Abstract

Molecular Detection and Evolutionary Study of Oral *Veillonella* Species in the Saliva of the Children

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Oral *Veillonella* species such as *V. atypica*, *V. denticariosi*, *V. dispar*, *V. parvula*, *V. rogosae*, and *V. tobetsuensis* play a central role in oral biofilm formation at the early stage. However, the concrete roles of oral *Veillonella* in biofilm formation at the species level have not been elucidated yet. Also, many studies have reported that unclassified strains displaying the characteristic of the genus *Veillonella* have been found in oral cavity and may contribute to oral biofilm formation. Meanwhile, there are no reliable reports on the phylogenetic characterization of the unclassified strains from different intra-oral sites.

The aim of this study was to molecularly detect oral *Veillonella* species in the saliva of children with different oral hygiene statuses and to evaluate the phylogenetic diversity of the unclassified *Veillonella* strains isolated in this study.

Saliva samples were collected from 107 Thai children divided into three groups based on their oral hygiene status (good [n = 27], moderate [n = 35], and poor [n = 45]). *Veillonella* isolates were identified at the species level by one-step PCR using species-specific primer sets based on the sequence of *rpoB*. In the phylogenetic studies, 22 or 23 unclassified *Veillonella* strains were chosen for the study. PCR-based amplification and sequence analyses of 16S rRNA, *rpoB*, and *dnaK* were performed using previously described primers.

We found that oral *Veillonella* isolates were twice more likely to be detected in subjects with poor oral hygiene than in those with good or moderate oral hygiene. The detection rates of *V. rogosae* decreased from good to poor oral hygiene groups (73.2%, 69.6%, and 58.6%, respectively). The *V. parvula* detection rate was low in the good oral hygiene group, but increased in the moderate and poor oral hygiene groups (6.3%, 7.0%, and 16.9%, respectively). The detection rate of *V. tobetsuensis* was 14.3% in the moderate oral hygiene group and at 17.8% in the poor oral hygiene group. Our results indicated that the ratio of oral *Veillonella*

species, such as *V. rogosae*, *V. parvula*, and *V. tobetsuensis* in the saliva could serve as an index of the oral hygiene status of children.

In the phylogenetic tree, based on *rpoB* within the genus *Veillonella*, the 23 isolates formed a distinct cluster with robust bootstrap values. Although the most closely related species was *V. dispar*, the cluster was comprised of three taxa in different locations. In the *dnaK* tree, the 23 isolates were also divided into three distinct taxa. Two of the three taxa were closely related with *V. parvula*. Another taxon was closely related with *V. dispar* and *V. atypica*. Moreover, the 22 isolates formed a distinct cluster compared to the established *Veillonella* species based on the 16S rRNA phylogenetic tree. The most closely related species were *V. dispar* and *V. tobetsuensis*.

To the best of our knowledge, this is the first report indicating that oral *Veillonella* species could be useful bio-indicators of the oral hygiene status in children. Furthermore, the phylogenetic study of these unclassified strains isolated from saliva samples suggested a novel species of the genus *Veillonella* in the oral cavity of children.