Impact of 16S rRNA gene redundancy and primer pair selection on the quantification and classification of oral microbiota in next-generation sequencing

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Research

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Abstract

Background: The identification, at least at the species level, is highly desirable in 16S rRNA sequencing-based studies of the oral microbiota. However, no study in the oral microbiology field has examined the impact of which primer pair is selected to detect redundant and matching amplicons. Consequently, our aims were to: 1) evaluate the number of 16S rRNA genes in the complete genomes of all the bacterial and archaeal species ever detected in the human oral cavity; and 2) assess how the use of different primer pairs would affect the detection and classification of redundant amplicons and matching amplicons (MA) from different taxa.

Results: A total of 709 complete genomes (518 bacteria, 191 archaea) were downloaded from the NCBI database, and their complete 16S rRNA genes were extracted. 94.1% of oral bacteria and 52.59% of oral archaea had more than one 16S rRNA gene in their respective genomes. Next, 33 primer pairs identified in previous research and 6 commonly used in the literature were used against all the genomes to obtain amplicons. Between 46.67%-1.29% of the bacterial species and between 38.89%-4.65% of the archaeal species had MA, affecting relevant genera present in the oral environment such as Actinomyces, Fusobacterium, Lactobacillus, Methanosarcina, Staphylococcus, and Streptococcus. The best primer pairs were (the species coverage with no MA values, SC-NMA; region; primer pair position for Escherichia coli J01859.1): KP_F048-OP_R030 for bacteria (93.55%; 3-7; 342-1079), KP_F018-KP_R063 for archaea (89.63%; 3-9; undefined-1506), and OP_F114_OP_R121 for both bacteria and archaea (92.52%; 3-9; 340-1405).

Conclusions : In addition to the 16S rRNA gene redundancy, the considerable presence of matching amplicons must be controlled to ensure the accurate interpretation of microbial diversity data. The SC-NMA is a more useful parameter than the conventional coverage percentage for selecting the best primer pairs. The performance of the primer pairs to detect no MA species increases as the average length of the amplicons increases; none of these being the most widely used primer pairs in the oral literature. The choice of primer pair affects significantly diversity estimates and taxonomic classification, conditioning the comparability of oral microbiome studies using different primer pairs.

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