16S rRNA gene amplicons and taxonomic classification of oral microbiome

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Introduction

The term microbiota refers to a set of microorganisms, considered as a living ecosystem, undergoing continuous changes in the growth and survival of all its members. The microbiome consists of a set of microorganism genomes, which are estimated to be around 10^{14} commensal bacterial cells in humans. Genome-based methods are effective for bacterial classification and for understanding the functional role of the microbiota and its interaction with the host.
In this study we explored the capability of a gene-based sequencing method to classify bacteria of the human oral microbiome, the second largest microbial community in the human body, after the gut. We evaluated the capability in detecting sequence variants in the bacterial 16S rRNA gene (length ~1500bp), present in al bacterial genomes, consisting of 9 hypervariable regions (V1-V9). At least 2 hypervariable regions are generally studied. We investigated all the 9 hypervariable regions, divided in 6 amplicons, to characterize overall taxa at different taxonomic layers, and point out the specificity and sensibility of each hypervariable regions (or their combination) to identify bacterial species.

Results

Our analysis detected 2600 unique ASVs in 4 samples, of which 1147 were successfully classified at the species taxonomic layer.

ASVs classification rate: 44.1%

	Kingdom	Phylum	Class	Order	Family	Genus	Species
ASVs	2600	2570	2522	2510	2492	2422	1147

Table a. Number of ASVs classified at each taxonomic layer. As resolution increases, not all ASVs are able to be classified through trained database. At Species level, almost 55% of ASVs remain without taxonomic nomenclature

In the details, we identified about 90 genera and more than 200 species; out of 9 identified phyla, Proteobacteria resulted to be the most abundant phylum (~ 56%), figure 1.

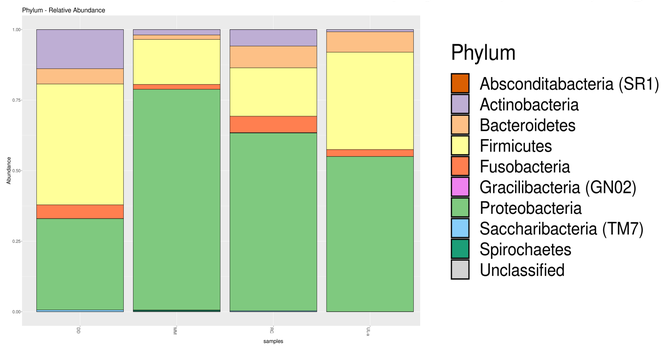


Figure1. Barplot representing the relative abundance of the 9 phyla in 4 healthy individuals.

The V1-V2 and V2-V3 amplicons recognized the highest number of species compared to the others, about 134 and 135 different species, respectively, of which 101 species in common.
The comparison analysis confirmed that each region resulted to be able to detect specific bacterial species that were not detectable by the other 16S regions.

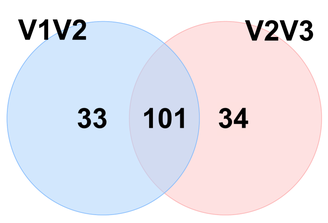


Figure2. Venn Diagram of shared species recognized by V1-V2 and V2-V3.

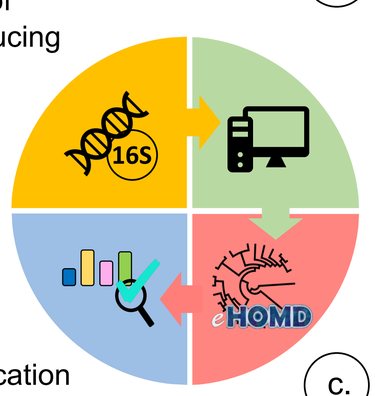
a.

V2-V3	V3-V4	V4-V5	V5-V7	V7-V9	
101	84	60	75	67	V1-V2
	92	65	77	71	V2-V3
		74	83	67	V3-V4
			66	60	V4-V5
				60	V5-V7

Table a. Number of common species detected by two amplicons.

Methods and Materials

a. 16S rRNA gene sequencing of 4 buccal swab samples of healthy individuals, producing ~200,000 PE reads per sample.



b. ASVs analysis (DADA2) in two ways:

Method1: Pooling data from all amplicons of the 16S regions together;

Method2: Combining data from each amplicon after individual processing.

c. Taxonomic classification by Bayesian Classification (Human Oral Microbiome Database, version 15.1).

d. Ascertainment of classification efficiency and accuracy (at genus or species layer) of every ASVs belonging to the different hypervariable regions.

Aim of the project

- To capture the most 16S gene variability in oral microbiome.
- To explore the capability of a gene-based sequencing method to classify bacteria of the oral microbiome.

Overall, 204 different species were recognized with the entire set of combined amplicons (method 1), whereas 206 different species were identified by the combined results of single amplicons (method 2).

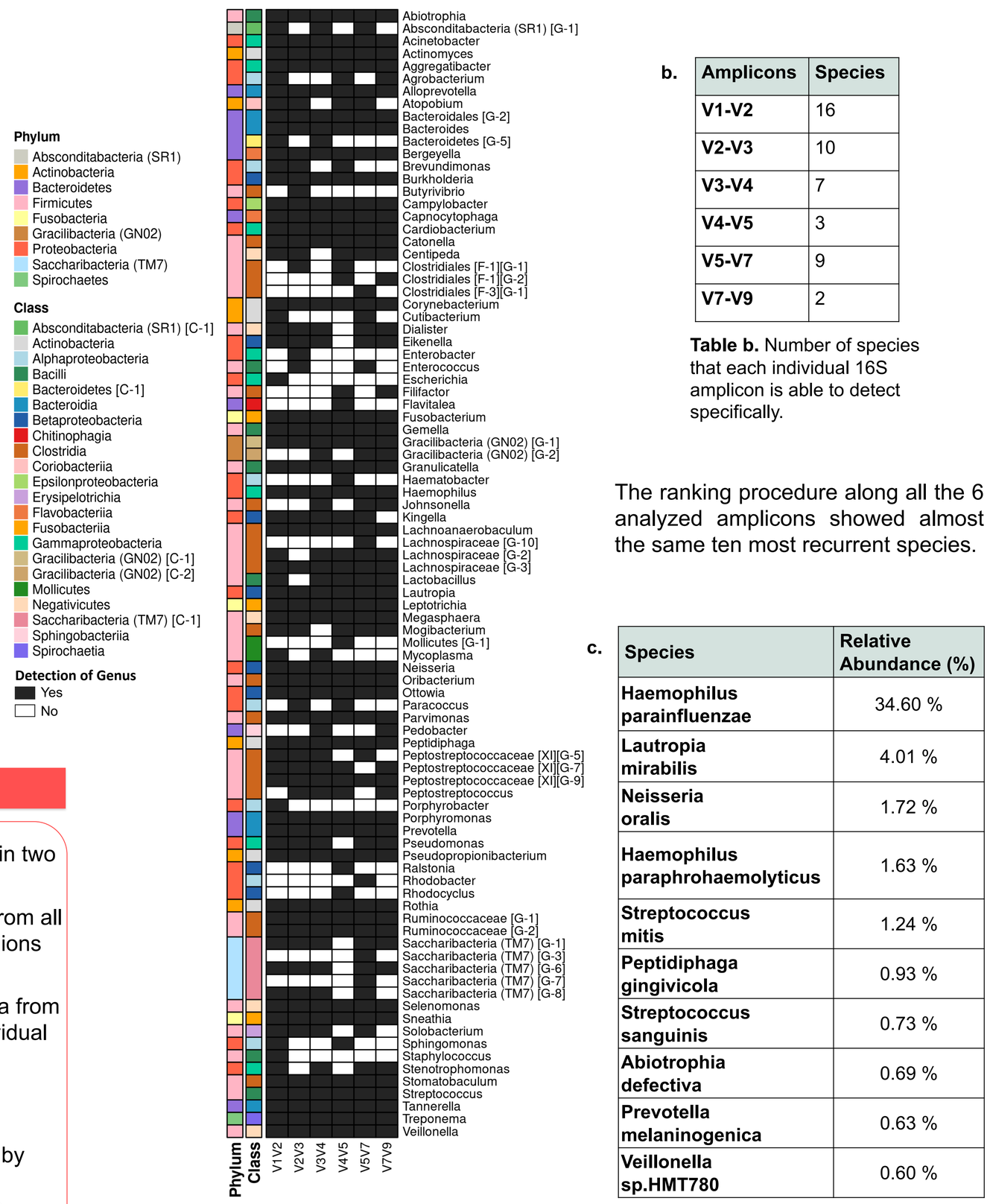


Figure3. Heatmap representing the 90 bacteria detected at Genus taxonomic layer by the analysis of the 6 amplicons.

b.

Amplicons	Species
V1-V2	16
V2-V3	10
V3-V4	7
V4-V5	3
V5-V7	9
V7-V9	2

Table b. Number of species that each individual 16S amplicon is able to detect specifically.

The ranking procedure along all the 6 analyzed amplicons showed almost the same ten most recurrent species.

c.

Species	Relative Abundance (%)
Haemophilus parainfluenzae	34.60 %
Lautropia mirabilis	4.01 %
Neisseria oralis	1.72 %
Haemophilus paraphrohaemolyticus	1.63 %
Streptococcus mitis	1.24 %
Peptidiphaga gingivicola	0.93 %
Streptococcus sanguinis	0.73 %
Abiotrophia defectiva	0.69 %
Prevotella melaninogenica	0.63 %
Veillonella sp.HMT780	0.60 %

Table c. Relative abundance of the first 10 species detected.

Conclusions

- Studying all the 9 16S gene regions is ~1.7 times more informative than the analysis of the individual amplicons, increasing the sensibility of the standard analysis.
- Using this method, the ~50% of the ASVs was not classified at Species taxonomic layer.
- For the study of the oral microbiome, we observed that V1-V2 and V2-V3 were the most informative individual amplicons.

References

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