq Ready-To-Go PCR Beads (GE Health Bioscience, Tokyo, Japan) were used for the PCR, which was performed in a GeneAmp PCR System 9700 (PE Applied Biosystems, Tokyo, Japan) using standard PCR conditions. PCR products were purified using a QIAquick Spin Column (Qiagen, Tokyo, Japan). Plasmid extraction from *L. lactis* ssp. *cremoris* MG1363 was performed using a Plasmid Miniprep kit (Bio-Rad, Tokyo, Japan). DNA sequencing was performed by an industrial sequence commission (Hokkaido System Science, Sapporo, Japan). The nucleotide sequence surrounding *tepA* was determined by primer walking, using

**Table 1** Bacterial strains and plasmids used in this study

Strains and plasmids	Description	Source or reference
<b>Strains</b>		
<i>Streptococcus thermophilus</i> SBT1277	Thermophilin 1277 producer	SBT
<i>Lactococcus lactis</i> ssp. <i>cremoris</i> MG1363	Plasmid-free derivative of NCDO712	Gasson (1983)
<b>Plasmids</b>		
pIL253-P32	Emr: pIL253 derivative with P32 promoter	Kemperman <i>et al.</i> (2003)
pTEPI	Emr: pIL253-P32 derivative carrying <i>tepI</i>	This study

Emr, erythromycin resistance; SBT, Snow Brand culture collection.

the total DNA of *Strep. thermophilus* SBT1277 as the template.

### Computer analysis

Open reading frames (ORFs) were identified using an ORF finder (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>). Sequence similarity searches of nucleotide or protein sequences were performed using the BLAST program in the DDBJ databases. Transmembrane regions in peptides and proteins were deduced using the SOSUI program (<http://bp.nuap.nagoya-u.ac.jp/sosui/>) (Hirokawa *et al.* 1998). G+C content was calculated using a web tool (<http://www.bioinformatics.org/JaMBW/3/1/9/index.html>).

### Cloning of recombinant carrying putative immunity peptide for thermophilin 1277

The *tepI* region was amplified using PuReTaq Ready-To-Go PCR Beads (GE Health Bioscience) with the primers 5'CGCGGATCCAAAGGCTCGAAAAATGGGTTA3' and 5'GCAGGACGTCTCCCAATCGCTAAAGCTAC3' (the *Bam*HI and *Pst*I sites were underlined in the primer sequences, respectively), and the genomic DNA of *Strep. thermophilus* SBT1277 was used as a template. After digestion of the PCR products with *Bam*HI and *Pst*I, the *tepI* fragment was ligated into pIL253-P32 and digested with the same restriction enzymes. The ligation mixture was used to transform pIL253-P32 into *L. lactis* ssp. *cremoris* MG1363, and the resultant plasmid was named pTEPI.

### Broth-based assay with recombinants of *Lactococcus lactis* ssp. *cremoris*

Bacteriocin tolerance was assayed using the agar well diffusion methods (Kabuki *et al.* 2007), which are briefly described later. The GM17 culture supernatant of *Strep. thermophilus* SBT1277 containing thermophilin 1277 was serially diluted by the 50 mmol l<sup>-1</sup> sterile phosphate buffer (pH 6.8). The GM17 agar plate was overlaid with a soft agar lawn (15 ml), which was inoculated with a diluted overnight culture of each indicator strain at 1%

(v/v). Wells, 6.88 mm in diameter, were cut from the plates, and 40 µl of serially diluted bacteriocin sample was added to each well. *Lactococcus lactis* ssp. *cremoris* MG1363, MG1363 (pIL253-P32) and MG1363 (pTEPI) were used as indicator strains. The diameters of the halos around wells of GM17 agar plates were measured after incubation at 30°C for 18 h. The experiments were performed in triplicate.

### Nucleotide sequence accession number

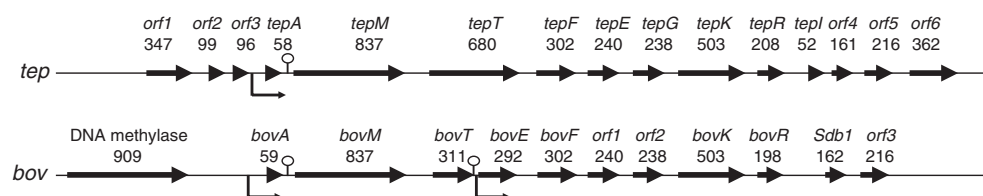
The nucleotide and protein sequences of the thermophilin 1277 region have been assigned GenBank accession number AB434921.

## Results

### Cloning and sequencing of the gene cluster for thermophilin 1277 production

The nucleotide sequence of a 16 402-bp region flanking the structural gene of thermophilin 1277 (*tepA*) was determined using a primer-walking method. The G+C content (33%) of the total region was different from the average G+C content of *Strep. thermophilus* (39%) (Hols *et al.* 2003; Bolotin *et al.* 2004). Using computer analysis, 15 ORFs were detected; nine ORFs were designated *tepM*, *tepT*, *tepF*, *tepE*, *tepG*, *tepK*, *tepR*, *tepI* and *ORF4* in a 9.9-kb region containing *tepA*. The amino acid sequences of the seven *tep* gene (*tepAMTEGKR*) products among nine putative thermophilin 1277 biosynthesis genes show the highest similarity (98–100%) with those of the respective gene products of bovicin HJ50 produced by *Strep. bovis* HJ50 (Liu *et al.* 2009) (Fig. 1, Table 2).

*TepM* (837 amino acids), which is present downstream of *tepA*, showed 99% similarity to *bovM* (ACA51935) of *Strep. bovis* HJ50. The gene clusters of AII- and AIII-type lantibiotics contain a *lanM* (-like) gene encoding 900–1000 amino acid residues, which has been proposed to be involved in post-translational modification of lantibiotics such as lactosin S and lacticin 481. The function of LanM for AII- and AIII-type lantibiotic is dehydration of amino



**Figure 1** Comparison of the gene clusters for thermophilin 1277 and bovicin HJ50. The names of genes and a protein are shown with the amino acid length. Predicted promoters and a terminator are represented by angled arrows and an omega-like symbol, respectively.

**Table 2** Deduced peptides and proteins derived from *tep*

ORF	Length (amino acids)	Proposed function	Best homologue [identities (%), no. of amino acids], GenBank accession no.	Localization (TMS no)*	pI	Molecular weight (kDa)
<i>ORF1</i>	347	Unknown	Helicase, <i>Streptococcus equi</i> ssp. <i>zooepidemicus</i> (95%, 2281 aa), YP002745281	Soluble (0)	5.30	41.0
<i>ORF2</i>	99	Unknown	Conserved domain protein, <i>Streptococcus suis</i> 89/1591 (88%, 99 aa), ZP03624205	Soluble (0)	4.15	12.0
<i>ORF3</i>	96	Unknown	Membrane protein, <i>Strep. equi</i> ssp. <i>zooepidemicus</i> (73%, 89 aa), YP002745279	Membrane (3)	6.07	10.9
<i>tepA</i>	58	Prethermophilin 1277	BovJH, <i>Streptococcus bovis</i> HJ50 (100%, 58 aa), AAP23217	Soluble (0)	4.44	6.1
<i>tepM</i>	837	Post-translational modification	BovM, <i>Strep. bovis</i> HJ50 (99%, 837 aa), ACA51935	Soluble (0)	5.71	97.6
<i>tepT</i>	680	Processing and secretion ABC transporter.	BovT, <i>Strep. bovis</i> HJ50 (99%, 311 aa), ACA51936, BovE, <i>Strep. bovis</i> HJ50 (98%, 292 aa), ACA51937	Membrane (6)	8.38	77.3
<i>tepF</i>	302	Subunit of ABC transporter involved in immunity. ATP-binding protein	Lantibiotic transport ATP-binding protein, <i>Strep. suis</i> SC84 (63%, 304 aa), YP003024866	Soluble (0)	5.70	33.8
<i>tepE</i>	240	Subunit of ABC transporter involved in immunity. ATP transporter	Unknown, <i>Strep. bovis</i> HJ50 (98%, 240 aa), ACA51938	Membrane (7)	9.30	27.4
<i>tepG</i>	238	Subunit of ABC transporter involved in immunity. ATP transporter	Unknown, <i>Strep. bovis</i> HJ50 (98%, 238 aa), ACA51939	Membrane (6)	9.11	27.3
<i>tepK</i>	503	Histidine kinase	BovK, <i>Strep. bovis</i> HJ50 (99%, 503 aa), ACA51940	Membrane (8)	8.06	58.1
<i>tepR</i>	208	Response regulator	BovR, <i>Strep. bovis</i> HJ50 (98%, 198 aa), ACA51941	Soluble (0)	4.99	24.1
<i>tepl</i>	52	Immunity protein	Not hit	Membrane (2)	10.02	6.0
<i>ORF4</i>	161	Unknown	Sdb1, <i>Strep. bovis</i> HJ50 (100%, 161 aa), ACA51942	Membrane (1)	5.27	18.0
<i>ORF5</i>	216	Unknown	Unknown. <i>Strep. bovis</i> HJ50 (99%, 216 aa), ACA51943	Membrane (1)	5.44	24.4
<i>ORF6</i>	362	Unknown	DNA primase. <i>Strep. suis</i> BM407 (78%, 358 aa), NP735785	Soluble (0)	5.97	41.7

\*TMS, transmembrane segment number, deduced using the sosui program.

acid and formed the thioether bridges in the molecule. On other hand, the function of Lan C for AI-type lantibiotic is to form the thioether bridges in the molecule. Uguen *et al.* (2000) identified 11 and eight conserved domains (N1–11 and C1–8) in the N- and C-terminal parts of LanM containing those from lacticin 481 groups, respectively. The LanM and LanC gene products shared some structural motifs, such as GXAHG, WCXG and CHG, in which histidine and cysteine residues are well conserved. These motifs might have some relevance in the catalytic function of these enzymes (Paul *et al.* 2007). The N-terminal region of TepM showed homology between six conserved domains of LanM proteins (positions 222–261, 274–284, 350–372, 385–398,

437–459 and 467–495). The C-terminal regions of TepM showed high similarity to the five domain motifs (positions 547–558, 661–673, 703–715, 752–758 and 787–816) including the consensus sequences of GXAHG (position 662), WCXG (position 706) and CHG (position 753) of LanM and lanC, which are predicted to form a thioether bridge-forming enzyme for lantibiotics (Li *et al.* 2006; Li and van der Donk 2007; Zhang *et al.* 2007). These findings suggest that TepM catalyses the reaction of prelantibiotic post-translation modification, which results in the formation of  $\beta$ -methylanthionine residues.

TepT (680 aa) is deduced to possess an N-terminal proteolytic domain for cleavage of the leader sequence from

the prepeptide at the two conserved sequence motifs: QX4D/ECX2AX3MX3MX4Y/FGX4L/L and HY/FY/VV-X10L/LXDP (Havarstein *et al.* 1995) at positions 10 and 88, respectively. TepT also contains an ATP-binding domain, with Walker A motifs, GXXGXGKS/(position 498), and Walker B motif, *hhhh*DEP/A (*h*: a hydrophobic amino acids; positions 621) in a C-terminal domain (Fath and Kolter 1993). *tepT* has 99 and 98% similarity with *bovT* (ACA51936) and *bovE* (ACA51937) of *Strep. bovis* HJ50, respectively (Liu *et al.* 2009). These findings suggest that TepT has dual functions – the cleavage of the leader peptide (by the N-terminal domain) and the secretion of thermophilin 1277 as an ABC transporter (by the C-terminal domain).

The three ORFs, *tepF*, *tepE* and *tepG*, encode 302, 240 and 238 amino acids, respectively. TepF exhibits high similarity (39–63%) with proteins of immunity gene clusters for lactacin 481-type lantibiotics possessing the consensus ATP-binding domain with Walker motifs A and B at positions 36 and 156, respectively. TepE is predicted to be a membrane protein containing seven transmembrane segments. TepG was also a membrane protein containing six transmembrane segments. These results suggest that TepF, TepE and TepG are associated in an ABC transporter-like complex that functions in the immunity of *Strep. thermophilus* 1277 against thermophilin 1277.

*tepKR* encodes regulatory proteins for a typical two-component signal transduction system; TepK (503 aa) and TepR (208 aa) exhibited 99% similarity to BovK, a membrane-bound sensor histidine kinase, and BovR, a response regulator, of *Strep. bovis* HJ50.

TepI, a putative small peptide consisting of 52 amino acids, showed no similarity to any reported proteins encoded by lantibiotic gene clusters. The hydrophobic profile revealed that TepI is a highly cationic peptide with two putative membrane-spanning domains (Fig. 2). The ORF4 encoding 161 amino acid residues was identified as

the disulfide oxidoreductase (Sdb1; ACA51942) by *Strep. bovis* HJ50 (Liu *et al.* 2009), which has a thioredoxin domain and exhibits 50% similarity to BlpGst from *Strep. thermophilus* LMD-9 (Gasson 1983) and BdbB from *Bacillus subtilis* 168 (Dorenbos *et al.* 1998; Pag *et al.* 1999) containing the conserved thioredoxin motif (CXXC). The formation of a disulfide bond in thermophilin 1277 might be related to the function of ORF4 (Sdb1). The ORF5 encoding 216 amino acid residues was 99% similarity to unknown protein from *Strep. bovis* HJ50. ORF5 and ORF6 (both downstream of ORF4), which had no similarity to protein by lantibiotic gene cluster reported, appeared to be unrelated to the biosynthesis of thermophilin 1277.

A putative promoter was located upstream of *tepA* (Kabuki *et al.* 2009). A deduced terminator was predicted to be present downstream of *tepA*. However, deduced terminator sequences were not found downstream of other regions related to bacteriocin synthesis. The G+C content of the area between ORF1 and ORF3 and that between *tepA* and *orf4* was 39 and 29%, respectively. ORF4, which is downstream of the putative *tep* operon, contained 33% G+C. On the other hand, the downstream region of ORF4 exhibited 37% G+C content.

#### Heterogenous expression of the putative immunity peptide for thermophilin 1277

*In silico* analysis indicated that TepI – a novel hydrophobic peptide with the cationic region – is the immunity-like peptide for thermophilin 1277. Figure 2 shows the primary sequence and deduced transmembrane regions of the small immunity peptides for the lantibiotics. The peptides, TepI (for thermophilin 1277), EciI (for epicidin 280), PepI (for Pep5), LtnI (lactacin 3147) and NukI (nukacin ISK-1), ranging in size from 52 to 116 amino acid residues, have two or three transmembrane segments.

**Figure 2** Primary sequences for (putative) immunity peptides of LAB bacteriocins. TepI, EciI, PepI, LtnI and NukH are immunity peptides for thermophilin 1277, epicidin 280, Pep5, lactacin 3147 and nukcin ISK-I, respectively. The transmembrane regions deduced using *sosui* program are underlined. The residue positions and lengths of the region are shown within parentheses.

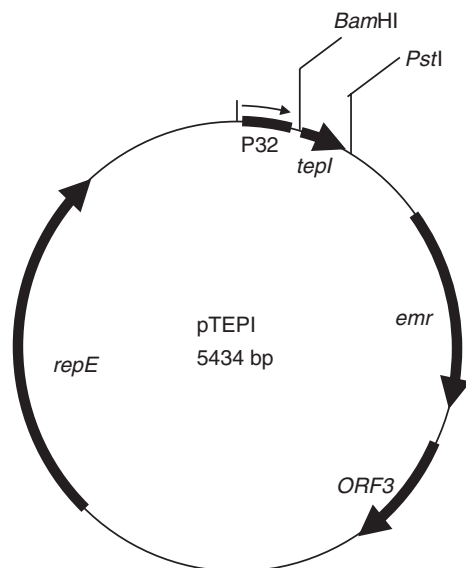
TepI (52aa)	<u>MKKYIPLICFLFLFIVFLGITVRAFLADKTLMVADGLLSIVFFISFLITRKKL</u> (4–25, 22) (27–48, 22)
EciI (62aa)	<u>MNIYLKIVITALFFESSIIFTVTYVSSKNLGMSSLFGLLSFIANLVYDYVMGASERKSKKD</u> NK (3–25, 23) (27–49, 23)
PepI (69aa)	<u>MNIYLVKIVLTSFFALIIIFVTYIITTKQWGTSLGFSSLSFIGNFIYDYSTKLSDDKYEKR</u> INSNKKDKL (3–25, 23) (29–51, 23)
LtnI (116aa)	<u>MKNENINTFSESFLYSLKEWAEGSANNYNILLGLSFIFVMSSVVFVVTISTKIGKKDERT</u> <u>TKISLSAYCVLITLIIICDIIFFPKGYLIQPPFMLKYGFSCLVGGIYCLLKYIKDNK</u> (64–86, 23) (90–112, 23)
NukH (96aa)	<u>MKRKDYLFVIALLLPLISLLLOLMKISLIHNYQSFFSIVNIICILEFTIAYSIILVINSK</u> <u>KNNLQKTILLLSIIYVILTLIFISFGVIINMFN</u> (7–29, 23) (66–88, 23) (35–56, 22)

*tepI* was cloned downstream of the lactococcal P32 promoter in pIL253-P32, and heterologous expression of *tepI* from the resulting plasmid, pTEPI (Fig. 3), was examined in *L. lactis* ssp. *cremoris* MG1363. The recombinant strain, *L. lactis* ssp. *cremoris* MG1363 (pTEPI), was at least 1.3 times more resistant to the GM17 culture supernatant of *Strep. thermophilus* SBT1277 than the control strain carrying pIL253-P32 (Fig. 4).

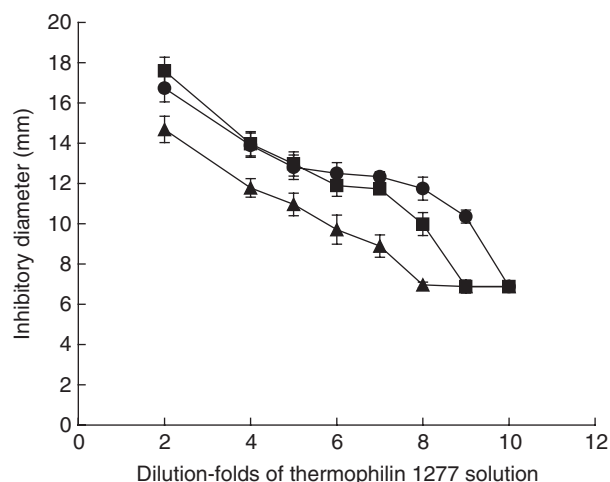
## Discussion

By sequencing the gene cluster containing the structural gene (*tepA*) for a lantibiotic, thermophilin 1277, a modification enzyme gene (*tepM*), an ABC transporter gene (*tepT*), self-protection genes (*tepFEGI*) and two-component regulation genes (*tepRK*) were deduced. These ORFs (except for *tepI*) are commonly found in the biosynthetic gene clusters of lactacin 481-type lantibiotics (Dufour et al. 2006).

Thermophilin 1277 is identical to bovicin HJ50 of *Strep. bovis* HJ50 in the structural genes. The biosynthetic gene cluster of bovicin HJ50 (c. 9.9 kbp) (Liu et al. 2009) contains at least 10 ORFs (*bovA*, *M*, *T*, *E*, *F*, *ORF1*, *ORF2*, *K*, *R* and *ORF3*) involved in biosynthesis, regulation and immunity, which correspond to each gene of *tepAMTFEGRK-ORF4*, except for *tepI*. However, the putative *tepT* product consisted of 680 aa, instead of 311 aa from the putative *bovT* product. This shows that



**Figure 3** Plasmid map of the *tepI* expression vector pTEPI. P32, the lactococcal constitutive promoter; *tepI*, the putative thermophilin 1277 immunity gene; *emr*, the erythromycin resistance gene; *ORF3*, an uncharacterized ORF in the vector pIL253; and *repE*, the replication protein E gene.



**Figure 4** Immunity assay for the recombinants of *Lactococcus lactis* ssp. *cremoris* MG1363 against thermophilin 1277. The immunity levels of *L. lactis* ssp. *cremoris* MG1363 (●), MG1363 (pIL253-P32) (■) and MG1363 (pTEPI) (▲) were investigated by agar-well diffusion assay using serially diluted culture supernatants of *Streptococcus thermophilus* SBT1277 (thermophilin 1277 solution).

*TepT* has both a proteolytic domain in its N-terminal and an ATP-binding domain in the C-terminal corresponding to *BovT* that has a proteolytic domain and *BovE* (292 aa) that has an ATP-binding domain, respectively. Based on the molecular size and estimated function difference between *BovT* and *TepT*, we conclude *TepT* may possess the activities of both *BovT* and *BovE*. *TepF*, *E* and *G* are the putative immunity genes corresponding to the deduced genes of bovicin HJ50, *bovF*, *ORF1* and *ORF2*, respectively. *TepI* product has no similarity to other bacteriocin biosynthesis protein. As the homology analysis of *orf5* and *orf6* products show no similarity to the bacteriocin biosynthesis protein, these protein might be not involved with thermophilin 1277 expression.

Two immunity (self-protecting) systems for lantibiotics have been reported. The first consists of individual immunity proteins (generically termed LanI or LanH) that are located in the cytoplasmic membrane or at the surface and prevent interaction of bacteriocin(s) with the membrane. The other consists of the ABC transporters (usually composed of two or three subunits, generically termed (LanFE(G)), which actively export intracellularly accumulated bacteriocins to the outside (e.g., NisFEG). LanI (LanH) and LanFE(G) may act alone, or in combination, which results in cooperative functionality (Draper et al. 2008)

A LanI immunity system has been detected in some major lantibiotics, such as nisin (NisI, 245 residues), subtilin (SpaI, 165 residues), Pep5 (PepI, 69 residues), lactacin 3147 (LtnI, 116 residues), epicedin 280 (EciI, 62 residues)

and cytolysin (CylI, 327 residues) (Kuipers *et al.* 1993; Klein and Entian 1994; Qiao *et al.* 1995; Paik *et al.* 1998; McAuliffe *et al.* 2000; Stein *et al.* 2003, 2005; Hoffmann *et al.* 2004; Reis *et al.* 2004). NisI and SpaI are lipoproteins that are anchored in the cytoplasmic membrane by their lipid motifs; they capture lantibiotics to avoid bacteriocin action. LtnI contains a leucine zipper motif and three hydrophobic domains, which suggests that it is a transmembrane protein that forms homodimers (McAuliffe *et al.* 2000).

LanFEG is the only immunity system for the lactacin 481 families, such as lactacin 481 (Piard *et al.* 1992), mutacin II (Woodruff *et al.* 1998) and streptococcin A-FF22 (McLaughlin *et al.* 1999). Aso *et al.* (2005) reported a novel type of lantibiotic-binding peptide, NukH, which contributes to the immunity of nukacin ISK-1. NukH (92 aa) has three putative membrane-spanning domains (Fig. 4) and is similar to the LanI peptide for lactacin 3147. Together, the two immunity systems of NukH and NukFEG provide a much greater degree of immunity function than each system alone. NukH has been referred to as an accessory factor, which acts as an ancillary protein for the assembly of the functioning ABC transporter.

We demonstrated the heterologous expression of *tepI* with the P32 promoter in *L. lactis* ssp. *cremoris* MG1363. The recombinant strain is not completely resistant to thermophilin 1277, but is at least 1.3 times more resistant to thermophilin 1277 than the control strains carrying pIL253-P32. This result shows that the *tepI* product is an immunity peptide against thermophilin 1277 and might cooperate with the *tepFEG* products for full immunity function. This suggests that the immunity system of thermophilin 1277 slightly differs from that of bovicin HJ50.

The ORF4 downstream of *tepI* encodes a putative disulfide oxidase-like protein (161 aa). BlpGst of *Strep. thermophilus* LMD-9 (Fontaine and Hols 2008) and BdbB of *B. subtilis* 168 (Dorenbos *et al.* 1998) have been reported to be disulfide oxidases involved in bacteriocin production. Bovicin HJ50, which is identical to thermophilin 1277 with regard to the primary amino acid structure, has a thiol-disulfide oxidoreductase gene in the bacteriocin loci (Liu *et al.* 2009). Gene disruption analysis showed that the thiol-disulfide oxidoreductase encoded by *sdb1* is not involved in bovicin HJ50 biosynthesis. The inhibitory activity of the reduced form of bovicin HJ50 treated by dithiothreitol (DTT) neither decreased nor increased (Xiao *et al.* 2004). On the other hand, our previous result shows that the antimicrobial activity of thermophilin 1277 disappears after treatment with DTT. It can be speculated that ORF4 functions in the formation of intra- and/or intermolecular disulfide bridges between

the cysteine residues of thermophilin 1277. The function of ORF4 products is being investigated.

The G+C content of *tepAMTFEGRKI-ORF4* (29%) is lower than the average genome G+C content (*c.* 38%) of *Strep. thermophilus* (Hols *et al.* 2003; Bolotin *et al.* 2004). Horizontal gene transfer (HGT) can be defined as the exchange of genetic material between phylogenetically unrelated organisms (Jain *et al.* 2002). Mechanisms of intercellular DNA transfer include conjugation, phage transduction and transformation. HGT events can be detected using phylogenetic and compositional approaches. Information on the gene transfer mechanisms for genes, such as transposase- or phage-related genes found in the neighbourhood of the target genes, can improve the prediction of HGT events; there are many reports regarding the HGT events indicated. The operon of lactacin 481, which is 23% similar to thermophilin 1277, contains part of Tn5721 – a potentially mobile DNA sequence in a 70-kbp plasmid (Dufour *et al.* 2006). In contrast, no plasmids were detected in the strains of *Streptococcus pyogenes* and *Streptococcus agalactiae* producing streptococcin A-F22 and salivaricin A1 (45 and 42% similarity to thermophilin 1277, respectively). This indicates that the lantibiotic loci are chromosomal in these species (Wescombe *et al.* 2006). It should be noted that a transposase gene lies downstream of the streptococcin A-F22 gene cluster in *Strep. pyogenes* (McLaughlin *et al.* 1999), which might explain plasmid–chromosome and/or chromosome–plasmid exchanges. The other gene clusters of the lactacin 481 group are chromosomally located. Transposase genes have been found in the mutacin II cluster (Cheng *et al.* 1999), as well as between the *macA* and *macR* genes for macedocin (Papadelli *et al.* 2007). Consistently, the G+C content of the macedocin gene cluster (29.6%) is low compared to the average genome G+C content of *Strep. macedonicus* species (38%) (Papadelli *et al.* 2007). The surrounding region of the macedocin gene cluster is involved in the three putative mobilization elements: relaxase, transposase and resolvase; this suggests that *Strep. macedonicus* might be able to gain the macedocin genes by horizontal transfer of DNA from other organisms. These findings might indicate the possibility of horizontal transfer for the thermophilin 1277 gene cluster in *Strep. thermophilus* SBT1277.

*Streptococcus thermophilus* is of major importance to the food industry; it is the second most important industrial dairy starter after *L. lactis* (Hols *et al.* 2003). *Streptococcus thermophilus* SBT1277 is a food-grade GRAS microorganism, which is isolated from milk and is routinely used as a starter organism by various sectors of the food industry. The safety status of *Strep. thermophilus* SBT1277 will be of considerable advantage in the application of this bacteriocin-producing organism as a biopreservative.

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