J Infect Chemother 23 (2017) 634–641

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Original Article

Inhibiting effects of fructanase on competence-stimulating peptide-dependent quorum sensing system in *Streptococcus mutans*

Yusuke Suzuki [a, b], Ryo Nagasawa [b], Hidenobu Senpuku [b, *]

[a] *Department of Maxillofacial Surgery, Nihon University Graduate School of Dentistry at Matsudo, Chiba, Japan*
[b] *Department of Bacteriology I, National Institute of Infectious Diseases, Tokyo, Japan*

A R T I C L E I N F O

Article history:
Received 12 October 2016
Received in revised form
22 May 2017
Accepted 16 June 2017
Available online 17 July 2017

Keywords:
Streptococcus mutans
Biofilm formation
Quorum sensing system
Competence-stimulating peptide
Fructanase

A B S T R A C T

Streptococcus mutans produces glucosyltransferases encoded by the *gtfB* and *gtfC* genes, which synthesize insoluble glucan, and both insoluble and soluble glucans by conversion of sucrose, and are known as principal agents to provide strong biofilm formation and demineralization on tooth surfaces. *S. mutans* possess a Com-dependent quorum sensing (QS) system, which is important for survival in severe conditions. The QS system is stimulated by the interaction between ComD {Receptor to competence-stimulating peptide (CSP)} encoded by the *comD* and CSP encoded by the *comC*, and importantly associated with bacteriocin production and genetic competence. Previously, we found enzyme fructanase (FruA) as a new inhibitor for the glucan-dependent biofilm formation. In the present study, inhibiting effects by FruA on glucan-independent biofilm formation of *S. mutans* UA159, UA159.*gtfB*$^-$, UA159.*gtfC*$^-$, and UA159.*gtfBC*$^-$ were observed in sucrose and no sucrose sugars-supplemented conditions using the plate assay. The reduction of UA159.*comC*$^-$ and UA159.*comD*$^-$ biofilm formation were also observed as compared with UA159 in same conditions. These results suggested that inhibitions of glucan-independent and Com-dependent biofilm formation were involved in the inhibiting mechanism by FruA. To more thoroughly investigate effects by FruA on the QS system, we examined on CSP-stimulated and Com-dependent bacteriocin production and genetic transformation. FruA inhibited bacteriocin production in collaboration with CSP and genetic transformation in bacterial cell conditions treated with FruA. Our findings show that FruA has multiple effects that inhibit survival functions of *S. mutans*, including biofilm formation and CSP-dependent QS responses, indicating its potential use as an agent for prevention of dental caries.

© 2017 Japanese Society of Chemotherapy and The Japanese Association for Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

1. Introduction

Streptococcus mutans is a member of the group of etiological bacteria that cause human dental caries [1–3]. Oral biofilm formation is associated with bacterial attachment to the acquired enamel pellicle, which includes salivary components, as well as ingestion and fermentation of foods containing sugar [2,3]. Colonization and maturing of bacteria are dependent on extracellular polysaccharide (EPS) [4] for bacterial survival in low pH conditions, and when anti-bacterial agents are introduced into the oral cavity [5,6]. *S. mutans* produces glucosyltransferases (GTFs) that form insoluble (α(1,3)-linked) and soluble (α(1,6)-linked) glucans resulting in the formation of sticky biofilm on tooth surfaces [7]. GTF I and GTF SI, which encode the *gtfB* and *gtfC* genes, synthesize both insoluble and soluble glucans by conversion of sucrose, and are known as principal agents to provide strong biofilm formation on tooth surfaces [7–9].

For survival, several different bacteria employ a quorum sensing (QS) system, which interacts with various environmental conditions in the oral cavity and functions as a bacterial intercellular signal mechanism for controlling gene expression in response to population density [10–12]. The Com-dependent QS system of *S. mutans* is known to be stimulated by the competence-stimulating peptide (CSP; amino acid sequence, SGSLSTFFRLFNRSFTQALGK) in conditions of high cell density [10,13]. Com-dependent QS systems mainly consist of various pathways that appear following bacteriocin production, genetic transformation, and acid tolerance, thus

* Corresponding author. Department of Bacteriology I, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo, 162-8640, Japan.
E-mail address: hsenpuku@nih.go.jp (H. Senpuku).

http://dx.doi.org/10.1016/j.jiac.2017.06.006
1341-321X/© 2017 Japanese Society of Chemotherapy and The Japanese Association for Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

QS pathways are controlled by various *com* genes, such as *comA*, *comB*, *comC*, *comD*, and *comE*, as well as others [10,14–18]. The ComCDE system in streptococci has been reported to be a very important model for genetic competence [11,14,15]. In this system, the CSP precursor is translated by ComC and released to outside of the cell. Then, CSP is sensed by a two-component system composed of the sensor-histidine kinase to ComD and response regulator to ComE [13,14,17,18]. Thereafter, it is activated by autophosphorylation and transfers the phosphoryl group to the ComE response regulator, after which response signals are continued for expression of cell activities [18].

Streptococcus salivarius is a numerically predominant oral bacteria and presented as a healthy commensal bacteria in human oral cavity. *S. salivarius* has been noted as an important source of safe and efficacious probiotics, capable of fostering more balanced, and health-associated oral microbiota [19,20]. Clinical trials using *S. salivarius* as a probiotic showed to reduce plaque formation and to lower colonization of *S. mutans* in primary school-aged children [19]. Our recently reported findings indicate that supernatant proteins from *S. salivarius* induce inactivation of CSP-dependent genetic transformation, and also inhibit glucan-dependent biofilm formation and bacteriocin production by *S. mutans* [21,22]. The protein that inhibited biofilm formation in our experiments was identified as fructanase (FruA), an exo-β-D-fructosidase enzyme encoded by the *fruA* gene [22–24]. FruA digests sucrose which is utilized as a substrate to form insoluble (α(1,3)-linked) and soluble (α(1,6)-linked) glucans, and fructan which is β(2,1)- and β(2,6)-linked extracellular fructose polymers, in *S. mutans* [22,25]. However, the function of FruA in the QS system has not reported. In the present study, to clarify the multiple effects of FruA in detail in regard to biofilm formation under various sugar conditions, CSP-dependent bacteriocin production and genetic transformation were examined in conditions with and without FruA. Our results show that FruA has multiple effects related to survival functions of *S. mutans*, including biofilm formation, bacteriocin production, and genetic transformation.

2. Materials and methods

2.1. Bacterial strains and culture conditions

The bacterial strains used in this study are described in Table 1. All strains were grown in Brain Heart Infusion broth (BHI; Difco Laboratories, Detroit, MI) in an aerobic atmosphere of 5% CO_2, 75% N_2, and 20% O_2 (GasPack CO_2, Becton/Dickinson, Sparks, MD) at 37 °C before use in the experiments.

2.2. Construction of mutants

The *gtf* and *com* genes were identified in the *S. mutans* UA159 database (http://www.genome.jp/kegg/), and mutants were constructed using double-crossover homologous recombination via insertion of the resistance determinants kanamycin (Kan; Sigma-Aldrich, St. Louis, MO) and erythromycin (Erm; Sigma-Aldrich) into each gene, as previously described [26]. For polymerase chain reaction (PCR) assays, fragments of the upstream and downstream regions of the *gtf* and *com* genes were amplified with primer pairs (Table 2).

2.3. Human saliva collection

Whole saliva samples were collected from 3 healthy human volunteers (27–32 years old) after stimulation by chewing paraffin gum and pooled into ice-chilled sterile bottles over a period of 5 min. The samples were clarified by centrifugation at 10,000×*g* for 10 min at 4 °C, sterilized using a 0.22-μm Millex-GP Filter Unit (Merck Millipore, Darmstadt, Germany), and coated onto wells in plates (Sumitomo Bakelite, Tokyo, Japan) for biofilm formation assays.

2.4. Fructanase

For the present study, we used a FruA mixture (Megazyme, Wicklow, Ireland) that included exo-inulinase (FruA)-to-endo-inulinase unit ratios of 10:1 or less of FruA activity. FruA is the sole enzyme involved in utilization of β(2,1)- and β(2,6)-linked extracellular fructose polymers by *S. mutans* [27]. Furthermore, this enzyme has an ability to digest sucrose to fructose and glucose, as well as convert fructan to fructose by hydrolyzation, and is also an important component for the virulence of *S. mutans*.

2.5. Biofilm formation assays

Biofilm formation of by each of the examined strains was assayed using a method previously described, with slight modifications [22]. Briefly, 96-well flat-bottom microtiter plates were coated with whole human saliva and incubated at 4 °C for 60 min. Saliva was removed and the wells were rinsed twice with sterile phosphate-buffered saline (PBS). To evaluate the effects of FruA on biofilm formation by each bacterial cell suspension, the cell suspension was adjusted to $OD_{600} = 0.4$ using tryptic soy broth without dextrose (TSB; Difco Laboratories), supplemented with 0.25% sucrose (TSBs), 0.25% glucose (TSBg), or 0.25% fructose (TSBf). Thereafter, 20 μl of each cell suspension was mixed with 20 μl of FruA (final concentration, 57 unit/ml) in 160 μl of TSB, TSBs, TSBg, or TSBf in human saliva-coated 96-well flat-bottom microtiter plates. Each biofilm formation assay was performed at 37 °C for 16 h under an aerobic condition with 5% CO_2. After the plates were incubated,

Table 1
Bacterial strains.

Strain	Genotype or phenotype	Reference
Streptococcus mutans		
UA159	Erm, serotype c human isolate	
gtfB mutant	Erm, UA159 derived, *gtfB* deficient	Ref. 26
gtfC mutant	Kan, UA159 derived, *gtfC* deficient	Ref. 26
gtfBC mutant	Erm, Kan, UA159 derived, *gtfBC* deficient	Ref. 26
comC mutant	Erm, UA159 derived, *comC* deficient	Ref. 26
comD mutant	Erm, UA159 derived, *comD* deficient	Ref. 26
GS5	Erm, serotype c human isolate	
comC mutant	Erm, GS5 derived, *comC* deficient	Ref. 26

Table 2
Primers for the construction of mutans.

Primer	Nucleotides sequence (5'→3')	Amplicon
GtfBuF	ccccgaattcACAGTTGACAAAACTTCTGAAGC	*gtfB*
GtfBuR	ccccggtaccGCTCTGTGCAGAGCGATCATAAAC	*gtfB*
GtfBdF	cccctctagaTGATGATACAAGTAATCAATTGC	*gtfB*
GtfBdR	ccccaagcttATAGTGTTATCAGCTGTATATC	*gtfB*
GtfCuF	cccccgaattcTGAGTGGTGTATGGCGTCAC	*gtfC*
GtfCuR	cccccggtaccGACCGTTAATGGTTCTGGC	*gtfC*
GtfCdF	ccccctctagaAAACTCTGACTGCTACTGATAC	*gtfC*
GtfCdR	cccccaagcttGAGCAAAGCTGTTAGTGTTATCA	*gtfC*
ComCuF	cccccgaattcAAATCTGAACAAGCAGGGG	*comC*
ComCuR	cccccggtaccGATAGTGTTTTTTTCATTTTATATCTCC	*comC*
ComCdF	ccccctctagaGCCTATCAACATTTTTCCGGC	*comC*
ComCdR	cccccaagcttCCACTAAAGGCTCCAATCGC	*comC*
ComDuF	cccccgaattcCCATTCATCTGAAACTCAGT	*comD*
ComDuR	cccccggtaccAACAGGCAGCAGACCATAA	*comD*
ComDdF	ccccctctagaGGCGGGCAATCATATTCTT	*comD*
ComDdR	cccccaagcttTCCTGCAATTGATGTCCTG	*comD*

Download English Version:

https://daneshyari.com/en/article/5668843

Download Persian Version:

https://daneshyari.com/article/5668843

Daneshyari.com