Advances in
Biotechnology & Microbiology
ISSN 2474-7637

Juniper Publishers
key to the Researchers

Short Communication
Volume 5 Issue 4 – August 2017
DOI: 10.19080/AIBM.2017.05.555667

Adv Biotech & Micro

Prospects of Novel Species of Oral *Veillonella* in Human Saliva

Citra F Theodorea[1,2], Izumi Mashima[1,3,4] and Futoshi Nakazawa[1]*

[1]*Department of Oral Microbiology, Health Sciences University of Hokkaido, Japan*

[2]*Department of Oral Biology, Universitas Indonesia, Indonesia*

[3]*Postdoctoral Fellow of Japan Society for the Promotion of Science, Japan*

[4]*Department of Oral Biology, University at Buffalo, The State University of New York, USA*

Submission: August 3, 2017**; Published:** August 22, 2017

***Corresponding author:** Futoshi Nakazawa, MS, Ph D, Department of Oral Microbiology, School of Dentistry, Health Sciences University of Hokkaido, 1757 Kanazawa, Ishikari-Tobetsu, Hokkaido, 061-0293, Japan, Tel +81-133-23-2484; Fax: +81-133-23-1385; Email: nakazawa@hoku-iryo-u.ac.jp

Abstract

Although 13 species of bacteria have been established in the genus *Veillonella*, only 6 have currently been isolated from human oral cavities (*V. atypica, V. denticariosi, V. dispar, V. parvula, V. rogosae*, and *V. tobetsuensis*). Oral *Veillonella* have played a central role as early colonizers that establish multispecies oral biofilms that cause many oral infectious diseases in humans such as periodontitis and dental caries. Our previous study isolated 1,609 strains (confirmed by PCR with the genus-specific primer set) of the genus *Veillonella* from saliva samples of 107 Thai children. Although 167 strains could not be classified under any oral *Veillonella* species, 1,442 strains were linked to the 6 oral *Veillonella* species by PCR with species-specific primer sets. To reveal the phylogenetic characteristics of these unclassified *Veillonella* strains, PCR-based amplifications and sequence analyses of *rpoB* genes were performed for 23 representative strains. When the phylogenetic tree was examined based on the obtained sequence data, these 23 strains formed a cluster distinct from the 13 established species of the genus *Veillonella* with a robust bootstrap value. Thus, the phylogenetic study demonstrated the prospects of several novel species of oral *Veillonella* in human saliva.

Keywords: Novel species; Oral biofilm; Oral *Veillonella*; Phylogenetic tree; *rpoB* gene

Keywords: PCR: Polymerase Chain Reaction

Introduction

The genus *Veillonella* consists of small, strictly anaerobic, gram-negative cocci lacking flagella, spores, and capsules that are characterized by their ability to obtain energy from short-chain organic acids [1,2]. Although 13 species have currently been established in the genus *Veillonella*, only 6 species (*V. atypica*, *V. denticariosi, V. dispar, V. parvula, V. rogosae,* and *V. tobetsuensis*) have been isolated from human oral cavities [2-9]. The main habitats of the oral *Veillonella* are tongue biofilms, dental biofilms, buccal mucosa, and saliva [10-12]. Oral *Veillonella* has been found in severe early childhood caries [13], apical root canals [14], and dental tubules [15]. Additionally, oral *Veillonella* are also predominantly found in saliva [16] and subgingival biofilm specimens [17] from patients who have chronic periodontitis.

It was reported that oral *Veillonella* play a central role as early colonizers that establish multispecies oral biofilm communities [18]. Oral biofilms are known to cause many oral infectious diseases, such as periodontitis and dental caries in humans [2,19]. It was reported that oral *Veillonella*, found throughout the entire oral cavity, comprise as much as 10% of the bacterial community initially colonizing the enamel [20, 21], and often form biofilms with the *Streptococcus* species [22]. Some studies have also shown that during the formation of early dental plaque, the ratio of *Veillonella* to *Streptococcus* species varied in mixed-microbial colonies [23].

The identification of *Veillonella* isolates at the genus level is relatively simple, but identification at the species level remains difficult because of the lack of conventional phenotypic and biochemical tests [24]. However, molecular methods based on the 16S rRNA gene sequencing have previously been used to identify *Veillonella* strains at the species level [25,26]. Unfortunately, recent studies have demonstrated that using 16S rRNA gene sequencing exclusively for identification purposes is unreliable because of the high levels of sequence conservation

in the 16S rRNA gene sequence among several *Veillonella* species [5,8,9,25]. To overcome this difficulty, Beighton et al. [27] used the *rpoB* gene sequence to identify isolates of the genus *Veillonella* from the human tongue at the species level. Furthermore, our previous study established the first successful one-step PCR method using species-specific primer sets based on the highly variable region of the *rpoB* gene of 6 species of oral *Veillonella* (positions 2,500-3,100)[12]. This one-step PCR method is easier and more effective than the previous molecular methods described above in identifying oral *Veillonella* at the species level.

Our previous study examined the distribution and frequency of oral *Veillonella* species in the saliva of 107 Thai children with varying oral hygiene statuses [28]. PCR with the genus-specific primer sets confirmed 1,609 strains, isolated in the study, as members of the genus *Veillonella* [27]. Furthermore, 1,442 strains were identified by the one-step PCR method as *V. atypica, V. denticariosi, V. dispar, V. parvula, V. rogosae*, or *V. tobetsuensis*. However, 167 strains could not be classified into any oral *Veillonella* species. This short communication seeks to report the phylogenetic position and diversity of 23 of the 167 unclassified *Veillonella* strains by phylogenetic analysis with the *rpoB* gene sequence.

23 unclassified *Veillonella* strains-S22108-3a, S34277-12b, S42329-13a, S31177-14a, S12034-12, S11011-11d, S44377-5b, S13062-15, S13054-9, S44377-5a, S34277-12a, S22118-11a, S33222-2c, S42329-8b, S43355-15, S34284-15, S14073-19a, S24170-5, S31180-9b, S14073-19c, S32198-4a, S42329-8a, S31177-14b-were selected as representative strains and cultured in BactoTM Brain Heart Infusion (Difco Laboratories, BD) broth supplemented with 5% (v/v) defibrinated sheep blood (BHI agar), hemin (10μg/mL), and menadione (5μg/mL) under anaerobic conditions with 80% N2, 10% CO2, and 10% H2 at 37 °C for 5d.

Genomic DNA was extracted from individual bacterial cells using an Insta Gene Matrix Kit (Bio-Rad) and PCR-based amplification and sequence analyses of *rpoB* gene was performed using previously described primers [27]. The sequences were determined by an ABI PRISM 310 Genetic Analyzer (Applied Biosystems) and were aligned and connected by using SEQMAN Pro of the LASERGENE 14 program (DNASTAR). The programs MEGALIGN including CLUSTAL W using the neighbor-joining (NJ) method [29] were used to compare sequences and to reconstruct an evolutionary tree. The *rpoB* sequences of the 23 unclassified *Veillonella* strains were aligned against the sequences of the representative *Veillonella* type strains retrieved from GenBank.

Alignment of the *rpoB* gene sequences showed 97.6-100% similarity among the sequences of the unclassified 23 *Veillonella* strains. These results indicate that these 23 strains are closely related. The evolutionary tree obtained for the type strain of the established species of genus *Veillonella* and the unclassified 23 *Veillonella* strains is shown in Figure 1. Based on the tree, the 23 unclassified *Veillonella* strains formed a cluster distinct from the established *Veillonella* species. Although the most closely related species was *V. dispar*, the robust bootstrap value of 99.9% indicated that these 23 strains formed a taxon distinct from *V. dispar.*

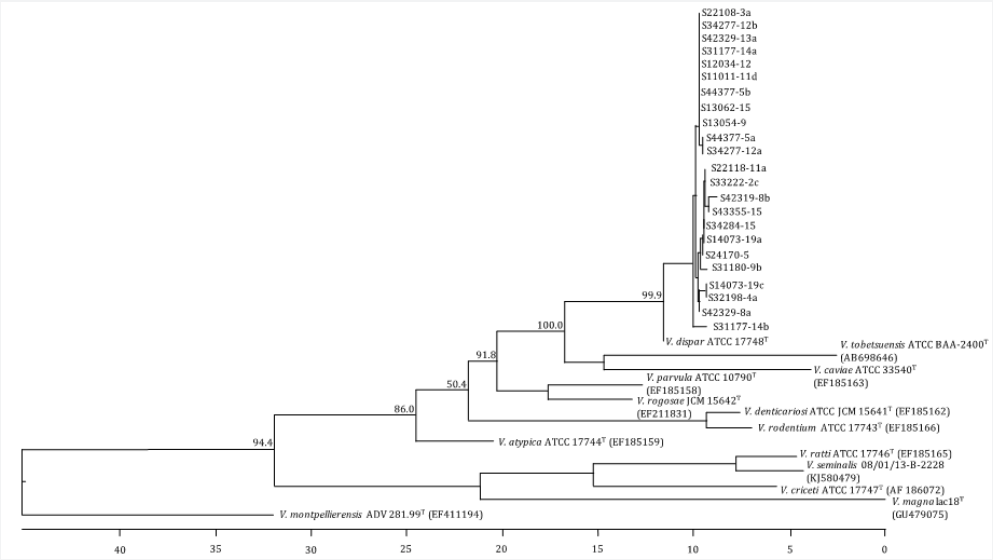

Figure 1: Neighbor-joining tree-based analysis of the *rpoB* gene sequences showing the relationship between the 23 unclassified *Veillonella* strains and the type strains of the established species of genus *Veillonella*. Accession numbers for *rpoB* gene sequences are given for each established strain. Bootstrap values are indicated at corresponding nodes. Nucleotide Substitution per 100 residues.

Conclusion

This study demonstrated prospective novel oral *Veillonella* species in human saliva. It is expected that the new perspective of oral biofilm communities at early stages would be investigated by this discovery of novel *Veillonella* species.

References

1. Carlier JP (2015) *Veillonella*. Bergey's Manual of Systematic of Archaea and Bacteria, Wiley online library, USA, pp. 1-11.

How to cite this article: Citra F T, Izumi M, Futoshi N. Prospects of Novel Species of Oral *Veillonella* in Human Saliva. Adv Biotech & Micro. 2017; 5(4): 555667. DOI: 10.19080/AIBM.2017.05.555667

2. Delwiche EA, Pestka JJ, Tortorello ML (1985) The *Veillonellae*: gram negative cocci with a unique physiology. Annu Rev Microbiol 39: 175-193.

3. Rogosa M (1965) The genus *Veillonella* IV. serological groupings, and genus and species emendations. J Bacteriol 90(3): 704-709.

4. Jumas-Bilak E, Carlier JP, Jean-Pierre H, Teyssier C, Gay B, et al. (2004) *Veillonella montpellierensis* sp. nov., a novel, anaerobic, Gram-negative coccus isolated from human clinical samples. Int J Syst Evol Microbiol 54(Pt 4): 1311-1316.

5. Byun R, Carlier JP, Jacques NA, Marchandin H, Hunter N (2007) *Veillonella denticariosi* sp. nov., isolated from human carious dentine. Int J Syst Evol Microbiol 57(Pt 12): 2844-2848.

6. Arif N, Do T, Byun R, Sheehy E, Clark D, et al. (2008) *Veillonellarogosae* sp. nov., an anaerobic, Gram-negative coccus isolated from dental plaque. Int J Syst Evol Microbiol 58(Pt 3): 581-584.

7. Kraatz M, Taras D (2008) *Veillonella magna* sp. nov., isolated from the jejunal mucosa of a healthy pig, and emended description of *Veillonellaratti*. Int J Syst Evol Microbiol 58(Pt 12): 2755-2761.

8. Mashima I, Kamaguchi A, Miyakawa H, Nakazawa F (2013) *Veillonellato betsuensis* sp. nov., an anaerobic, gram-negative coccus isolated from human tongue biofilms. Int J Syst Evol Microbiol 63(Pt 4): 1443-1449.

9. Aujoulat F, Bouvet P, Jumas-Bilak E, Jean-Pierre H, Marchandin H (2014) *Veillonella seminalis* sp. nov., a novel anaerobic Gram-stain-negative coccus from human clinical samples, and emended description of the genus *Veillonella*. Int J Syst Evol Microbiol 64(Pt 10): 3526–3531.

10. Arif N, Sheehy EC, Do T, Beighton D (2008) Diversity of Veillonella spp. from sound and carious sites in children. J Dent Res 87(3): 278–282.

11. Hughes CV, Kolenbrander PE, Andersen RN, Moore LV (1988) Coaggregation properties of human oral *Veillonella* spp.: relationship to colonization site and oral ecology. Appl Environ Microbiol 54(8):1957–1963.

12. Mashima I, Theodorea CF, Thaweboon B, Thaweboon S, Nakazawa F (2016) Identification of *Veillonella* Species in the Tongue Biofilm by Using a Novel One-Step Polymerase Chain Reaction Method. PLoS One 11(6): e0157516.

13. Kanasi E, Dewhirst FE, Chalmers NI, Kent R, Moore A, et al. (2010) Clonal analysis of the microbiota of severe early childhood caries. Caries Res 44(5): 485-497.

14. Baumgartner JC, Falkler WA (1991) Bacteria in the apical 5mm of infected root canals. J Endod 17(8): 380-383.

15. Peters LB, Wesselink PR, Buijs JF, van Winkelhoff AJ (2001) Viable bacteria in root dentinal tubules of teeth with apical periodontitis. J Endod 27(2): 76-81.

16. Takeshita T, Nakano Y, Kumagai T, Yasui M, Kamio N, et al. (2009) The ecological proportion of indigenous bacterial populations in saliva is correlated with oral health status. ISME J 3(1): 65-78.

17. Heller D, Silva-Boghossian CM, do Souto RM, Colombo AP (2012) Subgingival microbial profiles of generalized aggressive and chronic periodontal diseases. Arch Oral Biol 57(7): 973-980.

18. Periasamy S, Kolenbrander PE (2010) Central role of the early colonizer *Veillonella sp*. in establishing multispecies biofilm communities with initial, middle, and late colonizers of enamel. J Bacteriol 192(12): 2965-2972.

19. Mashima I, Fujita M, Nakatsuka Y, Kado T, Furuichi Y, et al. (2015) The Distribution and Frequency of Oral *Veillonella spp.* Associated with Chronic Periodontitis. Int J Curr Microbiol App Sci 4(3): 150–160.

20. Aas JA, Paster BJ, Stokes LN, Olsen I, Dewhirst FE (2005) Defining the normal bacterial flora of the oral cavity. J Clin Microbiol 43 (11): 5721-5732.

21. Diaz PI, Chalmers NI, Rickard AH, Kong C, Milburn CL, et al. (2006) Molecular Characterization of Subject-Specific Oral Microflora during Initial Colonization of Enamel. Appl Environ Microbiol 72(4): 2837-2848.

22. Olson JC, Cuff CF, Lukomski S, Lukomska E, Canizales Y, et al. (2011) Use of 16S ribosomal RNA gene analyses to characterize the bacterial signature associated with poor oral health in West Virginia. BMC Oral Health 11: 7.

23. Chalmers NI, Palmer RJ Cisar JO, Kolenbrander PE (2008) Characterization of a *Streptococcus sp.-Veillonella sp.* Community Micromanipulated from Dental Plaque. J Bacteriol 190(24): 8145-8154.

24. Kolenbrander PE, Moore LVH (1992) The genus *Veillonella.* In: The Prokaryotes (2nd edn), Springer, New York, USA, pp. 2034-2047.

25. Marchandin H, Teyssier C, Siméon De Buochberg M, Jean-Pierre H, Carriere C, et al. (2003) Intra-chromosomal heterogeneity between the four 16S rRNA gene copies in the genus *Veillonella*: implications for phylogeny and taxonomy. Microbiology 149(Pt 6): 1493–1501.

26. Sato T, Sato M, Matsuyama J, Hoshino E (1997) PCR-restriction fragment length polymorphism analysis of genes coding for 16S rRNA in *Veillonella* spp. Int J SystBacteriol 47(4): 1268-1270.

27. Beighton D, Clark D, Hanakuka B, Gilbert S, Do T (2008) The predominant cultivable *Veillonella* spp. of the tongue of healthy adults identified using *rpoB* sequencing. Oral Microbiol Immunol 23(4): 344-347.

28. Theodorea CF, Mashima I, Thaweboon B, Thaweboon S, Nakazawa F (2017) Molecular Detection of Oral *Veillonella* species in the Saliva of Children with Different Oral hygiene Statuses. Int J Curr Microbiol Appl Sci 6(7): 449-461.

29. Saitou N, Nei M (1987) The neighbor-joining method: a new method for reconstructing phylogenetic trees. MolBiol Evol 4(4): 406-425.

DOI: 10.19080/AIBM.2017.05.555667

How to cite this article: Citra F T, Izumi M, Futoshi N. Prospects of Novel Species of Oral *Veillonella* in Human Saliva. Adv Biotech & Micro. 2017; 5(4): 555667. DOI: 10.19080/AIBM.2017.05.555667