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Molecular Genetics, Genomics and Biochemistry of Mutacins

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ABSTRACT

Bacteriocins are proteinaceous antibacterial substances produced by bacteria. Mutacins are bacteriocins produced by *Streptococcus mutans*. Four groups of mutacins have been described to date: lantibiotic monopeptides (bacteriocin Class Ia, Ib) including mutacins B-Ny266, H-29B, K8 (MukA), I, II, III, and 1140; lantibiotic dipeptides (bacteriocin Class Ic) grouping mutacins GS5 (SmbA, SmbB) and BHT-A (BHT-Aα, BHT-Aβ); non-lantibiotic monopeptides (bacteriocin Class IIa, IIb) including mutacins BHT-B, F-59.1, I-T9, N and V; and the non-lantibiotic dipeptide (bacteriocin Class IIc) mutacin IV (NlmA, NlmB). Bioinformatic analyses of the *S. mutans* UA159 genome have revealed genes potentially coding for bacteriocin-like peptides. Lateral gene transfer and recombination contributed to mutacin gene distribution and divergence among strains. Screening of large numbers of isolates has revealed a high polymorphism in the genes encoding mutacin-like inhibitory substances. This emphasises the diversity of antimicrobial substances produced by *S. mutans*. A relationship between bacteriocins and competence for transformation is emerging. In the future, more research is required to explore the role of mutacin production in the ecology of the oral ecosystem.

Keywords: bacteriocin, competence development, lantibiotic, mutacin, quorum sensing, *Streptococcus mutans*

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INTRODUCTION

Bacteriocins are ribosomally synthesised proteinaceous antibacterial substances produced by bacteria (Jack *et al*. 1995; Cotter *et al*. 2005) and probably by all prokaryotic species (Riley and Chavan 2007). Proposed classifications of bacteriocins are based on their molecular structures and mechanisms of action (Klaenhammer 1993; Nes *et al*. 1996). We will adopt here the most recent classification into four distinct classes (Cotter *et al*. 2005, 2006; Heng and Tagg 2006)

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Invited Review

Table 1 Classes of bacteriocins from Gram-positive bacteria (adapted from Cotter *et al*. 2005, 2006; Heng and Tagg 2006).

	Class I-lantibiotics	Class II	Class III	Class IV
Definition	Post-translationally modified peptides containing lanthionines and/or unsaturated amino acids	Non-lanthionine containing, heat-stable peptides	Large heat-labile proteins ("colicin-like")	Cyclic peptides (N and C termini covalently linked)
Specific Molecular Weight	< 5 kDa	< 10 kDa	> 10 kDa	No specification
Subgroups	**Type Ia:** Elongated peptides with a net positive charge *Subtype AI*: Nisin-like *Subtype AII*: SA-FF22-like **Type Ib:** Globular peptides with a net negative charge or no charge **Type Ic:** Multi-component	**Type IIa:** Pediocin-like with antilisterial activity and a YGNGVXC N-terminal sequence **Type IIb:** Miscellaneous **Type IIc:** Multi-component	**Type IIIa:** Bacteriolytic **Type IIIb:** Non-lytic Little known group	None
Examples[1]	Nisin, lacticin 481, mersacidin, lacticin 3147, **mutacins B-Ny266, B-JH1140, H-29B, I, II(J-T8), III, Smb, BHT-A and K8**	Pediocin PA1, thermophilin 13, aureocin A53, **mutacins F-59.1, I-T9, IV, V, N and BHT-B**	Lysostaphin, helveticin J	Enterocin AS-48

[1] Examples of mutacins are in bold.

(**Table 1**).

Mutacins, bacteriocins produced by *Streptococcus mutans* (Hamada and Ooshima 1975a), were first studied by Kelstrup and Gibbons (1969). Their possible use as anti-caries agents and the role they play in the colonisation of the oral cavity have raised much interest (Hamada and Ooshima 1975a, 1975b; Weerkamp *et al*. 1977). They have also been studied as a bacterial epidemiological fingerprinting tool and to assess the distribution of mutacin-producing strains (Rogers 1976; Groonroos *et al*. 1998). New interests are developing for mutacins, as potential food preservatives (Nicolas *et al*. in press) and as new effective antibiotics (Mota-Meira *et al*. 2005).

There are many reports showing that *S. mutans* produces bacteriocin-like inhibitory substances (BLIS) (Jack *et al*. 1995), but not many of these have been isolated and characterised as mutacins (Parrot *et al*. 1989, 1990; Morency *et al*. 1995; Mota-Meira *et al*. 1997; Morency *et al*. 2001; Nicolas *et al*. 2006) and only a few have undergone complete genetic characterisation (Woodruff *et al*. 1998; Qi *et al*. 1999a, 2000; Bekal-Si Ali *et al*. 2002; Yonezawa and Kuramitsu 2005). The diversity of antimicrobial substances produced by *S. mutans* is supported by genetic screening of large numbers of isolates which has revealed considerable polymorphism in the genes encoding mutacin-like inhibitory substances among strains isolated around the world (Bekal-Si Ali *et al*. 2002; Longo *et al*. 2003). Furthermore, bioinformatic analysis of the *S. mutans* UA159 genome has revealed more than 10 potential bacteriocin-like peptide-coding genes (Dirix *et al*. 2004). Many mutacins thus remain to be investigated.

CLASSIFICATION OF MUTACINS

Chikindas *et al*. (1997) reviewed some of the mutacins that were well-characterised before 1997. Since then, more biochemical and genetic information has been acquired and four types of mutacins have been defined: lantibiotic monopeptides (bacteriocin Class Ia, Ib), lantibiotic dipeptides (bacteriocin Class Ic), non-lantibiotic monopeptides (bacteriocin Class IIa, IIb), and non-lantibiotic dipeptides (bacteriocin Class IIc) (**Tables 2, 3, 4** and **5**).

Lantibiotic monopeptides

Mutacin I

Mutacin I is produced by *S. mutans* CH43 and UA140 (Qi *et al*. 2000, 2001). The mature peptide is composed of 24 amino acids with a molecular mass of 2364 Da. Mutacin I belongs to the lantibiotic type AI and epidermin group (**Table 2**). The biosynthetic operon was reported to encompass 14 ORFs (**Fig. 1**). Upstream of the biosynthetic genes is the alanyl t-RNA synthetase *ats* (SMU.650) gene (Qi *et al*. 2000). Mutacins I and III harbour some similarities with slight differences in the hinge region of the peptides (**Table 2**) conferring different hydrophobic properties and different levels of antibacterial activity (Qi *et al*. 1999a, 2000) (**Table 3**). A common origin has been suggested for the operons encoding mutacins I and III. Duplication of the prepropeptide-coding gene *mutA* as *mutA'* (in mutacins I and III (Qi *et al*. 1999a, 2000)) and *lanA* as *lanA'* (in mutacin B-Ny266 (Bekal-Si Ali *et al*. 2002)) appears to be a common response in bacteria exposed to different selective pressures (Yamanaka *et al*. 1998).

Mutacin II and H-29B

Mutacin II (alias J-T8) produced by *S. mutans* T8 is a hydrophobic peptide with a molecular mass of 3245 Da made of 27 amino acids (Novak *et al*. 1993, 1994; Krull *et al*. 2000). The producing strain was isolated in Australia by Rogers (1976). Post-translational modifications include two lanthionines (Lan), one methyllanthionine (MeLan) and one α, β-didehydro residue (Novak *et al*. 1996) (**Table 3**). Mutacin II belongs to the type AII lantibiotic and the lacticin 481 group (Krull *et al*. 2000; Chatterjee *et al*. 2005; Dufour *et al*. 2006). The complete sequence of the mature peptide was deduced from genetic characterisation of the *mutA* gene coding for prepromutacin II (Woodruff *et al*. 1998). The gene *mutA* encodes a prepropeptide of 53 amino acids including an N-terminal leader peptide of 26 amino acids. Mutacin II harbours a double glycine-type leader sequence (Chen *et al*. 1998b; Woodruff *et al*. 1998) (**Table 2**). The specificity of some amino acids in mutacin II has been analysed revealing that didehydro amino acids, thioether bridges and the hinge region were essential for the biological activity and exportation of the peptide (Chen *et al*. 1998b). Structural analysis of residues 1 to 8 showed that the peptide forms an α-helix connected to the C-terminus part by a hinge region and the importance of this hinge region has been demonstrated by the mutation Pro(9)Ala that leads to the loss of antimicrobial activity (Chen *et al*. 1998b). Furthermore, replacement of the double Gly motif in position –1 and –2 with two Ala residues resulted in a complete loss of production of bioactive peptides; premutacin accumulated intracellularly (Chen *et al*. 1998b, 2001). Mutacin production was not affected by substitution of other conserved residues with similar amino acids. Only two mutations close to the propeptide (Ile(-4)Asp and Leu(-7)Lys) failed to produce mutacin and the prepeptides could not be detected.

Genes encoding mutacin II production are located on the bacterial chromosome (Caufield *et al*. 1990b; Woodruff *et al*. 1998). Seven genes organised in an operon are necessary to achieve mutacin II production (*mutRAMTFEG*) (**Fig. 1**; **Table 5**). The operon is flanked upstream by a *tra* gene (transposase) and downstream by the *fba* gene (fructose biphosphate aldolase) (Chen *et al*. 1999; Qi *et al*. 1999b). Chen *et al*. (1999) reported that this operon can be transferred to a non-producing strain, converting it to a mutacin

Table 2 Peptide sequence of known mutacins.

Mutacin	Bacteriocin class	Accession No[a]	Amino acid sequence Leader peptide + **mature peptide**	Ref. *
I	Ia	AAF99577	MSNTQLLEVLGTETFDVQEDLFAFDTTDTTIVASNDDPDTR**FSSLSLCSLGCTGVKNPSFNSYCC**	1
II (J-T8)	Ia	AAC38144	MNKLNSNAVVSLNEVSDSELTILGG**NRWWQGVVPTVSYECRMNSWQHVFTCC**	2
	Subtype AII			
III	Ia	AAD56142	MSNTQLLEVLGTETFDVQEDLFAFDTTDTTIVASNDDPDTR**FKSWSLCTPGCARTGSFNSYCC**	3
IV				
NlmA	IIb or IIc[b]	AAN57926	MDTQAFEQFDVMDSQTLSTVEGG**KVSGGEAVAA**IGICATASAAIGGLAGATLVTPYCVGTWGLIRSH	4
NlmB		AAN57927	MELNVNNYKSLTNDELSEVFGG**DKQAADTFLS**AVGGAASGFTYCASNGVWHPYILAGCAGVGAVGSVVFPH	
V	IIb	AAN59525	MNTQAFEQFNVMDNEALSAVEGG**GRGWNCAAGIALGAGQGYMATAGGTAFLGPYAIGTGAFGAIAGGIGGALNSCG**	5
NlmC				
1140	Ia	AAC18827	MSNTQLLEVLGTETFDVQEDLFAFDTTDTTIVASNDDPDTR**FKSWSLCTPGCARTGSFNSYCC**	6
B-Ny266	Ia	P80666	MSNTQLLEVLGTETFDVQENLFTFDTTDTIVAESNDDPDTR**FKSWSFCTPGCAKTGSFNSYCC**	7
BHT-Aα	Ic	AAZ76603	MKEIQKAGLQEELSILMDDANNLEQLTAG**IGTTVVNSTFSIVLGNKGYICTVTVECMRNCQ**	8
BHT-Aβ		AAZ76602	MKSNLLKINNVTEVEKDMVTLIKDEDMELAGG**STPACAIGVVGITVAVTGISTACTSRCINK**	
BHT-B	IIb	AAZ76605	**MWGRILAFVAKYGTKAVQWAWKNKWFLLSLGEAVFDYIRSIWGG**	8
F-59.1	IIa	-	**KYYGNGVTCGKHSXSVDWXKXTXXX**	9
H-29B	Ia	P84110	**NRWWQGVVPTVSYECRMNSWQHVF**	10
	Subtype AII			
K8				11
MukA1	Ia	ABK59354	MKNTTNEMLELIQEVSLDELDQVIGG**MGKGAVGTISHECRYNSWAFLATCCS**	
MukA2	*Subtype AII*	ABK59355	MKQSDEMLELIQEVSLDELDQVIGG**AGNGVIRTITQGCRMPNNMQVLFTC**	
MukA3		ABK59356	MKKGTQLYLEALEALQEIKVEELDTFIGG**MGKGAVGTISHECRYNSWAFLATCCS**	
N	IIb	-	MNVEENIMSFDVNSYNDLTRDELSQTIGG**SRQAADTFLSGAYGAAKGVTARASTGVYVVPATLVALGVYGAGLNIAFP**	12
Smb				
SmbA	Ic	BAD72776	MKSNLLKINNVTEMEKNMVTLIKDEDMLAGG**STPACAIGVVGITVAVTGISTACTSRCINK**	13,
SmbB		BAD72777	MKEIQKAGLQEELSILMDDANNLEQLTAG**IGTTVVNSTFSIVLGNKGYICTVTVECMRNCSK**	14

[a] Swissprot accession numbers are given when GenBank accession numbers are not available.
[b] Mutacin IV was first proposed to consist of two peptides designated NlmA and NlmB (class IIc bacteriocin) (Qi *et al*. 2001). However, Hale *et al*. (2005a) showed that disruption of the *nlmB* gene has no impact on the activity spectrum of mutacin IV, placing mutacin IV in class IIb instead of IIc.
For mutacins IV and K8, the peptide portion determined by N-terminal sequencing is indicated in bold underlined letters.
For mutacin N, the sequence similarity with NlmB (mutacin IV) is shown in bold highlighted letters.
For mutacin F-59.1, X denotes N-terminal amino acids unidentified by sequencing. The typical N-terminal pediocin-like sequence is highlighted.
For mutacins 1140 and B-Ny266, the differing amino acids are highlighted.
***Ref.**: 1, Qi *et al.* 2000; 2, Novak *et al.* 1994; 3, Qi *et al.* 1999a; 4, Qi *et al.* 2001; 5, Hale *et al.* 2005a; 6, Hillman *et al.* 1998; 7, Mota-Meira *et al.* 1997; 8, Hyink *et al.* 2005; 9, Nicolas *et al*. 2007a; 10, Nicolas *et al.* 2006; 11, Robson *et al.* 2007; 12, Balakrishnan *et al.* 2000; 13, Yonezawa and Kuramitsu 2005; 14, Petersen *et al.* 2006.

Table 3 Biochemical properties of mature lantibiotic mutacins.

Mutacin	Nb aa	Mw[a]	Leader sequence motif[b]	Net charge	Modified amino acids[c]				
					Lan	MeLan	dhA	dhB	other
I	24	2364	FNLD	+1	3	0	1	0	0
II (J-T8)	27	3245	GG	+1	2	1	0	1	0
H-29B	24	3246	GG	+1	2	1	0	1	0
III	22	2266	FNLD	+3	2	1	1	1	AviCys
1140	22	2263	FNLD	+3	2	1	1	1	AviCys
B-Ny266	22	2270	FNLD	+3	2	1	1	1	AviCys
K8[e]									
MukA1/A3	26	2734	GG	+1	n.d.	n.d.	n.d.	n.d.	n.d.
MukA2	25		GG	+2	n.d.	n.d.	n.d.	n.d.	n.d.
Smb									
SmbA	30	2808	GG	+2	n.d.	n.d.	n.d.	n.d.	n.d.
SmbB[d]	33	3452	GG	+2	n.d.	n.d.	n.d.	n.d.	n.d.
Smb variant									
BHT-Aα	31	3451	GG	+2	n.d.	n.d.	n.d.	n.d.	n.d.
BHT-Aβ	30	2893	GG	+2	n.d.	n.d.	n.d.	n.d.	n.d.

[a] Molecular weight (Mw) determined by mass spectrometry.
[b] Leader sequence motif. GG: double glycine motif (GG/AG/GS); FNLD: serine protease recognition motif.
[c] Lan: lanthionine; MeLan: β–methyllanthionine; dhA: 2,3-didehydroalanine; dhB: (2)-2,3-didehydrobutyrine; AviCys: S-(2-aminovinyl)-D-cysteine residue.
[d] Deduced from the DNA sequence (Yonezawa and Kuramitsu 2005) and complemented by amino acid sequencing (Petersen *et al.* 2006).
[e] Deduced from the DNA sequence (Robson *et al.* 2007).
n.d.: not determined.

II producer.

Mutacin H-29B was shown to be identical to mutacin II (Nicolas *et al*. 2006). It can be produced in a low-cost growth medium made from whey permeate (Nicolas *et al*. 2004). The peptide was purified by successive hydrophobic chromatography steps and the first 24 amino acids revealed by Edman sequencing were found to be identical to those of mutacin II (**Table 2**). Characterisation of the coding gene will confirm if the prepeptide of mutacin H-29B is completely identical to that of premutacin II.

Mutacin III

Mutacin III is produced by *S. mutans* UA787 (Qi *et al*. 1999a). The complete sequence of the peptide was deduced from the sequence of the gene and the molecular mass was calculated to be 2266 Da. Biosynthesis of mutacin III is controlled by an operon composed of eight ORFs, namely *mutRAA'BCDPT* (**Fig. 1**). As for mutacin I, the alanyl t-RNA synthetase *ats* (SMU.650) gene is found upstream of the operon (Qi *et al*. 1999a, 2000). Mutacin III also shows high similarity with lantibiotics from the epidermin group (epidermin, gallidermin, mutacin B-Ny266, mutacin 1140) (**Table 2**). The sequence of the gene coding for mutacin III

Table 4 Biochemical properties of non-lantibiotic mutacins (determined with the ProtPram program from the ExPASy Proteomics Tools, http://ca.expasy.org/tools/).

Mutacin	Nb aa	Cys residus	Mw[a]	Mw[b]	pI	Q+ residues	Q- residues	Aliphatic Index	Instability Index[c]	GRAVY[d]
IV										
NlmA	44	2	4169	4171.8	8.06	2	1	108.86	4.59	0.911
NlmB	49	2	4826	4828.4	5.98	1	2	77.76	13.51	0.573
V[e]	53	2	-	4777.7	8.03	1	0	74.15	24.53	0.636
BHT-B	44	0	5195	5165.0	9.99	6	2	93.18	21.42	0.241
F-59.1	25	4	2720	2798.3	9.05	-	-	23.20	-11.07	-0.748
N	49	0	4806	4801.4	9.52	3	1	97.76	19.20	0.649

[a] Molecular weight (Mw) determined by mass spectrometry (MS).
[b] Mw calculated by the ProtParam program (differences observed with Mw determined by MS can be due to the presence of *N*-formylmethionine, disulfide bridges, or unidentified residues present in the query sequence).
[c] Instability index >50: unstable; <50: stable protein.
[d] Overall hydropathicity average.
[e] Deduced from the DNA sequence (Hale *et al*. 2005a).
-: Information cannot be determined.

Table 5 Summary of the characteristics of mutacin gene clusters.

Mutacin	Structural gene	Self-immunity genes	Export genes	Post-translational modification genes	Genes of unknown function	Regulator genes	External regulator genes[a]	Flanking genes	GenBank accession number	G+C ratio[b]
I	*mutA*	*mutF*, *mutE*, *mutG*	*mutP*, *mutT*	*mutBCD*	*mutA'*	*mutR*	*ciaH*, *luxS*, *hdrM*, *irvA*, *hrcA*, *adhE*, *vicK*, *pttB*, *hk03*	*ats*	AF238860	0.84
II (J-T8)	*mutA*	*mutF*, *mutE*, *mutG*	*mutT*	*mutM*	-	*mutR*	*dgk*	*tra*, *fba*	U40620	0.84
III	*mutA*	-	*mutP*, *mutT*	*mutBCD*	*mutA'*	*mutR*	-	*ats*	AF154675	0.81
IV	*nlmA*, *nlmB*	-	*nlmT*, *nlmE*	-	-	-	*comD*, *comE*	*	*	*
V	*nlmC*	-	*nlmT*, *nlmE*	-	-	-	*comD*, *comE*	*	*	*
1140	*lanA*	*lanB* (incomplete)	-	-	*orfY*	(*orfX*)	*fhs*	-	AF051560	0.80
B-Ny266	*lanA*	*lanB* (incomplete)	-	-	*lanA'*	-	-	-	AF338349	0.82
BHT-A[c]	*bhtAα*, *bhtAβ*	*bhtAF*, *bhtAG*	*bhtAT*	*bhtAM1*, *bhtAM2*	-	*bhtAR*	-	-	DQ145752	0.85
BHT-B[c]	*bhtB*	(*orf1*, *orf2*, *orf3*)	(*abc1*, *abc2*)	-	-	*mutR*	-	*ptsEII*, *α-amylase*	DQ145753	0.96
K8	*mukA1*, *mukA2*, *mukA3*	*mukF*, *mukE*, *mukG*	*mukT*	*mukM*	*mukA'*	*mukR*, *mukK*	-	putative maturase-related coding gene	EF060238	0.96
N	*mutN*	*blpI*	*nlmT*, *nlmE*	-	-	-	-	*	NA	-
Smb	*smbA*, *smbB*	*smbF*, *smbG*	*smbT*	*smbM1*, *smbM2*	-	-	*comD*, *comE*	*cyl*	AB179778	0.87

[a] Genes outside of the biosynthetic operon.
[b] The G+C mol % ratio of mutacin-coding operons was determined using the GC-Profile websoftware (http://tubic.tju.edu.cn/GC-Profile/) in comparison to the *S. mutans* G+C mol % content of 36.8%.
[c] Produced by *Streptococcus rattus* (Hyink *et al*. 2005).
- : Not elucidated.
* : Not determined as biosynthetic genes are apparently not clustered.
NA : Not available.
The function of genes in parentheses is hypothetical.

is identical to that of mutacin 1140 (Hillman *et al*. 1998; Qi *et al*. 1999a). Mutacin III thus belongs to the type AI lantibiotics and the epidermin group (Chatterjee *et al*. 2005).

Mutacin 1140

Mutacin 1140 is produced by *S. mutans* JH1140, reported to be a spontaneous mutant of *S. mutans* JH1000 (Hillman *et al*. 1998) and belongs to the lantibiotic class (type AI). Mutacin 1140 is composed of 22 amino acids with a molecular mass of 2263 Da, and the reported sequence makes it very similar to the lantibiotics belonging to the epidermin group, differing from mutacin B-Ny266 by only two amino acids (**Table 2**). The sequence of the putative immunity gene *lanB* has not been completed (**Fig. 1**). A three-dimensional structure and dynamic molecular model of mutacin 1140 have been determined by NMR (Smith *et al*. 2003). It was deduced from these studies that mutacin 1140 adopts a small and compact structure of multiple forms, which could confer an activity of membrane disintegration. The small size of the compact structure was thought not to conform to the classical mode of action of pore formation in the membrane by lantibiotics.

Mutacin B-Ny266

Mutacin B-Ny266 is produced by strain Ny266 (Parrot *et al*. 1990; Morency and Lavoie 1991), originally named T2 and isolated in Australia by Rogers (1976). The mature peptide is 22 amino acids long and possesses a calculated mass of 2270 Da. Mutacin B-Ny266 was the first mutacin lantibiotic to be completely sequenced (Mota-Meira *et al*. 1997). It possesses two Lan, one MeLan, one 2,3-didehydroalanine, one 2,3-didehydrobutyrine and one *S*-(*Z*)-2-aminovinyl-D-cysteine (**Tables 2, 3**). Only the 22nd amino acid could not be revealed upon sequencing because it is part of the *S*-(*Z*)-2-aminovinyl-D-cysteine. The mature peptide is related to the lantibiotics gallidermin and epidermin produced by *Staphylococcus gallinarum* and *Staphylococcus epidermidis*, respectively. Mutacin B-Ny266 belongs to type AI lantibiotics and the epidermin group (Chatterjee *et al*. 2005).

Genetic analysis of the operon for the biosynthesis of mutacin B-Ny266 has revealed that the *lanA* gene encodes a 63-amino acid protein that is 87% similar to LanA from the *S. mutans* strain JH1140 producing mutacin 1140 and to MutA from *S. mutans* UA787 producing mutacin III. A second open reading frame (ORF) encodes a 64-amino acid protein with 57.4% homology to LanA and was named *lanA'*. No ORF was identified as a regulator gene in the vicinity of *lanAA'* while many stop codons were found in all possible reading frames. A deletion of 50 bp was detected upstream from *lanA* in comparison with *lanA* and *mutA* from strains JH1140 and UA787 respectively. This region is A+T rich (>80%) and was proposed to be associated with a promoter activity for *lanA* expression (Bekal-Si Ali *et al*. 2002) (**Fig. 1**).

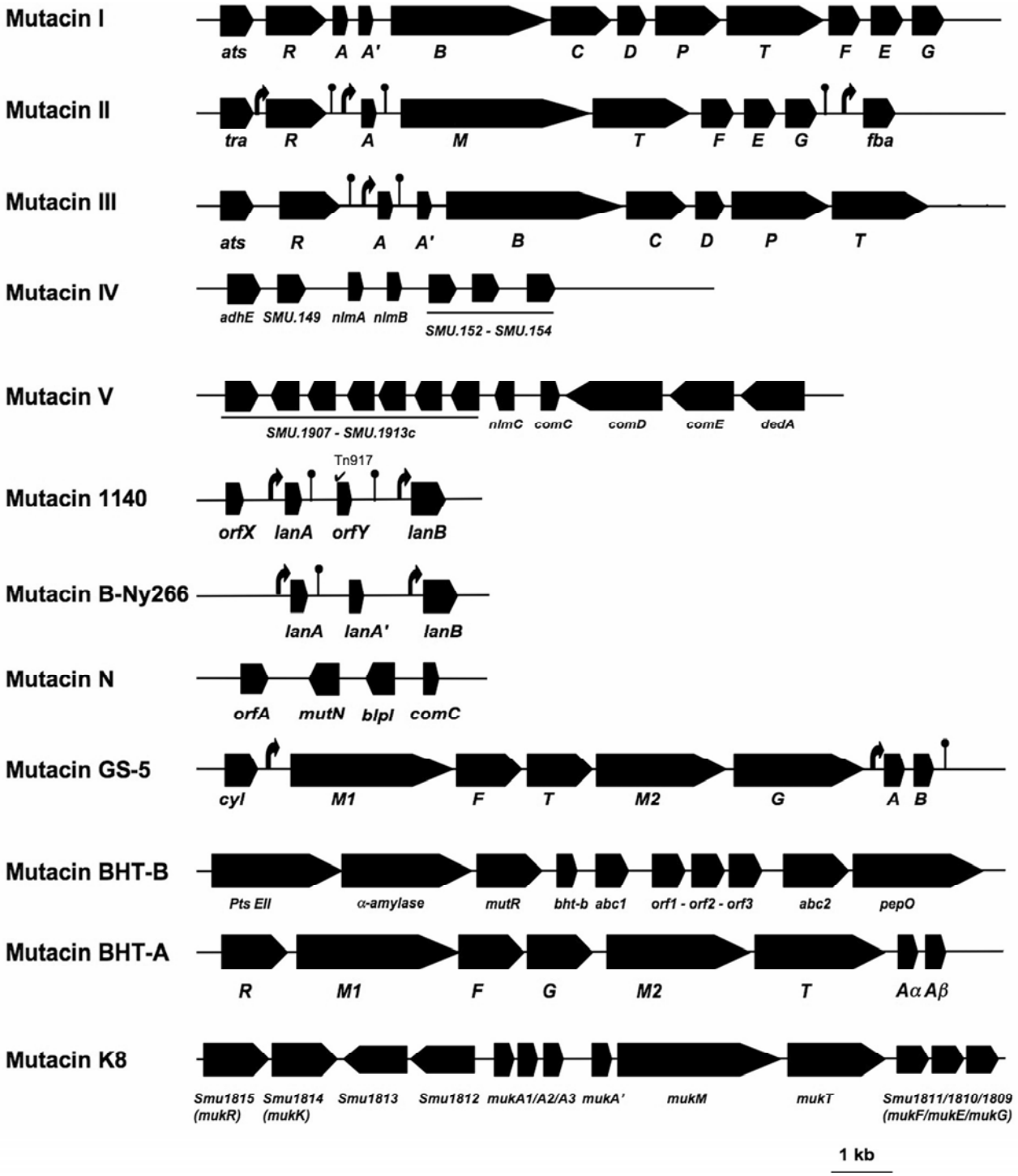

Fig. 1 Organisation of genes associated with mutacin production. Genes and symbols: Prepromutacin: *lanA*, *mutA*, *mutN*, *mukA1*, *mukA2*, *mukA3*, *smbA*, *smbB*, *bhtAα*, *bhtAβ*, *bht-b*, *nlmA*, *nlmB*, *nlmC*; duplicate: *lanA'*, *mutA'*, *mukA'*; putative regulator: *mutR*, *mukR*, *orfX*, *bhtAR*; putative associated histidine kinase: *mukK*; exportation proteins: *mutT*, *mukT*, *smbT*, *bhtAT*, *abc1*, *abc2*; post-translational modification enzymes: *mutBCD*, *mutM*, *mukM*, *smbM1*, *smbM2*, *bhtAM1*, *bhtAM2*; proteolytic processing and transport: *mutP*, *mutT*; immunity: *mutFEG*, *mukFEG*, *smbF*, *smbG*, *bhtAF*, *bhtAG*, *blpI*. Other annotated genes are: *adhE*: acetaldehyde alcohol deshydrogenase; *ats*: alanyl t-RNA synthetase; *comC*, *comD*, *comE*: competence stimulating peptide, associated histidine kinase and response regulator genes; *cyl*: leucyl t-RNA synthetase; *dedA*: putative associated membrane protein gene; *fba*: fructose biphosphate aldolase; *orf1*, *orf2*, *orf3*: putative genes encoding proteins involved in transport, maturation and immunity; *orfA*: ORF related to *Streptococcus agalactiae* transposase; *pepO*: oligopeptidase; *tra*: transposase. Promotors are shown as vertical arrows; potential transcriptional terminators are indicated by hairpins. The prefix '*mut*', '*smb*' and '*bht*' are omitted to simplify the annotation of genes for mutacins I, II, III, GS-5 and BHT-A operons. For non-annotated genes, nomenclature from the NCBI database is adopted (compiled from Woodruff *et al*. 1998; Hillman *et al*. 1998; Chen *et al*. 1999; Qi *et al*. 1999a, 1999b, 2000, 2001; Bekal-Si Ali *et al*. 2002; Hale *et al*. 2004, 2005a; Hyink *et al*. 2005; van der Ploeg 2005; Yonezawa and Kuramitsu 2005; Robson *et al*. 2007).

Mutacin K8

Mutacin K8 is a SA-FF22 lantibiotic-like mutacin produced by *S. mutans* strain K8 (Robson *et al*. 2007). The entire mutacin K8-encoding locus consists of 13 ORFs showing high homology with the ORFs encoding the *Streptococcus pyogenes* lantibiotic SA-FF22 that is found incomplete in the *S. mutans* reference strain genome (only five ORFs with homology to the SA-FF22 regulatory and immunity genes are found) (**Fig. 1**). The genes present are: a putative two-component regulatory system *mukR* (SMU.1815) and *mukK* (SMU.1814), followed by two transposases (SMU.1813 and

SMU.1812), four putative lantibiotic genes, named *mukA1*, *mukA2*, *mukA3* and *mukA'*, coding for prepropeptides that show identity with previously characterised lantibiotic prepropeptides (SA-FF22 and SA-M49 from *S. pyogenes*; Bvi79a and RumA from *Butyrivibrio fibrisolvens* and *Ruminococcus gnavus*, respectively). Finally three ORFs, *mukF* (SMU.1811), *mukE* (SMU.1810), and *mukG* (SMU.1809) encoding putative immunity proteins flank the locus downstream. A molecular mass of 2734 Da was determined for the purified putative mutacin K8 (MukA1 or MukA2 peptide). The first six amino acids were identified by Edman degradation and the complete peptide sequence was deduced from the DNA sequence of the *mukA1* and *mukA3* genes (**Tables 2, 3**).

Lantibiotic dipeptides

Mutacin GS-5 or Smb (for Streptococcus mutans bacteriocin) and mutacin BHT-A

Mutacin GS-5, produced by *S. mutans* GS-5, was preliminarily characterised by Paul and Slade (1975). Its molecular mass has been estimated at more than 20,000 Da and its activity was sensitive to trypsin and pronase E but insensitive to heat (100°C, 10 min at pH 2.0 to 7.0). At pH greater than 7.0, activity was reduced and abolished at pH greater than 11.0 (Paul and Slade 1975). Recently, Yonezawa and Kuramitsu (2005) identified in *S. mutans* GS-5, by insertional inactivation using *Tn916*, a cluster of genes encoding a bacteriocin, named Smb and belonging to the lantibiotic dipeptide class. The biosynthetic operon of Smb is composed of seven ORFs on a 9.5-kb fragment of chromosomal DNA. A leucyl t-RNA synthetase gene *cyl* is found upstream of the operon (Yonezawa and Kuramitsu 2005) (**Fig. 1**). The dipeptide Smb consists of peptides SmbA (30 amino acids) and SmbB (32 amino acids). The molecular masses of the SmbA and SmbB peptides were determined to be 2808 Da and 3452 Da, respectively. This indicates that Paul and Slade (1975) probably worked with a multimeric aggregate of these peptides. Peptide SmbB was slightly different from the predicted sequence proposed by Yonezawa and Kuramitsu (2005) as deduced from the DNA sequence. The presence of an isoleucine in position 1 in the SmbB peptide was confirmed by Edman degradation (Petersen *et al*. 2006) (**Tables 2, 3**). Hyink *et al*. (2005) also reported the purification of a variant of the two-peptide lantibiotic Smb, which is produced by *Streptococcus rattus* strain BHT and named mutacin BHT-Aαβ.

Non-lantibiotic monopeptides

Mutacin V

Mutational analysis of the *S. mutans* genome reference strain (UA159), known to produce the two-peptide non-lantibiotic mutacin IV (Qi *et al*. 2001), showed that this strain also produces an inhibitory activity encoded by the locus SMU.1914c (GenBank Accession number NC_004350, gene ID Smu.1738 in the Los Alamos Oral Pathogen Database), mainly active against non-streptococcal bacteria. SMU. 1914c was named *nlmC* and the product of the gene, mutacin V (Hale *et al*. 2005a). However, to our knowledge, mutacin V has never been purified and tested for its intrinsic antibacterial activity. Downstream of SMU.1914c are found *comCDE* genes encoding the competence stimulating peptide (CSP) with its concomitant histidine kinase sensor and response regulator proteins (Ajdic *et al*. 2002; Hale *et al*. 2005a; van der Ploeg 2005).

Mutacin BHT-B, or rather rattucin BHT-B

Streptococcus rattus BHT (previously *S. mutans* BHT) was reported to produce an antibacterial substance named mutacin b (Kelstrup and Gibbons 1969; Delisle 1975; Hamada and Ooshima 1975a; Rogers 1976; Delisle 1986). The activity of mutacin b was resistant to solvents (ethanol, acetone, butanol, chloroform (1% (v/v)) and to heat (100°C, 15 min at pH 3.0 and 7.0). It was sensitive to chymotrypsin and trypsin but resisted lipase, lysozyme, papain and filtered saliva (Delisle 1986). Later, Hyink *et al*. (2005) reported the production of the mutacin named BHT-B by *S. rattus* BHT. Mutacin BHT-B is a non-modified peptide of 5195 Da with similarities to the tryptophan-rich bacteriocin produced by *Staphylococcus aureus*, aureocin 53 (Netz *et al*. 2002) (**Tables 2, 4**). The operon encoding mutacin BHT-B has been characterised. Upstream of the *bht-b* gene, there is an ORF related to the receptor kinase gene *mutR* of the mutacin I operon and a gene encoding a protein involved in sugar metabolism. Downstream are genes related to ABC transporters (*abc1* and *abc2*), that encompass three ORFs (*orf1*, *orf2*, *orf3*) that could encode proteins involved in transport, maturation and immunity to BHT-B. A gene *pepO* encoding an oligopeptidase (PepO), a 68-kDa CSP-inactivating protein in *S. pneumoniae* (Berge *et al*. 2002), is located downstream of *abc2* (Hyink *et al*. 2005) (**Fig. 1**).

Mutacins F-59.1 and I-T9

Mutacins F-59.1 and I-T9 are produced by *S. mutans* strains 59.1 and T9, respectively (Nicolas *et al*. 2004). Mutacin F-59.1 was purified by hydrophobic chromatography and analysed by Edman degradation. Sequence comparison revealed that mutacin F-59.1 is related to the pediocin-like bacteriocins (Nicolas *et al*. 2007a) (**Table 2**). A similar molecular mass was measured for mutacin F-59.1 and I-T9, suggesting that the two are identical. They are the first pediocin-like mutacins ever reported (Nicolas *et al*. 2007a).

Mutacin N

Mutacin N produced by *S. mutans* N is a non-lantibiotic mutacin composed of 49 amino acids and has a molecular mass of 4806 Da. Structural homology of mutacin N was found with the protein IIc domain of a hypothetical sugar-phosphotransferase enzyme from *Mesoplasma florum* ATCC 33453. Similar inhibition spectra were observed for mutacin N and I while the molecules are clearly distinct (**Tables 2, 4**) (Balakrishnan *et al*. 2000; Qi *et al*. 2001; Balakrishnan *et al*. 2002). Hale *et al*. (2004) have cloned and sequenced the prepropeptide coding gene *mutN*. Three ORFs organised differently from those coding for mutacin I were found associated with *mutN* on an 8-kb DNA fragment (**Fig. 1**). One ORF (*orfA*) shows homology with a *Streptococcus agalactiae* transposase. Another shows homology with *blpI* for a bacteriocin-like peptide from *S. pneumoniae* and the last ORF is identical to *comC*, the gene coding the CSP that induces competence development. Hale *et al*. (2005b) have shown that secretion of mutacin N was carried out by the locus *nlmTE* encoding an ABC transporter similar to the one found in the UA159 strain that exports mutacin IV.

Non-lantibiotic dipeptides

Mutacin IV

Mutacin IV is produced by *S. mutans* UA140 and UA159. It was first proposed to consist of two non-modified peptides (Qi *et al*. 2001). Peptide A, encoded by *nlmA* for non-lantibiotic mutacin peptide A, is formed of 44 amino acids with a molecular mass of 4169 Da. Peptide B, encoded by *nlmB*, has 49 amino acids and a molecular mass of 4826 Da (**Tables 2, 4**). The complete sequences of the genes coding for the two peptides were determined as well as of all the genes necessary for the production of mutacin IV (**Fig. 1**) (Hale *et al*. 2005b). Upstream of the *nlmA* and *nlmB* genes is the acetaldehyde alcohol dehydrogenase gene (*adhE*). While mutacin IV has preliminarily been characterised as a two-peptide bacteriocin (Qi *et al*. 2001), Hale *et al*. (2005a) have recently questioned this two-component structure of the active mutacin IV. Disruption of the *nlmB* gene had no

impact on the activity spectrum of mutacin IV, suggesting that NlmA was sufficient for the complete activity of mutacin IV.

PRODUCTION, BIOSYNTHESIS AND REGULATION

Bacteriocin production is influenced by several environmental factors, such as pH, temperature, concentration of nitrogen and carbohydrate sources, and the presence of essential elements (vitamins, oligo-elements) (see review by Parente and Ricciardi 1999).

The difficulties in producing mutacins or in obtaining a good mutacin yield in liquid media have been reported in many studies (Rogers 1972; Kelstrup and Funder-Nielsen 1977; Parrot *et al*. 1989; Nicolas *et al*. 2004) and few mutacins have been purified from liquid cultures (Novak *et al*. 1993, 1994; Mota-Meira *et al*. 1997; Nicolas *et al*. 2006, 2007a). In each case, yeast extract seemed to be an activating element. Also, bacteriocin expression in *S. mutans* often requires high cell densities (reached only by growing cells on agar media) that hinder their easy purification (Kreth *et al*. 2005; Merritt *et al*. 2005). *S. mutans* generally grows as biofilms in nature, expressing a different gene profile compared to free-living cells that includes the expression of many loci putatively involved in bacteriocin biosynthesis (Motegi *et al*. 2006; Shemesh *et al*. 2007). Alternatives for mutacin characterisation have thus been sought using molecular genetic approaches. Caufield *et al*. (1990a) first attempted to locate genes for mutacin II production by *Tn916* random mutagenesis, which was also used more recently for characterising genes coding for mutacin Smb (Yonezawa and Kuramitsu 2005).

The bacteriocin operons are usually organised as a cluster of genes comprising the prepropeptide coding gene associated with genes for exportation and maturation, genes conferring immunity to the inhibitory activity and occasionally genes involved in regulation of the production of the bacteriocin. Bacteriocins are first synthesised as a prepropeptide that is then exported across the cytoplasmic membrane by dedicated transporters containing an ATP-binding cassette (ABC transporter), and are often processed by a specific protease. Occasionally, these two functions can be combined, depending on the presence of a typical motif in the prepeptide (Havarstein *et al*. 1995; Franke *et al*. 1999; McAuliffe *et al*. 2001). In many cases, the bacteriocin-encoding gene cluster contains one or more immunity proteins to prevent self-killing of the producing strain. Also, the expression of the bacteriocin gene cluster is sometimes under the control of a two-component signal transduction system that is usually part of the cluster. The inducer can be either the bacteriocin itself or a bacteriocin-like peptide.

The mutacin biosynthetic operons characterised to date have all been located on the chromosome and show slight variations in their organisation (**Fig. 1**). These operons are generally related to those coding for other lantibiotics with their prepropeptide-coding gene, along with regulator, maturation, exportation and immunity genes (McAuliffe *et al*. 2001; Chatterjee *et al*. 2005). Many lantibiotics are autoinducing peptides. Autoinducing ability involves activation of a two-component regulatory system, which is ensured by the presence of genes encoding a histidine kinase and a cognate response regulator in the operon (Kleerebezem *et al*. 1997). For class II bacteriocins, inducer peptides are referred to as pheromones and are not part of the biosynthetic operon. However, no mutacin lantibiotic has shown autoinducing activity so far. Furthermore, no genes encoding a histidine kinase and an associated response regulator have been identified in mutacin lantibiotic biosynthetic clusters excepted for the recently characterised mutacin K8, which harbours a *mukK* gene and its cognate *mukR* regulator gene (**Fig. 1**) (Robson *et al*. 2007). For mutacins I, II and III, the existence of a specific transcriptional activator *mutR* has been reported and identified in the biosynthetic operon (Chen *et al*. 1998a; Qi *et al*. 1999b, 2000). Mutacin II transcription is controlled by the promoters of *mutA* and *mutR*. The gene *mutR* encodes a protein homologous to the *rgg* family (regulator gene of glycosyltransferase) of transcription regulators. MutA promoter activation ensures transcription of the *mutAMTFEG* operon and is dependent on MutR as well as on currently unknown components in the growth medium (Qi *et al*. 1999b). Inactivation of MutR suppresses the transcription of the biosynthetic operon of mutacin II. DNA binding motifs are not found in MutR; only the homology with Rgg is consistent with its direct interaction with DNA. Also, direct binding of MutR to the *mutA* promoter has not been observed. To date, the *dgk* gene coding for gene diacylglycerol kinase has been shown to be required for mutacin II expression by strain T8 (Chen *et al*. 1998a). Deficient mutacin production in strain JH1005 was also reported following insertional inactivation of the *fhs* gene coding for formyl-tetrahydrofolate synthetase (Crowley *et al*. 1997).

Recent studies proved that additional genes are implicated in the mechanisms of regulation of the production of some mutacins (**Fig. 2**). A few of them were found to correspond to two-component regulatory systems involved in quorum sensing mechanisms. A two-component sensor, CiaH, and an interspecies signalling system, LuxS, have both been found to be involved in the regulation of the mutacin I operon (Qi *et al*. 2004; Merritt *et al*. 2005). However, inactivation of the putative response regulator CiaR of the histidine kinase sensor CiaH did not affect the production of mutacin I. Also, inactivation of *ciaH* has no influence on mutacin II or mutacin IV production by their respective producing strains (T8 and UA140) (Qi *et al*. 2004). The *luxS* mutation did not affect mutacin IV production either (Merritt *et al*. 2005). Merritt and co-workers (2005) also pointed out that the production of wide spectrum mutacin I appears to be regulated by the interspecies signalling mechanism of *luxS* while the narrow spectrum mutacin IV is controlled by intraspecies signalling through *comCDE*.

By random insertional mutagenesis, 25 additional genes/loci were found to be required for mutacin I production (Tsang *et al*. 2005). Putative assigned functions by the Los Alamos Oral Pathogen Sequence Database mainly identified these loci as two-component signal transduction systems (*vicRKX* and *hk03*/*rr03*), a stress response regulator (*hrcA*), metabolic enzymes (*pttB*, *adhE*), and a large conserved hypothetical protein (Smu.1281, Los Alamos Database). Tsang *et al*. (2006) reported that these multiple input signals for mutacin I production can be divided into two pathways regarding the induction of an 'inducible repressor of virulence' gene (*irvA*/Smu.1274): *irvA*-dependent and *irvA*-independent. IrvA has been identified as a putative repressor that seems to be implicated in the *luxS*-mediated mutacin I gene regulation pathway (Merritt *et al*. 2005). As for *luxS*, signals mediated through *vicK*, *pttB* and *hk03* exert their effect possibly through modulating *irvA* transcription, whereas signals mediated through *ciaH*, *hrcA*, *adhE*, and Smu.1281 exert their effect through an unknown mechanism independent of *irvA*. In the same way, Merritt *et al*. (2007) identified a putative membrane bound protein, encoded by *hdrM* and expressed under high cell density, that positively regulates expression of mutacin I, while having a negative effect on competence development. The gene *hdrM* is part of the two-gene operon formed by *hdrR* (SMU.1854, NCBI database/Smu.1689, Los Alamos database) and *hdrM* (SMU.1855/Smu.1690) that show similarity with the putative LytTR family regulators found in various Gram-positive bacteria and act as a two-component regulatory system. However, no kinase domain homology was found in the putative membrane bound protein sequence HdrM (Merritt *et al*. 2007).

In the reference strain UA159, the expression of several mutacin-like genes was found to be induced following addition of a competence stimulating peptide (CSP) (Kreth *et al*. 2005; van der Ploeg 2005). Other CSP-mediated phenotypes identified to date for *S. mutans* include competence, acid tolerance and biofilm formation (Li *et al*. 2002). The CSP coded by *comC* is synthesised as a prepeptide contain-

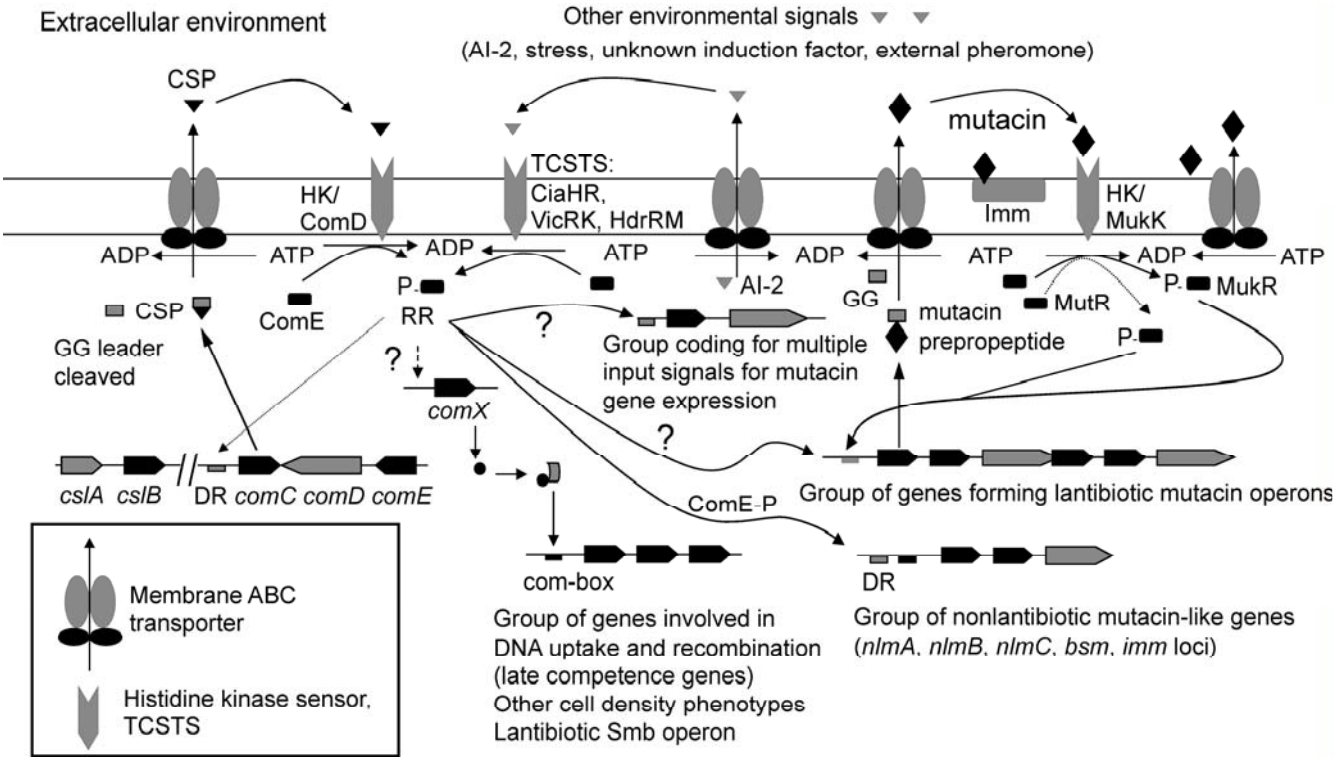

Fig. 2 Model of the current knowledge of *S. mutans* signal transduction pathways for competence, mutacin production and other cell density-dependent phenotypes and physiological processes. AI-2: autoinducer-2 signal molecule. CSP: competence stimulating peptide. HK: histidine kinase receptor. RR: response regulator. TCSTS: two-component signal transduction system formed by an HK and its cognate RR. *comD*: HK competence gene. *comE*: RR competence gene *comX*: alternate sigma factor gene. *cslAB*: ABC exporter gene for CSP. DR: direct repeat motif recognised by phosphorylated ComE. MutR: mutacin transcription regulator. MukRK: TCSTS for mutacin K8 autoinduction. Under high cell density, a threshold concentration of extracellular CSP induces autophosphorylation of a sensor HK (ComD), which phosphorylates a RR (ComE) that initiates either direct transcription of a group of mutacin-like genes or transcription of DNA uptake genes through ComX activation by an unknown mechanism. Autoregulation of the competence regulon is not supported. Environmental signals may trigger TCSTS (CiaHR, VicRK, HdrRM) that regulate the expression of genes ensuring competence, acid tolerance, biofilm formation and lantibiotic mutacin production. Lantibiotic mutacins can autoinduce their own biosynthesis through a quorum sensing mechanism similar to other lantibiotics. Question marks indicate that the mechanism and intermediates are unknown to date (Kleerebezem and Quadri 2001; Li *et al*. 2002; Qi *et al*. 2004; Tsang *et al*. 2005; van der Ploeg 2005; Kreth *et al*. 2005, 2006; Martin *et al*. 2006; Wang and Kuramitsu 2006; Kreth *et al*. 2007; Merritt *et al*. 2007).

ing a double-glycine (GG) leader motif at its N-terminus. The GG leader sequence of the CSP is cleaved off during export across the membrane by a dedicated ABC transporter, CslAB (Petersen and Scheie 2000; Li *et al*. 2002; Hale *et al*. 2005b). Following its secretion, CSP is recognised by the sensor kinase receptor ComD, which then autophosphorylates and by transferring the phosphoryl group, activates the ComE response regulator. Phosphorylated ComE activates expression of many mutacin-like and associated accessory genes (Li *et al*. 2002; Kreth *et al*. 2006), recognising an imperfect direct repeat DNA sequence positioned upstream of the transcription start site (van der Ploeg 2005; Kreth *et al*. 2007). The direct repeat motif is similar to the consensus binding sequence proposed for response regulators of the AlgR/AgrA/LytR family (Nikolskaya and Galperin 2002). ComE is thus reported as a transcriptional activator of mutacin production, but a repressor of CSP biosynthesis in *S. mutans* (Kreth *et al*. 2006, 2007). The CSP signal transduction system also activates an alternative competence-specific transcription factor, ComX, although not directly through ComE binding (Kreth *et al*. 2005). Generally in streptococci, ComX, in association with RNA polymerase, mediates expression of late competence genes *comYA-G* encoding proteins involved in the DNA uptake and recombination process of the competence state of bacterial cells (Martin *et al*. 2006). Many genes regulated by ComX have a com-box (also called a cin-box) with the consensus sequence TAC GAATA located in the -10 promoter region and such com-boxes have been identified close to numerous *S. mutans* open reading frames (Li *et al*. 2002). Coordinate expression of mutacin production and competence, along with other cell-density phenotypes, may thus be attained directly through the action of ComE (two-component signalling response), or through ComX (alternate transcription factor), as well as other signalling cascades (**Fig. 2**).

Mutacin lantibiotic Smb production was shown to be CSP dependent. Homology between promotor sequences of the Smb operon and the *comC* gene has been observed, suggesting a regulation of the Smb operon transcription dependent on *com* gene expression (Yonezawa and Kuramitsu 2005). The CSP was shown to be 21 amino acids in strain GS-5, but post-export cleavage of the three C-terminal residus showed a more potent inducing efficiency. This was exploited in order to purify the dipeptide lantibiotic Smb (Petersen *et al*. 2006).

Despite the genetic diversity of the competence gene locus (*comC*) in *S. mutans*, the allelic variation of *comC* seems to produce functionally equivalent ComC peptides. Pherotype specificity of ComC is thus not supported in *S. mutans* (Klein *et al*. 2006; Allan *et al*. 2007). In contrast, competence pherotype variability in *Streptococcus pneumoniae* depends on the variation of a hydrophobic patch in the CSP and on that of the sensor domain of the histidine kinase ComD (Iannelli *et al*. 2005; Johnsborg *et al*. 2006). *S. mutans* CSP presents no variability in its hydrophobic patch and structure-activity analysis of the peptide reveals that two distinct functional domains ensure binding to and activation of the two-component signal transduction system that triggers competence development (Syvitski *et al*. 2007; Allan *et al*. 2007). In addition, an overdose of CSP is lethal to *S. mutans* (Qi *et al*. 2005). The *S. mutans* competence regulon *comCDE-cslAB* shows similarity with the

blpABCDRH regulon that controls bacteriocin production by a quorum sensing mechanism in *S. pneumoniae* (Martin *et al*. 2006; Lux *et al*. 2007). Induction of bacteriocin production was thus proposed to be the primary functional role for *comDE* (Martin *et al*. 2006), and ComC would have the dual roles of competence stimulating peptide (CSP) and bacteriocin inducing peptide (BIP). This interdependence of competence development and bacteriocin production in *S. mutans* mediated by a quorum-sensing system appears to be characteristic in streptococci (Martin *et al*. 2006).

MUTACIN IMMUNITY

Bacteria that produce bacteriocins are protected against their cognate activity by expressing an immunity system. For lantibiotics, the immunity system depends on the expression of a set of three genes (*lanFEG* for nisin) that can be associated with the expression of a further gene *lanI*. The set of genes *lanFEG* encode a LanFEG immunity ABC transporter known to expel lantibiotics from the membrane into the external medium before peptides reach the necessary density required to form pores in the membrane. LanI is a lipoprotein that confers immunity by its membrane-bound specific lantibiotic-binding activity (Takala and Saris 2006). The two immunity systems function relatively specifically and can provide either a cooperative or an independent immunity against their cognate lantibiotic (Klein and Entian 1994; Stein *et al*. 2003; Aso *et al*. 2005; Chatterjee *et al*. 2005).

For class II bacteriocins, immunity is generally expressed by one gene (*pedB* for pediocin). However, their mode of action remains poorly understood. A cytosolic location of an immunity protein activity has been demonstrated (Quadri *et al*. 1995). Three-dimensional structures of immunity proteins for class IIa bacteriocins show very similar globular structures with a conserved left-turning four-helix bundle protein motif (Sprules *et al*. 2004; Johnsen *et al*. 2005a; Kim *et al*. 2007). The protective mechanism seems to involve an interaction between the C-terminal domain of the immunity protein and the C-terminal hydrophobic membrane-penetrating portion of its cognate bacteriocin that probably hinders the membrane insertion of the peptide (Johnsen *et al*. 2005b; Kim *et al*. 2007). Furthermore, several orphan immunity genes conferring resistance to some pediocin-like bacteriocins exist (Fimland *et al*. 2002). Another self-protective mechanism against class II peptide-bacteriocin activity resides in the interaction of the immunity protein with the IIC and IID components of the mannose phosphotransferase system (Diep *et al*. 2007).

Immunity for mutacins has been elucidated based on gene homology (**Fig. 1**). The lantibiotic immunity mechanism for mutacins I and II is based on the *mutFEG* genes that are closely related to the *lanFEG* lantibiotic immunity system (Qi *et al*. 1999b, 2000; McAuliffe *et al*. 2001). For the dipeptide lantibiotic Smb, genes *smbF* and *smbG* confer immunity to SmbA and SmbB peptides, respectively. SmbF shows homology to the SpaF immunity protein from *Bacillus subtilis* while *smbG* exhibits similarity with the *plnG* immunity gene from *Lactobacillus plantarum* (Diep *et al*. 1996; Stein *et al*. 2002; Yonezawa and Kuramitsu 2005). A similar argument has been applied to identify the immunity system of the dipeptide lantibiotic BHT-A, that shares more than 95% identity at the DNA level with the Smb operon (Hyink *et al*. 2005; Yonezawa and Kuramitsu 2005). No immunity genes have yet been identified in the vicinity of the biosynthetic gene clusters encoding mutacin lantibiotics from group B, as sequencing is incomplete (mutacin B-Ny266, mutacin III, mutacin 1140) (Bekal-Si Ali *et al*. 2002). However, immunity was demonstrated phenotypically (Morency *et al*. 2001). For the non-lantibiotic mutacin N, an ORF related to the bacteriocin-like peptide immunity gene *blpI* from *Streptococcus pneumoniae* has been identified near the *mutN* gene and the product of the gene was predicted to be involved in mutacin N immunity (Balakrishnan *et al*. 2000). Similarly, for the non-lantibiotic mutacin BHT-B, several ORFs in the biosynthetic operon encoding proteins sharing similarities with motifs common to ABC transporters were predicted to be involved in the immunity mechanism (Hyink *et al*. 2005). No cognate immunity genes have yet been identified for the non-lantibiotic dipeptide mutacin IV (Qi *et al*. 2001). We can speculate that cross-immunity could occur between bacteriocin immunity proteins and non-related bacteriocin-like peptides found in the UA159 reference strain genome. Furthermore, it is generally accepted that the bacteriocin immunity protein associated with bacteriocin activity cannot alone ensure self-protection for the producer cell. This suggests the existence of other immunity mechanisms that may be related to natural resistance mechanisms found in bacteria and that rely on either variation in membrane composition or modification of a specific target site (Fimland *et al*. 2002; Vadyvaloo *et al*. 2004; Kramer *et al*. 2006).

STREPTOCOCCUS MUTANS GENOMICS

Bacteriocin-coding genes can be found on plasmids, transposons and on the chromosome (Jack *et al*. 1995). However, mutacin genes have only been found on the chromosome so far (Caufield *et al*. 1990b; Woodruff *et al*. 1998; Yonezawa and Kuramitsu 2005). Gene clusters governing mutacin biosynthesis are sometimes close to or framed by transposase genes, indicating potential mobility for those operons through transposition events, and their G+C content can differ from that of *S. mutans* genome, also suggesting cross-species transfer (**Fig. 1**; **Table 5**).

The complete genome sequence of the *S. mutans* strain UA159 of serotype c (ATCC 700610) has been determined (Ajdic *et al*. 2002) (GenBank accession no. AE014133). A complete set of genes was found to ensure a natural competence phenotype for this strain of *S. mutans*. The bacterium is able to metabolise a large variety of carbohydrates such as mono- and disaccharides and its genome encodes many peptidases, proteases and other exoenzymes for action on various substrates found in the oral cavity. No temperate bacteriophage and no genes encoding toxins have been detected in the *S. mutans* genome and few virulence traits are reported for *S. mutans* compared to other opportunistic pathogenic streptococcal species (Mitchell 2003). While incomplete biosynthetic operons for bacteriocin production were first found in the genome of strain UA159 (Ajdic *et al*. 2002) and a preliminary study indicated that the strain did not possess bacteriocinogenic activity (Chen *et al*. 1999), one mutacin (mutacin IV) has been purified from the genome reference strain (Qi *et al*. 2001). More recent studies using genomic analysis have identified many putative genes in the genome of *S. mutans* that could be involved in producing other mutacin-like inhibitory substances (Dirix *et al*. 2004; Hale *et al*. 2005a; van der Ploeg 2005). Five genes for bacteriocin production similar to the regulation genes (*scnK*/SMU.1814 and *scnR*/SMU.1815) and to the immunity genes (*scnG*/SMU.1809, *scnE*/SMU.1810 and *scnF*/SMU.1811) belonging to the streptococcin A-FF22 operon from *Streptococcus pyogenes* were found in the genome of strain UA159 (McLaughlin *et al*. 1999; Ajdic *et al*. 2002) while the genes required for exportation and maturation (*scnM* and *scnT*) were not identified. Similarly, homologs of genes conferring immunity to lacticin 481 (*lctFEG*) (SMU.1148, SMU.1149, SMU.1150) and combined homologs of the two-component signal transduction systems genes, *rumRK* and *scnRK*, putatively involved in ruminococcin or streptococcin autoinduction, were also identified (SMU.1145c and SMU.1146c) (McLaughlin *et al*. 1999; Dufour *et al*. 2000; Ajdic *et al*. 2002; Gomez *et al*. 2002). These observations suggest the presence of partial bacteriocin synthesis operons that could complement some functions of compatible bacteriocin production. Robson *et al*. (2007) have identified a strain capable of producing a streptococcin AFF22-like mutacin, named mutacin K8. This study indicates that some *S. mutans* strains could produce a lacticin 481-like mutacin and to a lesser extent a rumino-

coccin-like mutacin.

A screening of the *S. mutans* genome identified a putative MutE1 (SMU.655) which probably belongs to an incomplete bacteriocin biosynthesis cluster also containing the immunity factors (MutF/SMU.654, MutE2/SMU.656, MutG/SMU.657), three putative ABC transporters (SMU. 653c, SMU.652c, SMU.651c), a putative histidine kinase with its cognate response regulator (SpaK/SMU.660, SpaR/SMU.659) similar to that of subtilin biosynthesis in *Bacillus subtilis* (Klein *et al*. 1993) followed by a putative member of the CAAX amino terminal protease family (SMU.662). This cluster of genes is framed upstream by an alanyl t-RNA synthetase gene (SMU.650), as was found for the biosynthesis clusters of mutacin I and III (Qi *et al*. 1999a, 2000). A *mutR* homolog is predicted in the *S. mutans* genome (SMU.110). This observation strongly suggests that the biosynthesis cluster of mutacin I could have been fragmented and dispersed as a mosaic on the *S. mutans* genome by various ancestral insertion and deletion events of mobile genetic elements. One other putative immunity bacteriocin protein is located on the *S. mutans* genome (SMU.2035) presenting homology to peptidase U61, LD-carboxypeptidase A from *Streptococcus suis* 89/1591 (EAP40801) and a putative protein for microcin C7 resistance from *Streptococcus sanguinis* SK36 (ABN45697) (Xu *et al*. 2007) as well as to the microcin immunity protein MccF VCA0439 from *Streptococcus agalactiae* COH1 (EAO76085) (Tettelin *et al*. 2005).

Genomic islands in *Streptococcus mutans* encompassing bacteriocin-encoding genes

Genomic Islands (GI) can represent mobile genetic elements that have been integrated into an organism's genome via lateral gene transfer (LGT) mechanisms (Jain *et al*. 2002). Transformation, conjugation, and transduction are genetic mechanisms that favour LGT of mobile elements between different microorganisms and/or strains. Acquisition of new genes by LGT is a predominant force in bacterial evolution (Jain *et al*. 2002; Pal *et al*. 2005; Mc Arthur 2006) and has been recently studied for streptococci (Marri *et al*. 2006). GIs encode a variety of different functions that depend largely on the environment of the organism, and can be involved in symbiosis or pathogenesis. Often, they increase the fitness of an organism to occupy a particular ecological niche (Hentschel *et al*. 2001). Evolving in a complex microbial environment, *S. mutans* could thereby be subject to genomic mixing. Myers and Kuramitsu (2006) reported in the Los Alamos Oral Pathogen Database that some bacteriocin-like genes have been located on two potential GIs in *S. mutans* UA159 (GI ID 12 and 15). GI 12 (57 kb) contains genes coding for bacitracin and gramicidin S synthesis as well as two ABC transporter cassettes. GI 15 (13.5 kb) could contain up to five genes potentially coding for bacteriocin-like peptides based on homology to *S. pneumoniae* Blp bacteriocin gene clusters. The bacteriocin-coding gene for the recently identified mutacin V (Smu1738/SMU. 1914c/*nlmC*) is located at one end of the GI 15 cluster of genes (Hale *et al*. 2005a; Myers and Kuramitsu 2006).

Bioinformatic genome screening algorithms for bacteriocin-related genes

Identification of putative bacteriocins can be done by screening a genomic DNA sequence for the presence of bacteriocin genes and biosynthetic operons using specifically designed algorithms. This represents an *in silico* identification of bacteriocins which might lead to the discovery of new types of bacteriocin-like peptides (Dirix *et al*. 2004; Nes and Johnsborg 2004; de Jong *et al*. 2006). Algorithms have been proposed to screen genomes of Gram-positive bacteria for double-glycine (GG)-motif-containing peptides, as it is known that the Gram-positive bacteria GG-motif plays a key role in many peptide secretion systems involved in quorum sensing and bacteriocin production (Kleerebezem and Quadri 2001; Dirix *et al*. 2004). The motif screening has been combined with the criterion of the presence of two types of proteins; (1) the peptidase C39 protein family domain, which is present in the cognate transporter responsible for the proteolytic removal of the GG-type leader peptide (Havarstein *et al*. 1995) and (2) the presence of a histidine kinase domain often involved in the autoinducing regulation of lantibiotic bacteriocin production (Kleerebezem *et al*. 1997; Kleerebezem and Quadri 2001). In addition to the GG motif characteristic of class IIa and IIb bacteriocin processing, de Jong *et al*. (2006) included other leader motifs in their search criteria (PR/PQ/GA/GS for lantibiotics and Class IIb bacteriocins).

Simple bioinformatic analyses have resulted in the identification of many putative mutacin-like bacteriocins by screening the genomic DNA sequence of the reference genome strain UA159. Dirix *et al*. (2004) previously identified 10 genes that could code for possible GG motif containing peptides (**Table 6**) with their putative cognate transporters. van der Ploeg (2005) also identified putative genes related to bacteriocin production in *S. mutans* (designated *bsmA* to *K*, except *bsmD* and *bsmJ*; **Table 6**) with two putative immunity factors (*immA*/SMU.925 and *immB*/SMU. 1913c), which were identified by Blast homology searching using NlmA and NlmB as query sequences.

Further putative bacteriocin-coding genes may be found by adding to the search criteria. Such an algorithm has been developed by de Jong *et al*. (2006), using seven criteria that were attributed priority weighting to give a final score. In addition to the presence of leader motifs, the criteria include the distance from putative genes coding for potential transporters, immunity and regulatory genes, as well as the leader sequence peptidase. The BAGEL software (MolGen; de Jong *et al*. 2006) is thus conceived to identify putative bacteriocin genes by evaluation of the genomic context and presence of accessory genes in the vicinity, along with the predicted protein characteristics themselves.

We used the BAGEL websoftware to locate putative bacteriocin gene clusters in the genome sequence of *S. mutans* strain UA159 (*Streptococcus mutans* genome NC_004350 as query entry with bacteriocin default as selected profile; **Table 6**). A total of 21 gene products scored significantly (BAGEL score over 185), while the scores of eight gene products were close to significance (between 165 and 175; only one does not contain a GG motif; **Table 6**) and 49 scored between 105 and 155. Of these 49 non-significant hits, nine gene products contain a GG leader motif, while 16 contain one of the PR/PQ/GA/GS leader motifs for lantibiotics and Class IIb bacteriocins (de Jong *et al*. 2006). Eleven of the gene products identified by the BAGEL search belong to GI 12, but none except one transposase fragment scored significantly (**Table 6**). Thus, the bacitracin synthesis gene cluster of GI 12 is effectively excluded by BAGEL. Among the 23 genes of GI 15 (13.5 kb), 11 were detected by BAGEL, while nine gene products scored significantly (**Table 6**). The gene coding for mutacin V (Smu 1738/SMU.1914c/*nlmC*) (Hale *et al*. 2005a) is the only recently identified mutacin located in this set of genes. A gene coding for a CSP, previously shown to regulate mutacin gene expression, scored a significant BAGEL hit, and this *comC* gene is located next to the bacteriocin-related GI 15. The ComC peptide contains a leader sequence that is highly similar to that of mutacin peptide NlmA, and contains the GG motif. The association between bacteriocin production and competence could possibly be explained by the function of bacteriocins in lysing neighbouring cells under high density conditions, which could thus release DNA for uptake by competent cells (Kreth *et al*. 2005; van der Ploeg 2005). In *S. pneumoniae*, competence-associated lysis of neighbouring non-competent cells is carried out by bacteriocins as well as amidases (Steinmoen *et al*. 2003; Guiral *et al*. 2005). In addition to the specific mechanism of DNA uptake encoded by late competence genes (ComG operon; Petersen *et al*. 2005), some mutacin molecules could created transient pores in the cytoplasmic membrane in order to

Table 6 Summary of bacteriocin-like gene products identified in the *S. mutans* UA159 genome (GenBank Acc. No. AE014133) using bioinformatic search algorithms (BlastP, GG motif and BAGEL).

Locus Tag (gene)	Size (amino acids)	Product[a]	Homology[b]	BAGEL Score[c]	Leader motif[d]	Origin or Reference[e]
SMU.27 (*acpP*)	82	acyl carrier protein	(1) AAL96853, putative acyl carrier protein	170	-	
SMU.33	93	hypothetical protein	none	170	GG	
SMU.150 (*nlmA*)	67	hypothetical protein	(1) AAG29818, bovicin 255 precursor	105	GG	Qi *et al*. 2001; Hale *et al*. 2005a; van der Ploeg 2005
SMU.151 (*nlmB*)	78	hypothetical protein	(1) AAA16637, lactacin F accessory protein	105	GG	Qi *et al*. 2001; Hale *et al*. 2005a; van der Ploeg 2005
SMU.281	92	hypothetical protein	(1) AAG18789, cationic amino acid transporter	225	GS	
SMU.283	72	hypothetical protein	(1) AAN57926, SMU.150 (*nlmA*)	225	GG	Dirix *et al*. 2004
SMU.299c	72	putative bacteriocin peptide precursor, BsmE	(1) AAG29818, bovicin 255 precursor	255	GG	van der Ploeg 2005
SMU.419	98	hypothetical protein	(1) ABJ65682, predicted nucleic acid binding protein; (2) AAB58316, halocin H4 precursor	155	-	
SMU.423	76	hypothetical protein, BsmC	(1) AAG29818, bovicin 255 precursor	225	GG	van der Ploeg 2005
SMU.513	77	hypothetical protein	(1) AAN58350, paralogous SMU.613	105	GS	
SMU.564	76	hypothetical protein	(1) AAK34305, COG4703	165	GA	
SMU.571	50	hypothetical protein	(1) ABJ65917, hypothetical protein	225	GA	
SMU.613	92	hypothetical protein	(1) AAB91455, ThmA precursor peptide; (2) CAE32973, linocin M18	155	GE	
SMU.616	82	hypothetical protein	(1) AAB91455, ThmA precursor peptide	No score	AG	
SMU.655 (*mutE1*)	82	Putative MutE	(1) AAF99692, MutE; ABC transporter, immunity	125	-	
SMU.865	91	30S ribosomal protein S16 HUC (3.7×10^{-12})	(1) ABN44709, putative 30S ribosomal protein S16	165	PR	
SMU.1127 (*rpsT*)	84	30S ribosomal protein S20 HUC (6.1×10^{-11})	(1) AAK99544, 30S ribosomal protein subunit S20	185	-	
SMU.1131c	87	hypothetical protein	none	225	GG	
SMU.1291c	90	hypothetical protein	(1) AAK19833, chorismate mutase-like protein; (2) AAF36414, rhizobiocin RzcB	155	-	
SMU.1354c	86	putative transposase fragment	(1) AAA74027, putative transposase	225	GS	Bacitracin gene cluster GI 12
SMU.1355c	97	putative transposase fragment	(1) ZP_00366060, transposase and inactivated derivatives	125		GI 12
SMU.1356c	96	putative transposase fragment	(1) AAD32432	185	-	GI 12
SMU.1357	78	putative transposase fragment	(1) AAC38767, putative transposase	185	-	GI 12
SMU.1358	33	putative transposase fragment	(1) AAC38767, putative transposase	125	-	GI 12
SMU.1359	36	hypothetical protein	none	125	-	GI 12
SMU.1360c	42	hypothetical protein	none	125	-	GI 12
SMU.1368	39	hypothetical protein	(1) AAN57950, paralogous SMU.175	125	-	GI 12
SMU.1369	40	hypothetical protein	none	125	-	GI 12
SMU.1372c	80	hypothetical protein	(1) AAN63779, tnpA-IS1253-like	125	-	GI 12
SMU.1373c	60	hypothetical protein	(1) AAN63779, tnpA-IS1253-like	125	-	GI 12
SMU.1641	56	hypothetical protein	(1) AAV60978, hypothetical protein, COG3237	165	GA	
SMU.1719c	82	hypothetical protein	(1) CAD47309, COG3763; (2) AAT85008, klebicin C	265	GG	
SMU.1818c	57	hypothetical protein	(1) ABF37350, transposase	165	GS	
SMU.1862	67	hypothetical protein	none	285	GS	
SMU.1882c	117	hypothetical protein	none	No score	GG	Dirix *et al*. 2004
SMU.1888	68	hypothetical protein	(1) EAP40958, integrase	185	-	
SMU.1889c	88	hypothetical protein, BsmF	(1) CAC03530, BlpU	345	GG	GI 15; Dirix *et al*. 2004; van der Ploeg 2005
SMU.1891c	60	hypothetical protein	none	245	-	GI 15
SMU.1892c	61	hypothetical protein, BsmG	none	355	GG	GI 15; Dirix *et al*. 2004; van der Ploeg 2005
SMU.1895c	53	hypothetical protein, BsmI	(1) CAC03521, BlpJ	225	GG	GI 15; Dirix *et al*. 2004; van der Ploeg 2005
SMU.1896c	100	hypothetical protein, BsmH	(1) CAC03530, BlpU	125	GG	GI 15; van der Ploeg 2005
SMU.1899	41	putative ABC transporter	(1) EDK62895, bacteriocin processing peptidase, ABC transporter	185	-	GI 15

Table 6 (Cont.)

Locus Tag (gene)	Size (amino acids)	Product[a]	Homology[b]	BAGEL Score[c]	Leader motif[d]	Origin or Reference[e]
SMU.1902c	47	hypothetical protein, BsmK	none	285	GG	GI 15; Dirix *et al*. 2004; van der Ploeg 2005
SMU.1903c	50	hypothetical protein	none	185	-	GI 15
SMU.1905c	62	putative bacteriocin secretion, BsmL	(1) E49786; bacteriocin secretion protein A2	285	GG	GI 15; Dirix *et al*. 2004; van der Ploeg 2005
SMU.1906c	70	hypothetical protein, BsmB	(1) CAC03530, BlpU	225	GG	GI 15; Dirix *et al*. 2004; van der Ploeg 2005
SMU.1914c (*nlmC*)	76	hypothetical protein, BsmA	(1) CAC03526, BlpO	165	GG	GI 15; Dirix *et al*. 2004; van der Ploeg 2005; Hale *et al*. 2005a
SMU.1915 (*comC*)	46	competence stimulating peptide precursor	(1) ABE02365, competence stimulating peptide precursor	175	GG	van der Ploeg 2005

[a] Hypothetical protein generally presenting no similarity with any other sequenced protein; Bsm is for bacteriocin from *Streptococcus mutans* as found by van der Ploeg (2005) using BlastP search for homology to NlmA and NlmB. Blp is for Bacteriocin-like peptide identified in the *Streptococcus pneumoniae* genome (de Saizieu *et al.* 2000). Significant FASTA scores of the gene product with the "Highly Unlikely Bacteriocin Candidates" HUC database are in parenthesis, while non-significant scores under 10^{-8} are not reported (de Jong *et al*. 2006).

[b] Homology refers to: (1) the best BlastP hit of the locus in GenBank (alignment scores <40 were ignored) and (2) the name of homologous gene from the bacteriocin database (http://bioinformatics.biol.rug.nl/bacteriocin/database.php). The cluster of orthologous group (COG) is given when homology corresponds to an uncharacterised conserved protein in bacteria.

[c] The BAGEL websoftware of MolGen (RuG) at http://bioinformatics.biol.rug.nl/websoftware/bagel (de Jong *et al*. 2006) was run with the bacteriocin default profile. The scores correspond to the sum of the seven weighted factors attributed to the peptide. Significant hits score above 175 (de Jong *et al*. 2006). "No score" indicates that the BAGEL algorithm did not identify the gene product under the default profile, but the gene product was identified using other search algorithms such as BlastP or leader motif searching.

[d] Leader sequence motifs are the GG-motif of Class IIa and IIb bacteriocins and PR/PQ/GA/GS for lantibiotics and Class IIb bacteriocins (de Jong *et al*. 2006).

[e] References indicate that the gene product was identified as bacteriocin-like in published studies. GI 12 is Genomic Island 12 containing the bacitracin synthesis gene cluster and GI 15 is the bacteriocin-related genomic island as listed on the Oral Pathogen Database site (http://www.oralgen.lanl.gov/oralgen/; Myers and Kuramitsu 2006).

enhance the entrance of exogenous DNA fragments during transformation, accompanied by the lysing of non-immune related cells. Mutacin-producing competent cells are protected from the lethal activity of the bacteriocin by expressing a cognate immune system. The existence of alternative mechanisms for DNA binding and uptake in streptococci has been proposed by Petersen *et al*. (2005), as transformation is not completely abolished when *comG* genes are inactivated (Lundsford and Roble 1997; Berge *et al*. 2002). However, whether bacteriocin pores are able to accommodate DNA uptake remains to be investigated.

The BAGEL search did not significantly score some of the genes reported by Dirix *et al*. (2004) and van der Ploeg (2005), in particular SMU.1882, SMU.1896c (BsmH), as well as mutacin V (SMU.1914c/*nlmC*) and the mutacin IV defined as a putative dipeptide bacteriocin encoded by SMU.150/*nlmA* and SMU.151/*nlmB* (these gene products all possess a GG leader motif, but are either not reported or reported only in the potential or not significant scores by the BAGEL search). In addition, BlastP using NlmA detects SMU.513, SMU.613 and SMU.616 as significant hits, while these gene products are not scored, or scored non-significantly by BAGEL, perhaps due to the absence of a known leader motif. Relaxing the search criteria allowed a higher number of false positive results, without improving the detection of known bacteriocin gene products. Two ribosomal protein encoding genes (SMU.1127/*rpsT* and SMU.865) were reported as significant or close to significant using the default BAGEL profile (**Table 6**), and could thus be false positive results, as the gene products have significant similarity to entries in the database of highly unlikely bacteriocin candidates (HUC, de Jong *et al*. 2006). However, some antimicrobial activity has actually been reported for peptide fragments similar to ribosomal proteins from bacteria (Park *et al*. 2005). These would thus be good candidates for *in vitro* peptide synthesis and testing for antimicrobial activity.

Three putative transposase fragments were scored as significant by the BAGEL search (SMU.1354c, SMU.1356c, SMU.1357) while two others were scored as non-significant (SMU.1355c, SMU.1358). Transposase genes are known to be associated with genomic islands, such as those linked to bacteriocin encoding genes in *S. mutans*. Integrases are known to mediate integration of DNA into the host chromosome and thus promote lateral gene transfer (Mazel 2006). Mobile genetic elements are often found flanking bacteriocin-coding gene loci, so these significant hits could be simply due to their proximity, and most probably represent false positive hits with respect to any putative bacteriocin activity.

Many gene products found by the BAGEL algorithm correspond to hypothetical proteins or peptides presenting no homology to any database entries. By screening the *S. mutans* genome, we observed that these loci are in some cases framed by putative transporter genes (SMU.286, SMU.1881c, SMU.1897, SMU.1898, SMU.1899, SMU.1900, SMU.1905c) and an immunity factor (SMU.1913c). The absence of transporter genes and immunity factors in the vicinity of some bacteriocin-like gene products could conceivably be complemented with those dedicated to other bacteriocins (van Belkum *et al*. 1997; Fimland *et al*. 2002; Matsumoto-Nakano and Kuramitsu 2006). Sometimes the specificity of transporter/exporter proteins is closely related to the leader sequence of the bacteriocin (Havarstein *et al*. 1995; Hale *et al*. 2005b). In this case, some loci encoding hypothetical peptides and harbouring significant similarities in their leader sequence with NlmA/SMU.150 (namely SMU.283, SMU.299c, SMU.423, SMU.1889c, SMU.1895c, SMU.1896c and SMU.1906c) could be exported by NlmTE (SMU.286/287), the dedicated transporter of mutacin IV (*nlmA*/SMU.150 and less probably *nlmB*/SMU.151) as well as of mutacin V (*nlmC*/SMU.1914c) (Hale *et al*. 2005a, 2005b). The other transporters identified by BAGEL (SMU.1897/1898) and named CslAB, are dedicated to CSP (*comC*/SMU.1915) exportation (Petersen and Scheie 2000; Hale *et al*. 2005b). The rest of the BAGEL search results (potential or not significant score) grouped gene products as either CSP (*comC*/SMU.1915), or other hypothetical proteins, such as an acyl carrier protein (SMU.27) (**Table 6**).

Some of the many hypothetical peptides identified by BAGEL as putative bacteriocin-like gene products with a nearly or not significant hit score present homology to peptides from the bacteriocin database (http://bioinformatics.biol.rug.nl/bacteriocin/database.php). These peptides are SMU.419, that has homology to the halocin H4 precursor (AAB58316) (Cheung *et al*. 1997), and SMU.613 with homology to linocin M18 (CAE32973) (Valdes-Stauber and Scherer 1996). SMU.613 and SMU.616 align with the NlmA leader peptide and their best BlastP hit (NCBI data-

base) corresponds to the amphipatic pore-forming peptide precursor ThmA (peptide A from thermophilin 13) produced by *Streptococcus thermophilus* (Marciset *et al*. 1997). SMU. 1291c presents similarity with rhizobiocin RzcB (AAF 36414) produced by *Rhizobium leguminosarum* (Oresnik *et al*. 1999) (**Table 6**).

The BAGEL algorithm thus provides convenient genomic screening consolidating important bacteriocin characteristics and adds potential candidates for genes that could be involved in bacteriocin production for future study. Combining more than one search algorithm is however necessary in order to complement the screening procedures, as no one method is able to identify all potential bacteriocin-like genes.

DIVERSITY OF MUTACINS

The use of deferred antagonism tests to group mutans streptococci on the basis of their bacteriocin production had only limited success, largely due to the lack of reproducibility of the technique, the putative sensitive strains used for the test that differ between laboratories, and the fact that one strain can produce more than one mutacin.

The diversity of mutacins has been reassessed on the basis of genetic analysis among a plethora of *S. mutans* strains from various origins. Bekal Si Ali *et al*. (2002) were the first to confirm the diversity and heterogeneity of mutacins by a genotypic analysis using specific probes derived from mutacin B-Ny266 (1140/III) and mutacin II (J-T8). Among 24 type strains (Morency *et al*. 2001), only the mutacin-producing strains CM7, Ny266, and 22A hybridised with the probe from group B mutacins while mutacin-producing strains 29B, UA96 and T8 hybridised with the probe from group J. From the seven distinct clusters of mutacin-producing strains defined by Morency *et al*. (2001), five were not genetically linked to mutacins from groups B and J. The existence of mutacins not related to the known lantibiotics (nisin, gallidermin, epidermin, subtilin and lacticin 481 group) was thus supported (**Fig. 3**). The genetic diversity of mutacins was confirmed for 19 clinical isolates by Longo *et al*. (2003) with a low detection frequency of the mutacin II *mutA* gene. They also showed that the inhibitory spectra of distinct *S. mutans* genotypes were independent of the genetic similarities observed between the strains as measured by RAPD (Random Amplified Polymorphic DNA). Kamiya *et al*. (2005a) evaluated the relationship between genetic diversity measured by genotyping by AP-PCR (arbitrary primed-PCR) and mutacin production in 319 clinical isolates of *S. mutans*. They observed different inhibitory spectra for mutacin-producing strains whether they were isolated from individuals with or without caries. No correlation between mutacin inhibitory spectra and the overall genetic similarities of the producing strains was observed. A total of 101 distinct genotypes against 48 different mutacin activity spectra were found. *S. mutans* strains with identical genotypes could have distinct mutacinotypes and vice versa. Kamiya *et al*. (2005b) also reported a low frequency of detection of genes encoding mutacin types I (III), II and IV in 63 different genotypes of *S. mutans*. Similarly, Li *et al*. (2005) showed a low frequency of detection of the structural genes *mutAII* and *mutAIII* in 200 isolates of *S. mutans*. Waterhouse and Russell (2006) also reported a low frequency of detection of mutacin I, II and Smb biosynthetic operons amongst 39 isolates of *S. mutans*.

These studies confirm the existence of a high genetic polymorphism of BLIS-encoding genes in *S. mutans* strains isolated around the world. So far, the diversity of mutacins has been highlighted as representing 40% of the listed streptococcal antimicrobial peptides, a prolific genus for the production of bacteriocins (Nes *et al*. 2006).

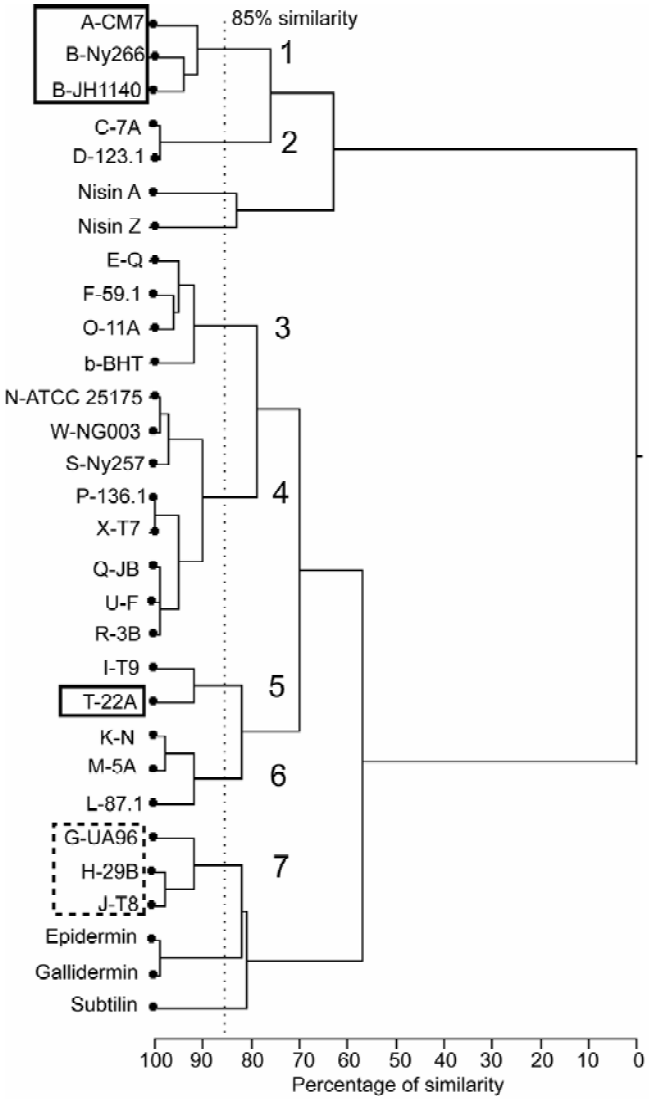

Fig. 3 Grouping of lantibiotic- or mutacin-producing strains according to the similarity of their activity spectra against pathogenic strains. In plain squares are mutacin-producing strains genetically related to those producing mutacins B-Ny266/1140/III. In dashed squares are the mutacin-producing strains genetically related to the strain producing mutacin II (J-T8). Clusters (1-7) are defined as mutacins with 85% or more similarity in their activity spectra against a panel of pathogenic bacteria (adapted from Morency *et al*. 1995, 2001; Bekal-Si Ali *et al*. 2002).

CONCLUDING REMARKS

Over the past five years, great progress has been made in bacteriocin research contributing to a better understanding of the molecular mechanism involved in their production, activity, structure-function relationships, and their biological functions. Some crucial studies have clarified the enzyme activity responsible for the modification reactions in the biosynthesis of lantibiotics and now *in vitro* synthesis of such molecules is conceivable (Xie *et al*. 2004; Li *et al*. 2006; Cheng *et al*. 2007). A more thorough understanding of the mechanisms involved in bacteriocin biosynthesis and immunity is also emerging (Cotter *et al*. 2005; Cheng *et al*. 2007; Diep *et al*. 2007). Bioinformatic and genomic analysis reveal evidence of BLIS diversity produced by *S. mutans*. Mutacins from class I (lantibiotics) and class II have been biochemically and genetically characterised while mutacins from class III and IV are, to date, only hypothetical. Coordination of the production of some mutacins and competence development exists in *S. mutans*. Mutacins exhibit activity against many Gram-positive bacteria, including multi-antibiotic-resistant pathogens and some Gram-negative pathogens (Mota-Meira *et al*. 2000, 2005). They not only represent efficient antimicrobial peptides produced by a human commensal bacterium, but also peptides governing important cellular processes. Continuing study of mutacins will contribute to understanding their important role in the ecology of *S. mutans*.

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