Phylogenetic and Ecological Study of Oral *Veillonella* in Biofilm Formation

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【Introduction】

Dental caries and periodontal diseases are caused by oral biofilms. It is already known that oral biofilms consist of various bacteria that form three-dimensional conformations and confer resistance against immunity and antimicrobial agents.

It has been suggested that oral *Veillonella* species have important roles in the early stages of the formation of oral biofilms (Saravanan & Kolenbrander, 2010). However, their role in biofilm formation has been not clarified in detail because of difficulties associated with their proper handling.

The aims of this study were to establish the phylogenetic relationships between oral *Veillonella* species and reveal their ecological roles in oral biofilm formation. Furthermore, this study aimed to establish a novel preventative method for dental caries and periodontal diseases by the regulation of oral biofilm formation at an early stage.

1. Phylogenetic Analysis of *Veillonella* Species in Human Oral Cavities

The genus *Veillonella* is composed of strictly anaerobic gram-negative cocci lacking flagella, spores, and capsules. The distribution and frequency of oral *Veillonella* species (*Veillonella atypica*, *Veillonella denticariosi*, *Veillonella dispar*, *Veillonella parvula*, and *Veillonella rogosae*) in human tongue biofilms of healthy young adults have been analyzed by PCR using species-specific primer sets to clarify the constitution of oral *Veillonella* during an early stage of biofilm formation (Mashima et al., 2011). In the process of above study, 12 strains that were not classified as any previously described *Veillonella* species. A sequence analysis of three genes, an analysis of cellular fatty acids, and various biochemical tests were carried out. As a result, the 12 strains were established as a novel *Veillonella* species, *Veillonella tobetsuensis* sp. nov., named after Tobetsu, the town where Health Sciences University of Hokkaido is located (Mashima et al., 2013). The type strain of *Veillonella*

tobetsuensis was deposited in the Japan Culture Microorganisms and American Type Culture Collection.

Furthermore, a species-specific primer pair for *V. tobetsuensis* was designed to estimate its distribution and frequency in the human oral cavities of healthy young adults (Mashima & Nakazawa, 2013). It was also reported that oral *Veillonella* might contribute to oral infectious diseases (Mashima & Nakazawa, 2013). In addition, the distribution and frequency of oral *Veillonella* species in periodontal pockets were revealed using PCR with 6 species-specific primer sets (Mashima et al., 2015).

2. The Ecology of Oral *Veillonella* in Biofilm Formation

In early ecological studies of biofilm formation, a novel method for biofilm assays *in vitro*, termed “the wire method,” was developed to overcome the disadvantages of plate assays and flow cell methods (Mashima & Nakazawa, 2012). This novel method enabled a quantitative analysis at the species level in biofilms formed by multiple bacterial species.

To determine the roles of oral *Veillonella* in the early stages of biofilm formation, biofilms formed by a combination of 4 *Streptococcus* species as the initial colonizers and 6 *Veillonella* species as early colonizers were analyzed. As a result, biofilm formations composed of 24 combinations of species were divided into 4 patterns, and *V. tobetsuensis* significantly stimulated the formation of biofilms with *Streptococcus gordonii* (Mashima & Nakazawa, 2014). Based on the results of these studies, the combination of *V. tobetsuensis* and *S. gordonii* is considered a model for biofilm formation at an early stage, and has been used in subsequent studies.

Although co-aggregation is one of the important factors in biofilm formation, it was not observed for *V. tobetsuensis* and *S. gordonii*. These results suggest that molecular factors produced by *V. tobetsuensis* stimulated biofilm formation with *S. gordonii*. Therefore, an

autoinducer-mediated quorum sensing system in *V. tobetsuensis* was the focus of this study. Autoinducer-1 (AI-1) and autoinducer-2 (AI-2) from *V. tobetsuensis* were detected and partially purified. Furthermore, the effects of AI-2 derived from *V. tobetsuensis* on *S. gordonii* biofilm formation were examined.

The highest AI-1 activity was observed during the late exponential phase. When the crude AI-1 obtained at the late exponential phase was analyzed by two-dimensional thin-layer chromatography, two spots were observed at a position similar to C_8-HSL. The highest *V. tobetsuensis* AI-2 activity was observed during the middle exponential phase. The partially purified AI-2 showed 10 times more AI-2 activity than the control strain. This result indicated that the partial purification of *V. tobetsuensis* AI-2 was successful.

Furthermore, the partially purified AI-2 from the supernatant of *V. tobetsuensis* significantly inhibited *S. gordonii* biofilm formation.

Many previous reports regarding AI-2, especially those related to oral bacteria, have indicated that it promotes biofilm formation. These results indicate that *V. tobetsuensis* AI-2 might be a new type of AI.

Further studies including a structural analysis of these AIs derived from *V. tobetsuensis* and a biofilm assay to examine their roles in oral biofilm formation are required.

【References】

Saravanan P, Kolenbrander PE. Central role of the early colonizer *Veillonella* sp. in establishing multispecies biofilm communities with initial, middle, and late colonizers of enamel. J Bacteriol 192:2965-2972, 2010.

Mashima I, Kamaguchi A, Nakazawa F. The distribution and frequency of oral *Veillonella* spp. in the tongue biofilm of healthy young adults. Curr Microbiol 63:403-407, 2011.

Mashima I, Nakazawa F. The wire method for generating experimental biofilms formed by

oral *Streptococcus* and *Veillonella* species, Dent J Heal Sci Univ Hokkaido 31: 73-80, 2012.

Mashima I, Nakazawa F. Identification of *Veillonella tobetsuensis* in Tongue Biofilm by Using a Species-Specific Primer Pair. Anaerobe 22:77-81, 2013.

Mashima I, Nakazawa F. A review on the characterization of a novel oral *Veillonella* species, *V. tobetsuensis*, and its role in oral biofilm formation, J Oral Biosci, 55:184-190, 2013.

Mashima I, Kamaguchi A, Miyakawa H, Nakazawa F. *Veillonella tobetsuensis* sp. nov., an anaerobic, Gram-negative coccus isolated from human tongue biofilms. Int J Syst Evol Microbiol 63: 1443-1449, 2013.

Mashima I, Nakazawa F. The influence of oral *Veilloenlla* species on biofilms formed by *Streptococcus* species. Anaerobe 28: 54-61, 2014.

Mashima I, Fujita M, Nakatsuka Y, Kado T, Furuichi Y, Herastuti, S, Nakazawa F. The distribution and frequency of *Veillonella* spp. associated with chronic periodontal diseases. IJCMAS, 4:150-160, 2015.