Can limited populations influence microbiome diversity?
A metagenomics study of the rural Amish and non-Amish oral cavity via Ilumina sequencing

Phillip Belder and **Debra Wohl**
Elizabethtown College, Elizabethtown, PA

Abstract: The human mouth hosts one of the most diverse microbiomes within the human body in terms of heterogeneous environments and bacterial species prevalence. Recent research using metagenomics has begun potentially shifting oral microbiology from an exclusively single-species pathogen model to microbial community-based disease aetiology. Characterizing the oral microbiome offers understanding in disease pathology, as well as potential methods for predictive diagnosis and customized therapeutic methodology based on individual microbial community distribution. Constant migration into the continental United States has generated an expansive genetic admixture, largely homogenous within the general population. However, several genetically closed populations, such as the Old Order Amish exist within the United States, isolated through geographical and cultural means. This study analyzes the oral microbiome of the rural Amish population and rural non-Amish population of Lancaster, Pennsylvania. We hypothesize that a significant difference in distribution of bacterial species within the isolated Old-Order Amish microbiome population via phylogenetic analysis. Illumina sequencing was performed on two subject groups of Amish (n=15) and non-Amish (n=25) patients within geographical control. Saliva and supragingival plaque samples were collected anonymously. Sample DNA was extracted, amplified using labelled prokaryotic 16S primers, sequenced using the Illumina MiSeq platform. Statistical analysis will be performed by the Qualitative Insights into Microbial Ecology (QIIME) statistical package. Ongoing sequencing analysis will offer novel characterization of the Amish oral microbiome. This characterization evaluates potential variability within the isolated oral microbial populations, influencing understanding of polymicrobial oral disease, predictive diagnosis, and oral health habit research.

Introduction:

- Dynamic relationship between bacterial composition and oral health within oral cavity microbiome[1,2]
- Oral bacterial pathogens and biofilm are linked to several systemic diseases, including diabetes and cardiovascular disease[2,3]
- Sequencing technology began characterization of an estimated 500-700 common oral bacteria within oral microbiome[1,4]
- Host genetic variation has been shown to impact microbiome composition across other body sites[4]
- Amish metagenomics investigations have been utilized to study the gut, but not the oral cavity[5]
- Epidemiological studies of Amish show significant rates of oral disease states[6]

Current Study Objectives:

- Novel characterization of Amish oral microbiome via culture-independent sequencing and collection of oral health habit data
- Explore taxonomical variation within oral microbiome due to oral health habits and community isolation.

Materials and Methods:

Sample Collection Protocol

- Saliva Sample: Rinse (0.85% Saline)
- Plaque Samples: Supragingival Curette

- Sample Groups: (Amish n = 15) (Non-Amish n = 25)
- Survey form establishing oral health habits

DNA extraction and 16S PCR to amplify V4 region

- Illumina tag RNA primers
- 2x pooled PCR product for Illumina MiSeq Platform

- Analysis via Qualitative Insights into Microbial Ecology statistical package

Expected Results:

- Phylogenetic analysis will yield variation between Amish and control non-Amish
- Incidence of periodontal disease and oral disease state higher in Amish population
- Significant microbial community to match expected core oral microbiome

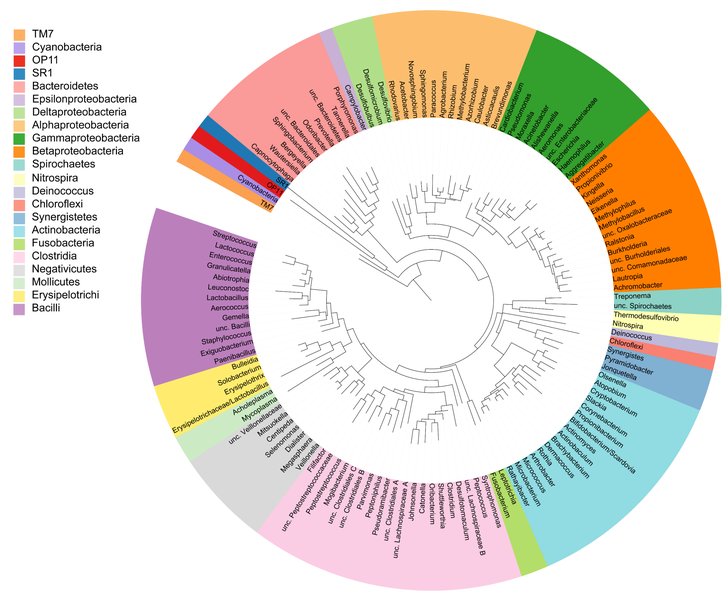

Figure 1: CORE Circular phylogenetic tree at level of genus, curated from 16s rDNA databases for oral microbiome[7]

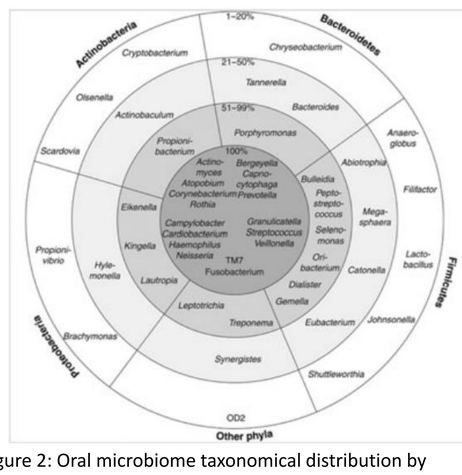

Figure 2: Oral microbiome taxonomical distribution by prevalence in patient pools via phylogenetic analysis[3]

Table 6. Odds ratios for periodontal disease

	Attachment loss ≥ 4 mm		Pocket depth ≥ 5 mm	
	odds ratio	*p*	odds ratio	*p*
Amish	2.27	0.0009	1.72	0.01
gender	2.32	0.0001	1.65	0.01
age (years)*				
25–34	3.82	0.0001	2.29	0.005
35–44	9.12	0.0001	1.88	0.03
45+	15.80	0.0001	2.08	0.01
Plaque	1.72	0.06	1.75	0.07
calculus	2.20	0.01	2.32	0.003
gingivitis	1.36	0.28	3.00	0.0005
behavior	1.09	0.28	N/A	N/A
disease knowledge	1.22	0.002	N/A	N/A

* Reference group was respondents 18–24 years old.

Figure 3: Epidemiological study of oral disease states incidence in Amish population compared to non-Amish control[46]

Current Status:

- Non-Amish population sample collection and processing complete
- Sequencing and data analysis forthcoming
- Sample collection via Amish Research Clinic ongoing

Statistical Analysis to Be Completed:

- Avg. Q score, alpha and beta diversity characterization of Amish oral microbiome
- Phyla and Genus abundance and distribution variation across Amish and non-Amish population
- Stratification by oral health habits and correlation analysis
- Comparison to proposed core microbiome studies to evaluate potential variations due to isolation.

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  HP-00068702 (Univ. of MD)

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