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Review Article

Role of *Streptococcus mutans* two-component systems in antimicrobial peptide resistance in the oral cavity☆

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Summary Approximately 100 trillion microorganisms exist in the oral cavity. For the commensal bacteria of the oral cavity, it is important to adapt to environmental stimuli, including human- or bacteria-derived antimicrobial agents. Recently, bacterial-specific signal transduction regulatory systems, called two-component systems (TCSs), which appear to be focused on sensing and adapting to the environment, were discovered. *Streptococcus mutans* is an oral commensal bacteria and is also known as a cariogenic bacteria. Although the virulence factors of *S. mutans* have been well demonstrated, the mechanism underlying the adaptation of the species to the oral cavity is poorly understood. *S. mutans* UA159 has 15 sets of TCSs. Among them, several have been demonstrated to be involved in acid tolerance, competence and biofilm formation. Recently, together with our findings, it was demonstrated that 5 TCSs were involved in resistance to antimicrobial agents. Furthermore, another TCS was associated with the production of bacteriocin. Six of 15 TCSs are associated with antimicrobial agents, implying that *S. mutans* can survive in the oral cavity by resisting various antimicrobial peptides.

In this review, we highlight the role of antimicrobial peptides in the oral cavity.
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☆ Scientific field of dental sciences: oral bacteriology.

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Contents

1. Introduction

Approximately 100 trillion microorganisms exist in the human body; however, little information about the role and character of these commensal bacteria has been reported.

Recently, it was proposed that the human body establishes a symbiotic relationship that contributes to the maintenance of immune function by providing a niche for commensal bacteria called as microbiome [1—3]. Currently, the study of microbiomes in the human body is progressing via comprehensive gene analyses, using samples collected from saliva, skin, and feces [4—6]. Because the constitution patterns of the intestinal flora are of an infinite variety among humans, microbiomes may affect human physical and mental health because of their diversity [3,7,8]. It can be said that the study of microbiomes has changed the traditional concepts of microbial research.

In the process of indigenous flora formation, it is necessary to colonize the host by adapting to external stresses, including the immune system. Furthermore, bacteria must also compete with other commensal bacteria.

In the oral cavity, several hundreds of bacterial species exist, forming a complex bacterial floral community [9]. Oral flora, as well as intestinal flora, are also expected to exert various effects on the host. Although many studies of oral bacteria have focused on the pathogenesis of cariogenic bacteria and periodontal bacteria, future research on the association between oral flora and the host has attracted attention.

We first focused on *Streptococcus mutans*, a cariogenic bacteria, and sought to elucidate the colonization mechanism of this organism in the oral cavity. *S. mutans* plays a key role in the formation of dental plaque, as well as in tooth decay, via the production of acids [10—12]. Although the mechanism underlying caries development due to *S. mutans* is known, it is not clear why this bacterium is able to reside in the oral cavity. There are numerous antimicrobial agents in the oral cavity, and resistance to these antimicrobial agents is largely responsible for the colonization of *S. mutans*. To form the biofilm (dental plaque) physical barrier to resist the antimicrobial agents. Furthermore, bacteria including *S. mutans* possess two-component systems (TCSs) which sense the environmental stimuli including antimicrobial agents and regulate the expressions of several factors to be responsible for the adaptation to the stimuli.

Two-component systems are prokaryote-specific signal transduction systems that comprise a sensory histidine kinase (HK) and a cognate response regulator (RR) [13]. The sensory HK undergoes autophosphorylation of a histidine residue in response to an environmental signal and relays the phosphate group to an aspartic acid residue on the cognate RR [14,15]. The phosphorylated RR then binds to target DNA elements with strong affinity, activating or repressing transcription of target genes (Fig. 1). We focused on the relationship between TCSs and resistance to antimicrobial peptides. Through these results, we have gained new knowledge of the above phenomena.

In this review, we present an overview of antimicrobial peptides and TCSs, including the results of our study regarding the acquisition of resistance mechanisms in *S. mutans* against human- and bacteria-derived antimicrobial peptides.

2. Human-derived antimicrobial peptides

In the oral cavity, there are many antimicrobial factors, such as antimicrobial peptides, lysozymes, hydrogen peroxide and lactoferrin [16,17]. Among these antibacterial factors, antimicrobial peptides are believed to have bactericidal activity against various oral bacteria, including cariogenic and periodontopathogenic bacteria [17]. Human-derived antimicrobial peptides originate from various sources, such as the saliva, gingival epithelium, mucosa, neutrophils and gingival crevicular fluid [18,19]. These peptides are considered the first defense against bacterial infections as a form of innate immunity. Fig. 2 shows the varieties of human-derived antimicrobial peptides.

2.1. Defensins

Defensins have three disulfide bonds among 6 cysteines in peptides, which distinguishes them from other antimicrobial

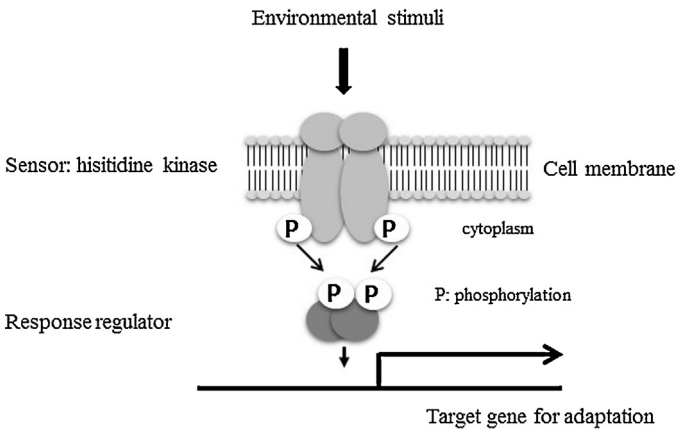

Figure 1 Scheme of the two-component system. The sensory histidine kinase undergoes autophosphorylation of a histidine residue in response to an environmental signal and relays the phosphate group to an aspartic acid residue on the cognate response regulator (RR). The phosphorylated RR then binds to target DNA elements with strong affinity, activating or repressing the transcription of target genes.

Group I: linear, α-helical peptides without cysteins

LL37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES

Group II: peptides with cysteins linked by disulfide bridges

α-defensins

HNP1	ACYCRIPACIAGERRYGTCIYQGRLWAFCC
HNP2	CYCRIPACIAGERRYGTCIYQGRLWAFCC
HNP3	DCYCRIPACIAGERRYGTCIYQGRLWAFCC
HNP4	VCSCRLVFCRRTELRVGNCLIGGVSFTYCCTRV
HNP5	ATCYCRTGRCATRESLSGVCEISGRLYRLCCR
HNP6	FTCHCRR-SCYSTEYSYGTCTVMGINHRFCCL
	-C-C----C---------C---------CC

ß-defensins

HBD1	GLGHRSDHYNCVSSGGQCLYSACPIFTKIQGTCYRGKAKCCK
HBD2	GIGDPVTCLKSGAICHPVFCPRRYKQIGTCGLPGTKCCKKP
HBD3	GIINTLQKYYCRVRGGRCAVLSCLPKEEQIGKCSTRGRKCCRRKK
HBD4	EFELDRICGYGTARC-RKKCRSQEYRIGRCP-NTYACCLRKWDESLLNRTKP
	-------C------C----C---------C------CC---

Group III: unusual high proportion of specific amino acids

Histatin-1	DSHEKRHHGYRRKFHEKHHSHREFPFYGDYGSNYLYDN
Histatin-3	DSHAKRHHGYRRKFHEKHHSHRGYRSNYLYDN

Figure 2 Antimicrobial peptides in humans. LL37 has a linear form. Defensins produced by humans are classified into two types: alpha- and beta-defensins. Defensins have three disulfide bonds among 6 cysteines in peptides. Histatins are salivary antimicrobial peptides and are a family of histidine-rich cationic peptides produced by parotid and salivary duct cells. Histatins are known to have antifungal activity. Histatin 5, in particular, has strong activity against fungi, including *Candida* species.

peptides. Defensins are further classified into three types: alpha-, beta- and theta-defensins, although theta defensins are not expressed in humans [18,20,21]. Differences in producing cells and the arrangement of the 3 disulfide bonds distinguish alpha- and beta- defensins.

Several alpha- defensins (human neutrophil defensin: HNP) have been identified. HNP1-4 is mainly localized in the granules of neutrophils, whereas HNP5 and 6 are localized in the Paneth cells of the small intestine [22]. HNPs demonstrate broad antimicrobial activity against Gram-positive and -negative bacteria, fungi and viruses. The activity of these peptides is decreased by high NaCl concentrations. In addition to antimicrobial activity, several HNPs are reported to have chemotactic activity as cytokines.

To date, 4 beta-defensins (human neutrophil defensing 1—4: HBD1-4) have been reported. These peptides are expressed in various epithelial tissues, such as the skin, trachea, gingiva and saliva [22—25]. In vitro experiments, HBD1 are constitutively expressed, while HBD2 and HBD3 are inducible [23—28]. Additionally, HBD1 and HBD2 have been demonstrated to have strong activity against Gram-negative bacteria, but not Gram-positive bacteria, while HBD3 and HBD4 have strong activity against both types of

Table 1 Classification of bacteriocins.

Class	Characteristics	Representative bacteriocins
I	Lantibiotics, small (<5 kDa) heat-stable peptides containing unsaturated amino acids, lanthionine and 3-methyllanthionine	
AI	More elongated peptides than Type-AII	Nisin, mutacin I, II, III, 1140, streptin
AII	A linear N-terminus and a globular C-terminus	Lacticin 481, nukacin ISK-1, salivaricin, mutacin K8
B	Globular peptide	Mersacidin, cinnamycin
others	Two peptide lantibiotics	Lacticin 3147, staphylococcin C55, Smb
II	Small (<10 kDa) heat-stable peptides formed by unmodified amino acids	
IIa	Anti-listerial peptides with a consensus sequence of YGNGVXC	Pediocin PA-1, Enterocin A
IIb	Two-peptide bactericins	Lactococcin G, Lactococcin Q, Enterocin 1071
IIc	Other bacteriocins	Enterocin B, Lactococcin A
III	High-molecular-weight (>30kDa), heat-labile proteins	Helveticin J, enterolysin A
IV	Complex bacteriocins containing lipid or carbohydrate moieties	Leuconocin S, Lactocin 27
V	Circular peptides	Enterocin AS-48, Lactocyclicin Q

bacteria [29—32]. Like HNPs, HBDs also have chemotactic activity against human cells [33,34].

2.2. Cathelicidins

The cathelicidin family features characteristic conserved domains known as cathelins (cathepsin inhibitors) in their N-terminal and variable regions and C-termini with antimicrobial activity. In humans, only one cathelicidin, known as LL37, has been identified [19,35]. LL37 is found in various cells, such as neutrophils, monocytes and various epithelial cells [35]. Unlike defensins, LL37 has no disulfide bonds and has a linear form. LL37 has strong antibacterial activity against Gram-negative and -positive bacteria [19,36]. LL37 is also known as LPS neutralizing factor, which binds LPS and inhibits endotoxin activity [19,37]. Moreover, LL37 has chemotactic activity against various cells, such as neutrophils, monocytes and mast cells [38].

2.3. Histatins

Histatins are salivary, cationic antimicrobial peptides and are a histidine-rich family produced by parotid and salivary duct cells [39]. Histatins are known to have antifungal activity. In particular, histatin 5 has strong activity against fungi, including Candida species [40].

3. Bacteria-derived antimicrobial peptides (bacteriocins)

The antibacterial agents produced by bacteria are called bacteriocins. Bacteriocins are ribosomally synthesized peptides, or proteins that exhibit antibacterial activity, mostly against bacterial species that are closely related to the bacteriocin producer [41,42]. Bacteriocins are mainly classified into classes I and II [43]. Class I bacteriocins (peptides <5 kDa) are called ''lantibiotics'' and contain a ring bridged by lanthionine and 3-methyllanthionine residues [44], whereas class II bacteriocins comprise unmodified amino acids [45]. Lantibiotics are further classified to Type A-I, Type A-II, and Type B by view of their similarities to peptides with established structures [43]. Table 1 shows the list of lantibiotics. Many bacteria produce bacteriocins to ensure their survival in this community [42,46]. To persist in the oral cavity, bacteria must possess several factors to resist not only host immune factors but also bacteriocins from other microfloral species. The mode of action of bacteriocins is believed to involve pore formation in the cell membrane or inhibition of cell wall synthesis. Fig. 3 shows the antibacterial mechanism of nisin A, which is a typical bacteriocin produced by *Lactococcus lactis*. Nisin A can bind to the lipid II moiety, which is related to the biosynthesis of cell wall peptidoglycan [42,43]. Therefore, the mode of action of nisin A is pore formation and inhibition of cell wall synthesis.

4. Two-component systems

4.1. General characteristic of TCSs

Recently, TCSs were reported to be associated with resistance to antibacterial agents, such as human- or bacteria-derived antimicrobial peptides. bacteria are able to quickly adapt to the external environment by regulating gene expression. Generally, bacteria possess multiple TCSs. It is believed that TCSs are involved in the adaptation to external stimuli, such as osmotic pressure, pH and temperature and virulence factor expression.

4.2. *S. mutans* TCSs

S. mutans UA159 possesses 15 sets of TCSs (Table 2). Genome analysis of several *S. mutans* strains showed that more than 12 TCSs are conserved among *S. mutans* strains [47]. Each

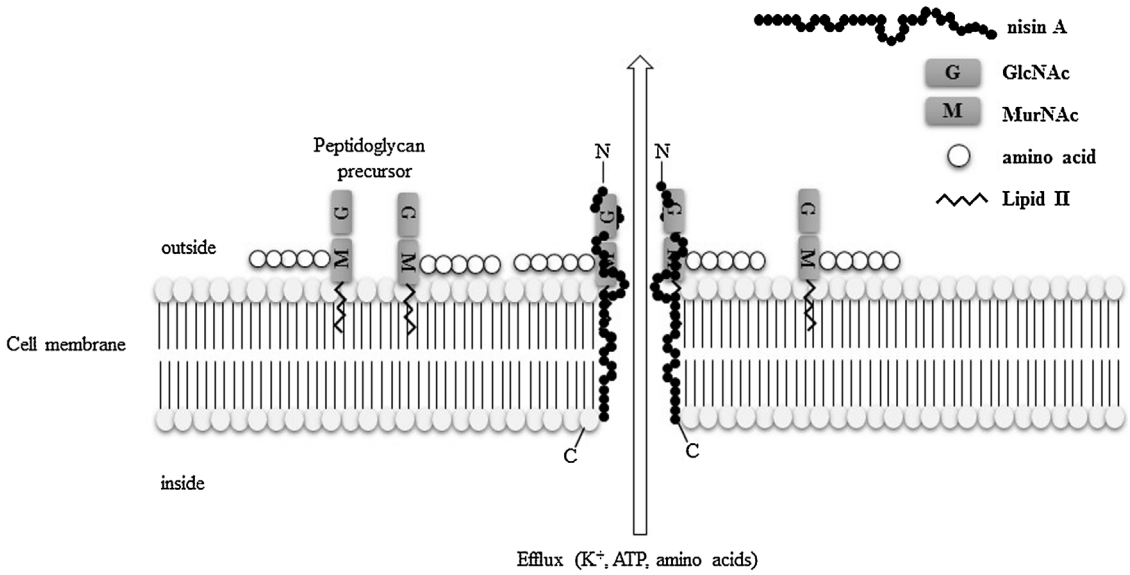

Figure 3 Antibacterial mechanism of nisin A. Nisin A interferes with cell wall biosynthesis and forms complexes with lipid I and lipid II. Subsequently, nisin affects the cytoplasmic membrane of susceptible bacteria and is able to form short-lived pores in the cell membrane. This effect leads to an efflux of small molecules (potassium, ATP, and amino acids) and dissipation of the membrane potential, resulting in the arrest of all cellular biosynthesis.

Table 2 Two-component systems in *S. mutans* UA159.

Gene ID	Gene name	Function
SMU_486-487	*liaRS*	Envelope stress, acid torelance, biofilm formation
SMU_577-576	unassigned	Unknown
SMU_660-659	*nsrRS*	Nisin resistance
SMU_928-927	unassigned	Low nutrition
SMU_1009-1008	*bceRS*	Bacitracin resistance
SMU_1037-1038	unassigned	Unknown
SMU_1128-1129	*ciaRH*	Acid tolerance,competence, resistance against cationic agents
SMU_1145-1146	*lcrRS*	Nukacin resistance
SMU_1516-1517	*vicRK*	Biofilm, oxidative stress
SMU_1548-1547	unassigned	Unknown
SMU_1814-1815	unassigned	Oxidative stress
SMU_1916-1917	*comDE*	MutacinIV production, competency
SMU_1965-1964	unassigned	Unknown
SMU_1924	*gcrR*	GbpC expression, biofilm formation
SMU_45-46	unassigned	Oxidative Stress

TCS is believed to have a role; however, the functions of some TCSs are unknown. ComDE is known as a quorum sensing system, which is a sensor of cell density in biofilm and is also related to competence [48,49]. VicRK is also conserved among many bacterial species and is related to virulence [50,51]. Due to the characteristics of *S. mutans*, the relationship between TCSs and biofilm formation and acid tolerance has been well-demonstrated [50,52—54]. To date, several TCSs have been reported to be associated with acid tolerance and biofilm formation, although the precise mechanism underlying these linkages has not been elucidated. Regarding antimicrobial agents, it was reported that ComDE (SMU. 1916—17) is associated with production of the bacteriocin known as mutacin IV [48].

5. Association of TCS with antimicrobial susceptibility in *S. mutans*

5.1. Resistance to human antimicrobial agents

First, we evaluated susceptibility to LL37 and β-defensins using 15 sets of TCS knockout mutants. As a result of this comprehensive analysis, one TCS (*ciaRH*) knockout mutant showed increased susceptibility to LL37 and beta-defensins (HBD1-3) in biofilm cells [55]. Further experiments showed that CiaRH regulates the *dlt* operon, which plays an important role in the acquisition of resistance to positively-charged antimicrobial agents, such as LL37 and β-defensins. In biofilm cells, *dlt* expression in a *ciaRH* knock-out mutant

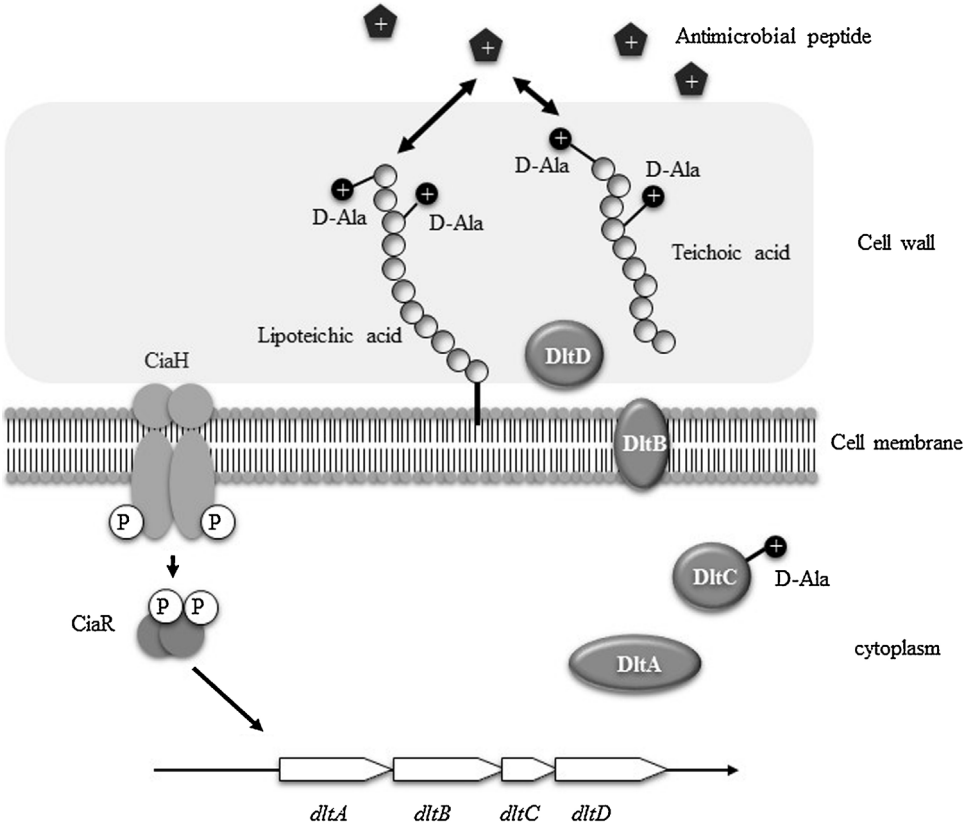

Figure 4 Scheme of the resistance mechanism against antimicrobial peptides via the CiaRH-Dlt system in *S. mutans*. CiaRH is related to the expression of *dlt* in biofilm cells and contributes to resistance to positively-charged antibacterial agents by weakening thenegative charge of the cell surface.

was decreased compared with the wild type. Normally, the bacterial cell surface is negatively charged, and positively charged antimicrobial peptides are attracted to the membrane. Dlt was reported to be a major contributor to the decreased negative charge of cell surfaces because the above factors are associated with the addition of alanine to teichoic acids [56—58]. Alanylation of teichoic acids in the cell wall conveys a positive charge to the bacterial cell surface, which results in a shift to a weak negative charge on the cell surface [57,58]. Our results suggest that CiaRH is related to the expression of *dlt* in biofilm cells and contributes to resistance to positively-charged antibacterial agents by weakening the negative change of the cell surface (Fig. 4).

5.2. Resistance to bacteria-derived antimicrobial agents (bacteriocins)

The susceptibility of the *S. mutans* wild type and its TCS knockout mutants was examined by direct methods using a variety of bacteriocin-producing bacteria. The susceptibility of three class I bacteriocins, including nisin A, nukacin ISK-1 and lacticin 481, and 3 class II bacteriocins, including types IIa-IIc, was evaluated. As a result, the knockouts of two unidentified TCSs (designated NsrRS and LcrRS) showed decreased susceptibility to bacteriocins, while the other mutants showed no change [59]. The knockout of the genes SMU.658—59, designated *nsrRS*, caused increased susceptibility to nisin A, a class I lantibiotic produced by *L. lactis*. In *nsrRS* mutants, the gene designated *nsrX* was not increased by the addition of nisin A, while *nsrX* expression was increased by nisin A in the wild type. Finally, we demonstrated that NsrX was expressed on the cell membrane and had binding affinity for nisin A, causing the trapping of nisin A to inhibit binding of nisin A to the target (Fig. 5).

The knockout of the genes SMU.1146—47, designated *lcrRS*, caused increased susceptibility to nukacin ISK-1, which is a class I lantibiotic produced by *Staphylococcus warneri*. The expression of the ABC transporter *lctFEG*, which is located downstream of *lcrRS*, was inhibited by exposing nukacin ISK-1 in In *lcrRS* knockout mutants. This result suggested that the expression of LctFEG was induced by nukacin ISK-1 via LcrRS (Fig. 5). Therefore, the functions of NsrX and LctFEG are thought to be different: NsrX binds nisin A and captures nisin A to avoid attachment to the cell membrane; however, LctFEG may export nukacin ISK-1. In other reports, BraRS and LiaRS were demonstrated to be involved in bacitracin and vancomycin susceptibility, respectively [60,61]. Also, ComDE was reported to be involved in Mutacin IV Production [48]. Therefore, totally 5 TCSs are associated with bacteriocin.

Deng et al. reported that VicRK, one of the TCSs in *S. mutans*, was associated with hydrogen peroxide (H_2O_2) stress [62]. Although this result was reconfirmed in our study, it is unclear which resistant factors are regulated by VicRK. It is known that *Streptococcus sanguinis* produces H_2O_2. This

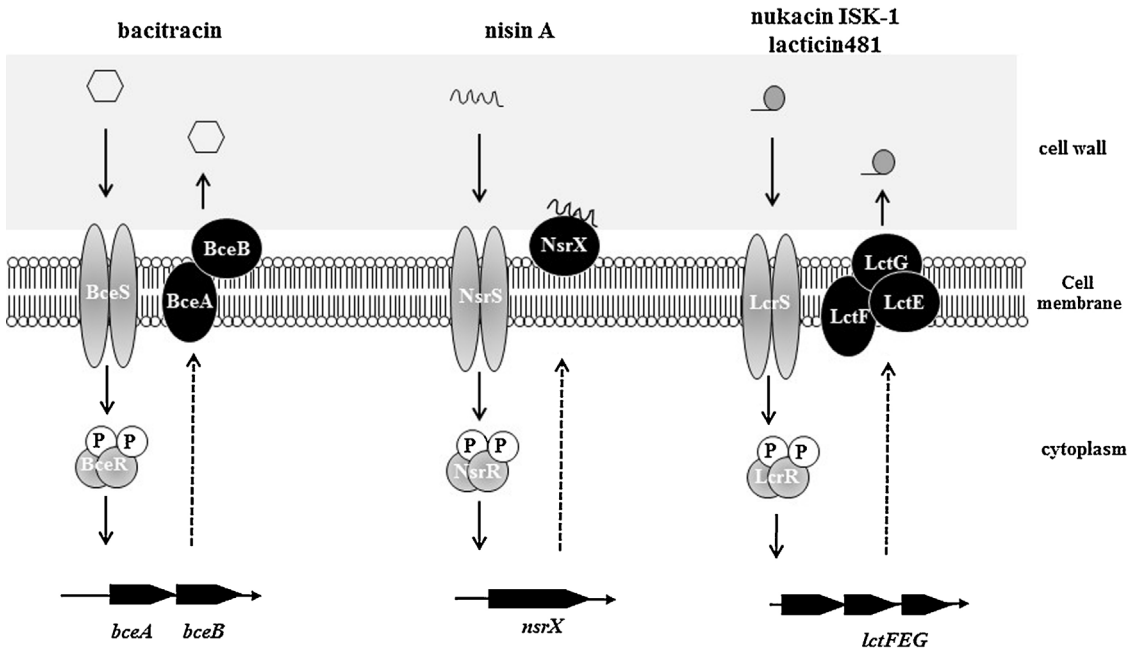

Figure 5 Proposed bacteriocin resistance mechanism mediated by TCSs in *S. mutans*. BceRS is related to bacitracin resistance by regulating BceAB ABC transporter. NsrRS is related to nisin A resistance by regulatiing NsrX which has ability to bind nisin A, suggesting trap nisin A. LcrRS is related to nukacin ISK-I and lacticin 481 resistance by regulating LctFEG ABC transporter.

bacteria is one of the major commensal bacteria in the oral cavity. We expected that VicRK systems in *S. mutans* play an important role in resisting the H_2O_2 produced by *S. sanguinis*.

5.3. Bacteriocin affects the proportion of bacteria when two bacteria are co-cultured

To determine whether bacteriocins are important for persistent survival in complex bacterial communities, we established a co-culture assay to evaluate each bacterial proportion when two bacterial strains were mixed and cultured [59]. When wild type *S. mutans* was co-cultured with bacteriocin-producing or nonproducing strains, the proportion of *S. mutans* co-cultured with bacteriocin-producing strains was decreased compared to that co-cultured with bacteriocin-nonproducing strains. Additionally, when the respective TCS mutants were co-cultured with bacteriocin-producing strains, the proportion was significantly decreased compared to the WT. These results indicate that bacteriocin and TCS-mediated bacteriocin resistance are important for survival in complex bacterial communities.

6. Conclusions

In our study, 3 of the 15 TCSs identified in *S. mutans* were demonstrated to be associated with resistance to human- or bacteria-derived antimicrobial peptides [55,59]. Furthermore, other researchers reported that 3 additional TCSs were associated with production of or resistance to antibacterial agents [48,60,61].

In particular, four TCSs have been identified as bacteriocin-related factors (Figs. 4 and 5). These results highlight the role of bacteriocins in the interactions among different species of oral bacteria and the importance of TCSs in these interactions. In the oral cavity, TCSs play an important role in acquiring resistance to human- or bacteria-derived antimicrobial agents.

It is suggested that bacterial TCSs may be key factors in understanding the colonization mechanisms of commensal bacteria in humans. In recent years, it has been reported that intestinal bacterial microbiomes have a large impact on human health. In the process of microbiome formation, we considered that TCSs play an important role in adapting to the environmental stimuli that arise from interactions between bacteria and the human body. In the future, we expect to examine whether TCSs are involved in microbiome formation.

In addition, in this study, it was suggested that antimicrobial factors, including human- and bacteria-derived antimicrobial peptides, are strongly associated with commensal flora formation, including oral flora formation. Further examinations are needed to analyze microbiome formation.

Conflicts of interest

None.

References

[1] Lee YK, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? Science 2010;330(6012):1768–73.

[2] Jones ML, Ganopolsky JG, Martoni CJ, Labbé A, Prakash S. Emerging science of the human microbiome. Gut Microbes 2014;5(4):446—57.
[3] Van Praet JT, Donovan E, Vanassche I, Drennan MB, Windels F, Dendooven A, et al. Commensal microbiota influence systemic autoimmune responses. EMBO J 2015;34(4):466—74.
[4] Shanahan F. The colonic microbiota in health and disease. Curr Opin Gastroenterol 2013;29(1):49—54.
[5] Wade WG. The oral microbiome in health and disease. Pharmacol Res 2013;69(1):137—43.
[6] Chen YE, Tsao H. The skin microbiome: current perspectives and future challenges. J Am Acad Dermatol 2013;69(1):143—55.
[7] Hsiao EY, McBride SW, Hsien S, Sharon G, Hyde ER, McCue T, et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. Cell 2013;155(7):1451—63.
[8] Ridaura V, Belkaid Y. Gut microbiota: the link to your second brain. Cell 2015;161(2):193—4.
[9] Grice EA, Segre JA. The human microbiome: our second genome. Annu Rev Genom Hum Genet 2012;13:151—70.
[10] Hamada S, Slade HD. Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiol Rev 1980;44(2):331—84.
[11] Takahashi N, Nyvad B. The role of bacteria in the caries process: ecological perspectives. J Dent Res 2011;90(3):294—303.
[12] Hamada S, Slade HD. Biology, immunology, and cariogenicity of *Streptococcus mutans*. Microbiol Rev 1980;44:331—84.
[13] Mitrophanov AY, Groisman EA. Signal integration in bacterial two-component regulatory systems. Genes Dev 2008;22:2601—11.
[14] Rampersaud A, Harlocker SL, Inouye M. The OmpR protein of *Escherichia coli* binds to sites in the ompF promoter region in a hierarchical manner determined by its degree of phosphorylation. J Biol Chem 1994;269:12559—66.
[15] Hoch JA. Two-component and phosphorelay signal transduction. Curr Opin Microbiol 2000;3:165—70.
[16] Tenovu J, Lumikari M, Soukka T. Salivary lysozyme, lactoferrin and peroxidases: antibacterial effects on cariogenic bacteria and clinical applications in preventive dentistry. Proc Finn Dent Soc 1991;87:197—208.
[17] Dale BA, Fredericks LP. Antimicrobial peptides in the oral environment: expression and function in health and disease. Curr Issues Mol Biol 2005;7(2):119—33.
[18] Ganz T, Lehrer RI. Defensins. Pharmacol Ther 1995;66:191—205.
[19] Larrick JW, Hirata M, Balint RF, Lee J, Zhong J, Wright SC. Human CAP18: a novel antimicrobial lipopolysaccharide-binding protein. Infect Immun 1995;63:1291—7.
[20] Ganz T, Selsted ME, Szklarek D, Harwig SS, Daher K, Bainton DF, et al. Defensins. Natural peptide antibiotics of human neutrophils. J Clin Invest 1985;76:1427—35.
[21] Cunliffe RN. Alpha-defensins in the gastrointestinal tract. Mol Immunol 2003;40:463—7.
[22] Mathews M, Jia HP, Guthmiller JM, Losh G, Graham S, Johnson GK, et al. Production of beta-defensin antimicrobial peptides by the oral mucosa and salivary glands. Infect Immun 1999;67(6):2740—5.
[23] Diamond G, Zasloff M, Eck H, Brasseur M, Maloy WL, Bevins CL. Tracheal antimicrobial peptide, a cysteine rich peptide from mammalian tracheal mucosa: peptide isolation and cloning of a cDNA. Proc Natl Acad Sci U S A 1991;88:3952—6.
[24] Stolzenberg ED, Anderson GM, Ackermann MR, Whitlock RH, Zasloff M. Epithelial antibiotic induced in states of disease. Proc Natl Acad Sci U S A 1997;94:8686—90.
[25] Krisanaprakornkit S, Weinberg A, Perez CN, Dale BA. Expression of the peptide antibiotic human beta defensin 1 in cultured gingival epithelial cells and gingival tissue. Infect Immun 1998;66:4222—8.
[26] Valore EV, Park CH, Quayle AJ, Wiles KR, McCray Jr PB, Ganz T. Human beta-defensin-1: an antimicrobial peptide of urogenital tissues. J Clin Invest 1998;101:1633—42.
[27] Krisanaprakornkit K, Kimball JR, Weinberg A, Darveau RP, Bainbridge BW, Dale BA. Inducible expression of human β-defensin-2 (hBD-2) by *Fusobacterium nucleatumin* oral epithelial cells: multiple signaling pathways and the role of commensal bacteria in innate immunity and the epithelial barrier. Infect Immun 2000;68:2907—15.
[28] Harder J, Bartels J, Christophers E, Schroder JM. Isolation and characterization of human betadefensin-3, a novel human inducible peptide antibiotic. J Biol Chem 2001;276:5707—13.
[29] Harder J, Bartels J, Christophers E, Schröder JM. A peptide antibiotic from human skin. Nature 1997;387(6636):861.
[30] Hoover DM, Rajashankar KR, Blumenthal R, Puri A, Oppenheim JJ, Chertov O, et al. The structure of human beta-defensin-2 shows evidence of higher order oligomerization. J Biol Chem 2000;275(42):32911—8.
[31] Hoover DM, Chertov O, Lubkowski J. The structure of human beta-defensin-1: new insights into structural properties of beta-defensins. J Biol Chem 2001;276(42):39021—6.
[32] Komatsuzawa H, Ouhara K, Kawai T, Yamada S, Fujiwara T, Shiba H, et al. Susceptibility of periodontopathogenic and cariogenic bacteria to defensins and potential therapeutic use of defensins in oral diseases. Curr Pharm Des 2007;13(30):3084—95.
[33] Niyonsaba F, Someya A, Hirata M, Ogawa H, Nagaoka I. Evaluation of the effects of peptide antibiotics human beta-defensins-1/-2 and LL-37 on histamine release and prostaglandin D(2) production from mast cells. Eur J Immunol 2001;31(4):1066—75.
[34] Yang D, Biragyn A, Kwak LW, Oppenheim JJ. Mammalian defensins in immunity: more than just microbicidal. Trends Immunol 2002;23(6):291—6.
[35] Frohm Nilsson M, Sandstedt B, Sorensen O, Weber G, Borregaard N, Stahle-Backdahl M. The human cationic antimicrobial protein (hCAP18), a peptide antibiotic, is widely expressed in human squamous epithelia and colocalizes with interleukin-6. Infect Immun 1999;67:2561—6.
[36] Zaiou M, Gallo RL. Cathelicidins, essential gene-encoded mammalian antibiotics. J Mol Med (Berl) 2002;80(9):549—61.
[37] Nagaoka I, Hirota S, Niyonsaba F, Hirata M, Adachi Y, Tamura H, et al. Augmentation of the lipopolysaccharide-neutralizing activities of human cathelicidin CAP18/LL-37-derived antimicrobial peptides by replacement with hydrophobic and cationic amino acid residues. Clin Diagn Lab Immunol 2002;9(5):972—82.
[38] Tokumaru S, Sayama K, Shirakata Y, Komatsuzawa H, Ouhara K, Hanakawa Y, et al. Induction of keratinocyte migration via transactivation of the epidermal growth factor receptor by the antimicrobial peptide LL-37. J Immunol 2005;175(7):4662—8.
[39] Troxler RF, Offner GD, Xu T, Vanderspek JC, Oppenheim FG. Structural relationship between human salivary histatins. J Dent Res 1990;69(1):2—6.
[40] Oppenheim FG, Xu T, McMillian FM, Levitz SM, Diamond RD, Offner GD, et al. Histatins, a novel family of histidine-rich proteins in human parotid secretion. Isolation, characterization, primary structure, and fungistatic effects on *Candida albicans*. J Biol Chem 1988;263(16):7472—7.
[41] Jack RW, Tagg JR, Ray B. Bacteriocins of gram-positive bacteria. Microbiol Rev 1995;59:171—200.
[42] Nissen-Meyer J, Nes IF. Ribosomally synthesized antimicrobial peptides: their function, structure, biogenesis, and mechanism of action. Arch Microbiol 1997;167:67—77.

[43] Bierbaum G, Sahl HG. Lantibiotics: mode of action, biosynthesis and bioengineering. Curr Pharm Biotechnol 2009;10:2—18.
[44] Nagao J, Asaduzzaman SM, Aso Y, Okuda K, Nakayama J, Sonomoto K. Lantibiotics: insight and foresight for new paradigm. J Biosci Bioeng 2006;102:139—49.
[45] Nes IF, Holo H. Class II antimicrobial peptides from lactic acid bacteria. Biopolymers 2000;55:50—61.
[46] Cotter PD, Hill C, Ross RP. Bacteriocins: developing innate immunity for food. Nat Rev Microbiol 2005;3:777—88.
[47] Song L, Sudhakar P, Wang W, Conrads G, Brock A, Sun J, et al. A genome-wide study of two-component signal transduction systems in eight newly sequenced mutans streptococci strains. BMC Genom 2012;4(13):128.
[48] van der Ploeg JR. Regulation of bacteriocin production in *Streptococcus mutans* by the quorum-sensing system required for development of genetic competence. J Bacteriol 2005;187(12):3980—9.
[49] Lemme A, Gröbe L, Reck M, Tomasch J, Wagner-Döbler I. Subpopulation-specific transcriptome analysis of competence-stimulating-peptide-induced *Streptococcus mutans*. J Bacteriol 2011;193(8):1863—77.
[50] Senadheera MD, Guggenheim B, Spatafora GA, Huang YC, Choi J, Hung DC, et al. A VicRK signal transduction system in *Streptococcus mutans* affects gtfBCD, gbpB, and ftf expression, biofilm formation, and genetic competence development. J Bacteriol 2005;187(12):4064—76.
[51] Senadheera DB, Cordova M, Ayala EA, Chávez de Paz LE, Singh K, Downey JS, et al. Regulation of bacteriocin production and cell death by the VicRK signaling system in *Streptococcus mutans*. J Bacteriol 2012;194(6):1307—16.
[52] Ahn SJ, Burne RA. Effects of oxygen on biofilm formation and the AtlA autolysin of *Streptococcus mutans*. J Bacteriol 2007;189(17):6293—302.
[53] Lévesque CM, Mair RW, Perry JA, Lau PC, Li YH, Cvitkovitch DG. Systemic inactivation and phenotypic characterization of two-component systems in expression of *Streptococcus mutans* virulence properties. Lett Appl Microbiol 2007;45(4):398—404.
[54] Liu Y, Burne RA. Multiple two-component systems modulate alkali generation in *Streptococcus gordonii* in response to environmental stresses. J Bacteriol 2009;191(23):7353—62.
[55] Mazda Y, Kawada-Matsuo M, Kanbara K, Oogai Y, Shibata Y, Yamashita Y, et al. Association of CiaRH with resistance of *Streptococcus mutans* to antimicrobial peptides in biofilms. Mol Oral Microbiol 2012;27(2):124—35.
[56] Peschel A, Otto M, Jack RW, Kalbacher H, Jung G, Götz F. Inactivation of the *dlt* operon in *Staphylococcus aureus* confers sensitivity to defensins, protegrins, and other antimicrobial peptides. J Biol Chem 1999;274:8405—10.
[57] Peschel A, Jack RW, Otto M, Collins LV, Staubitz P, Nicholson G, et al. *Staphylococcus aureus* resistance to human defensins and evasion of neutrophil killing via the novel virulence factor MprF is based on modification of membrane lipids with L-lysine. J Exp Med 2001;193:1067—76.
[58] Collins LV, Kristian SA, Weidenmaier C, Faigle M, Van Kessel KP, Van Strijp JA, et al. *Staphylococcus aureus* strains lacking D-alanine modifications of teichoic acids are highly susceptible to human neutrophil killing and are virulence attenuated in mice. J Infect Dis 2002;186:214—9.
[59] Kawada-Matsuo M, Oogai Y, Zendo T, Nagao J, Shibata Y, Yamashita Y, et al. Involvement of the novel two-component NsrRS and LcrRS systems in distinct resistance pathways against nisin A and nukacin ISK-1 in *Streptococcus mutans*. Appl Environ Microbiol 2013;79(15):4751—5.
[60] Suntharalingam P, Senadheera MD, Mair RW, Lévesque CM, Cvitkovitch DG. The LiaFSR system regulates the cell envelope stress response in *Streptococcus mutans*. J Bacteriol 2009;191(9):2973—84.
[61] Ouyang J, Tian XL, Versey J, Wishart A, Li YH. The BceABRS four-component system regulates the bacitracin-induced cell envelope stress response in *Streptococcus mutans*. Antimicrob Agents Chemother 2010;54(9):3895—906.
[62] Deng DM, Liu MJ, ten Cate JM, Crielaard W. The VicRK system of *Streptococcus mutans* responds to oxidative stress. J Dent Res 2007;86(7):606—10.