

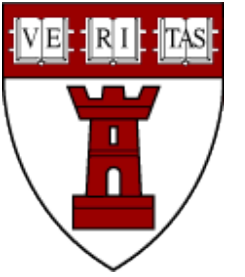


# Prevalence of Uncultivated Oral Taxa in Distinct Oral Niches

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## Abstract

**Objectives:** Of the 688 taxa currently in the Human Oral Microbiome Database (HOMD), 65% have been cultivated and 244 taxa (35%) are as-yet-uncultivated phylotypes. The goal of this study was to determine the prevalence of uncultivated taxa in various oral niches.

**Methods:** Ten adults who had not taken antibiotics within the past 3 months were sampled at 9 oral sites each: supragingival, subgingival (4 deepest sites), cheek, palate, tongue, tonsils (throat sampled for two subjects without tonsils). Soft tissue sites were sampled using nylon brushes, hard tissue sites with scalars. DNA was purified from the clinical samples, PCR amplified using 16S rRNA V3V4 primers, purified, and sequenced using an Illumina Miseq instrument. The 16S rRNA reads were parsed, and BLASTN analyzed using HOMD RefSeq Version 13.2.

**Results:** A total of 5,586,237 sequences (V3-V4 assembled overlapped paired reads) matched HOMD reference sequences at >98.5% BLASTN identity. The reads were identified to 481 HOMD taxa: 317 cultivated and 164 uncultivated phylotypes. The number of uncultivated taxa per sample ranged from 15-68 with a mean of 40.6. The total number of oral taxa/subject-site ranged from 91-251 with a mean of 182. The mean number of uncultivated taxa/individual sample at oral sites was as follows: cheek 45.9, palate 38.4, tongue 29.0, tonsils 46.3, throat 45.0, supragingival 39.8 and subgingival 39.6. Uncultivated taxa identified included 22 from the rare phyla: Chloroflexi, 1; Synergistetes genus *Fretibacterium*, 4; GNO2, 3; SR1, 3; and TM7, 11 taxa.

**Conclusion:** In screening just 10 subjects, we identified subject-sites with 164/244 (67%) of the uncultivated taxa currently in HOMD. The majority of these uncultivated taxa are thought to be uncultivable using standard cultivation methods; however, this study shows they are readily available for study and attempted cultivation.

## Introduction

Of the 688 taxa currently in the Human Oral Microbiome Database (HOMD), 344 are named cultivated taxa (50%), 100 are unnamed cultivated taxa (15%) and 244 as yet unnamed and uncultivated taxa (35%). Important for any effort to cultivate the previously uncultivated taxa is determining how many are commonly present in oral samples (and likely difficult to culture) and how many are rare (and probably uncultivated simply because of their rarity). It is also important to determine in which oral niche the uncultivated taxa most commonly reside. Finally, efforts to cultivate previously uncultivated taxa would benefit from establishing a set of subjects surveyed for site presence of particular uncultivated taxa who can be repeatedly sampled over the next five years. Therefore, ten Forsyth staff were recruited and nine oral sites examined by deep Illumina 16s rRNA sequencing.

## Methods

Ten adult (50% female; age range 24-72 years) Forsyth staff who likely would be available for repeated sampling over the next five years were recruited. All subjects signed the Forsyth IRB approved informed consent form prior to sampling. The only exclusion criteria was use of antibiotics within the past 3 months. Because some of the uncultivated taxa sought had been initially detected in subjects with periodontitis, halitosis or other oral diseases, subjects with signs of oral disease were intentionally included. The subjects were sampled at 9 oral sites each: supragingival, subgingival (4 deepest sites), cheek, palate, tongue, tonsils (throat sampled for two subjects without tonsils). Soft tissue sites were sampled using nylon brushes, hard tissue sites were sampled with scalars. DNA was purified from the clinical samples, PCR amplified using 16S rRNA V3V4 primers, purified, and sequenced using an Illumina Miseq instrument. The 16S rRNA reads were parsed, and BLASTN analyzed using HOMD RefSeq Version 13.2.

## Conclusions

In screening just 10 subjects, we were able to identify subject-sites with 164/244 (67%) of the uncultivated phylotypes currently in HOMD. The majority of these uncultivated taxa may well be cultivable using coculture or other innovative methods. This study shows bacteria from uncultivated oral taxa are readily available for study and attempted cultivation.

## References

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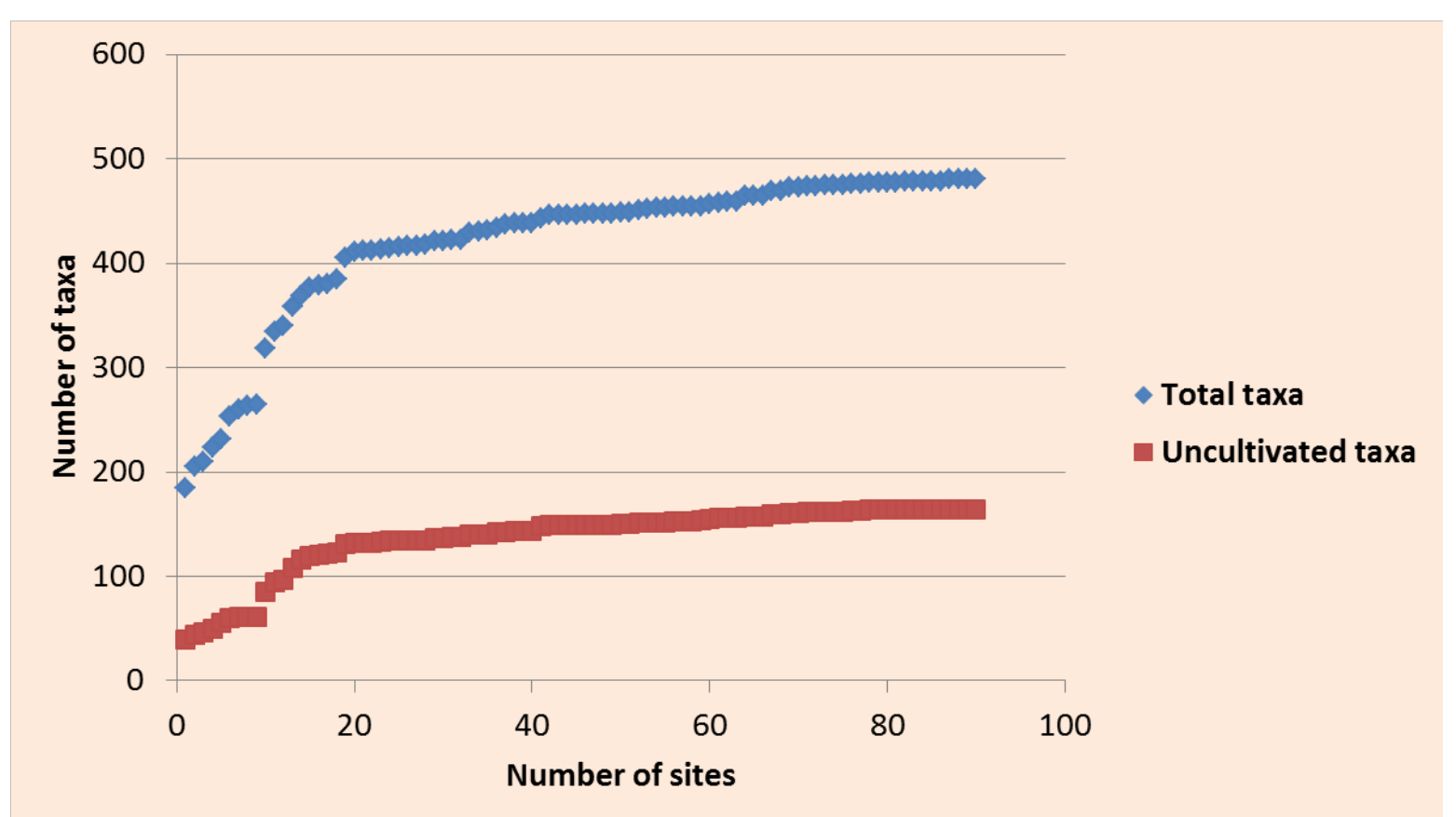
Segata N, Haake SK, Mannon P, Lemon KP, Waldron L, Gevers D, Huttenhower C, Izard J. 2012. Composition of the adult digestive tract bacterial microbiome based on seven mouth surfaces, tonsils, throat and stool samples. *Genome Biol.* **13**:R42

## Results

A total of 5,586,237 sequences, assembled from overlapped V3-V4 paired reads, matched HOMD reference sequences at >98.5% BLASTN identity. A total of 481 HOMD taxa were identified, 317 cultivated and 164 uncultivated. The number of uncultivated taxa per site sampled ranged from 15-68 with a mean of 40.6. The total number of taxa per site ranged from 91-251 with a mean of 182. The mean number of uncultivated taxa per individual sample at different oral niches was: cheek 45.9, palate 38.4, tongue 29.0, tonsils 46.3, throat 45.0, supragingival 39.8 and subgingival 39.6. Uncultivated taxa identified included 22 from the rare phyla: Chloroflexi (1); Synergistetes genus *Fretibacterium* (4); GNO2 (3); SR1 (3); and TM7 (11) taxa.

In Fig.1 below are collectors curves for the total and uncultivated number of taxa identified as a function of the number of sites examined. The nine sites in the first two subjects show the greatest increase in taxa seen, the remaining eight subjects producing a less dramatic increase in taxa detected.

Fig. 1. Collectors curve for taxa vs. sites sampled.



In Table 1. below are the top 50 as-ye-uncultivated taxa found in this study, with total reads, and the percentage of those reads in each of the 7 niches sampled. The niche with the highest percentage of reads is highlighted in yellow. Please note that most taxa appear to occur preferentially in a specific oral niche. Knowing which oral niche to sample will significantly facilitate attempts to cultivate uncultivated taxa.

Table 1. Uncultivated taxa reads from oral sites.

HOT ID	Species	Reads	Buc	Pal	Tng	Ton	Thr	Sup	Sub
HOT_313	Prevotella sp.	47539	1.7	2.7	5.8	49.2	40.2	0.1	0.3
HOT_417	Leptotrichia sp.	30612	1.6	2.9	9.4	14.9	67.8	2.4	0.9
HOT_177	Actinomyces sp.	24681	26.0	3.9	0.3	0.3	2.6	29.6	37.3
HOT_346	TM7 [G-1] sp.	21110	46.4	0.3	0.0	0.2	0.3	43.8	8.9
HOT_097	Moryella sp.	16256	4.6	7.6	19.2	21.5	38.8	5.5	2.8
HOT_221	Leptotrichia sp.	13974	1.7	24.9	43.2	21.7	6.5	0.5	1.6
HOT_203	Fusobacterium sp.	12874	36.3	2.7	1.2	2.1	17.4	19.5	20.9
HOT_392	Leptotrichia sp.	8190	17.7	2.1	0.7	7.8	1.5	27.4	42.7
HOT_172	Actinomyces sp.	7753	2.7	12.2	18.7	18.1	48.1	0.0	0.1
HOT_219	Leptotrichia sp.	6363	13.4	2.6	0.5	0.1	1.8	18.6	63.0
HOT_215	Leptotrichia sp.	5876	8.7	7.9	14.9	29.0	19.7	12.4	7.4
HOT_353	TM7 [G-1] sp.	5685	3.1	2.4	0.5	0.7	1.1	45.2	47.0
HOT_348	TM7 [G-1] sp.	5594	1.8	1.1	0.6	1.0	0.3	86.0	9.2
HOT_352	TM7 [G-1] sp.	5506	2.6	7.6	9.7	28.8	48.3	2.2	0.7
HOT_212	Leptotrichia sp.	5434	17.1	20.4	7.2	5.6	1.4	33.3	15.0
HOT_511	Bacteroidetes [G-5] sp.	5426	2.6	0.1	0.2	18.5	0.0	0.7	77.8
HOT_081	Peptostreptococcaceae [XI][G-7] sp.	5368	4.5	1.2	0.4	3.9	4.2	3.1	82.6
HOT_300	Prevotella sp.	5144	7.0	0.3	0.1	1.2	1.5	50.4	39.6
HOT_286	Tannerella sp.	5058	6.2	1.3	0.4	0.8	2.9	77.0	11.3
HOT_437	TM7 [G-5] sp.	5035	5.2	0.7	0.1	0.4	2.5	29.3	61.9
HOT_100	Lachnospiraceae [G-3] sp.	4783	11.1	3.3	3.7	0.7	5.5	31.4	44.2
HOT_452	Fretibacterium sp.	4130	9.5	0.8	0.3	2.2	2.1	1.9	83.2
HOT_035	Haemophilus sp.	3774	21.2	44.6	0.3	24.5	2.1	0.0	7.2
HOT_322	Bergeyella sp.	3115	21.7	16.1	6.2	5.6	1.9	23.7	24.8
HOT_308	Alloprevotella sp.	2291	3.9	12.9	8.3	32.6	41.1	0.3	0.9
HOT_231	Treponema sp.	2257	3.7	0.2	0.0	55.5	0.0	4.0	36.7
HOT_349	TM7 [G-1] sp.	2015	5.9	2.2	0.2	1.2	4.6	62.1	23.7
HOT_223	Leptotrichia sp.	1925	6.7	0.7	0.0	1.1	0.9	7.9	82.6
HOT_463	Leptotrichia sp.	1756	8.7	4.1	9.2	18.2	29.3	24.1	6.3
HOT_205	Fusobacterium sp.	1715	15.0	0.6	0.7	7.4	1.4	24.7	50.1
HOT_075	Clostridiales [F-2][G-1] sp.	1697	15.6	3.7	4.5	23.8	10.8	12.1	29.5
HOT_914	Alloprevotella sp.	1677	8.8	33.6	18.7	24.9	12.9	0.4	0.7
HOT_902	Capnocytophaga sp.	1661	2.6	1.5	0.0	0.5	0.0	75.2	20.2
HOT_526	Prevotella sp.	1603	4.6	1.5	1.2	0.5	4.2	9.1	78.9
HOT_347	TM7 [G-1] sp.	1548	7.1	1.6	0.7	1.6	0.0	28.4	60.6
HOT_278	Porphyromonas sp.	1380	7.9	10.4	0.0	1.8	0.9	30.9	48.1
HOT_044	Campylobacter sp.	1236	2.9	72.8	2.3	11.2	7.4	0.0	3.4
HOT_369	Peptostreptococcaceae [XI][G-4] sp.	914	7.9	1.2	0.0	20.6	4.6	3.4	62.3
HOT_168	Peptococcus sp.	852	6.8	8.2	11.5	45.3	4.7	8.2	15.1
HOT_237	Treponema sp.	851	2.5	0.4	0.0	9.4	0.0	2.5	85.2
HOT_310	Prevotella sp.	820	4.7	18.6	14.6	38.5	15.0	3.7	4.9
HOT_091	Peptostreptococcaceae [XI][G-2] sp.	773	8.7	3.9	6.5	12.5	11.3	3.5	53.6
HOT_218	Leptotrichia sp.	750	0.6	17.4	26.2	55.1	0.0	0.1	0.5
HOT_360	Fretibacterium sp.	674	11.1	0.5	0.0	1.3	0.0	6.6	80.5
HOT_113	Peptostreptococcaceae [XIII][G-1] sp.	659	17.9	2.9	1.2	28.6	10.3	3.5	35.7
HOT_910	Moryella sp.	645	10.6	1.4	0.0	7.0	1.4	52.2	27.5
HOT_500	Lachnospiraceae [G-8] sp.	595	20.5	5.9	1.7	1.2	26.4	5.0	39.3
HOT_900	Bergeyella sp.	594	0.9	0.2	0.0	0.3	0.0	89.4	9.2
HOT_132	Veillonellaceae [G-1] sp.	583	25.0	0.5	0.0	1.2	4.8	7.2	61.3
HOT_134	Selenomonas sp.	571	7.2	0.0	0.0	2.2	0.0	10.1	80.4

## Link

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