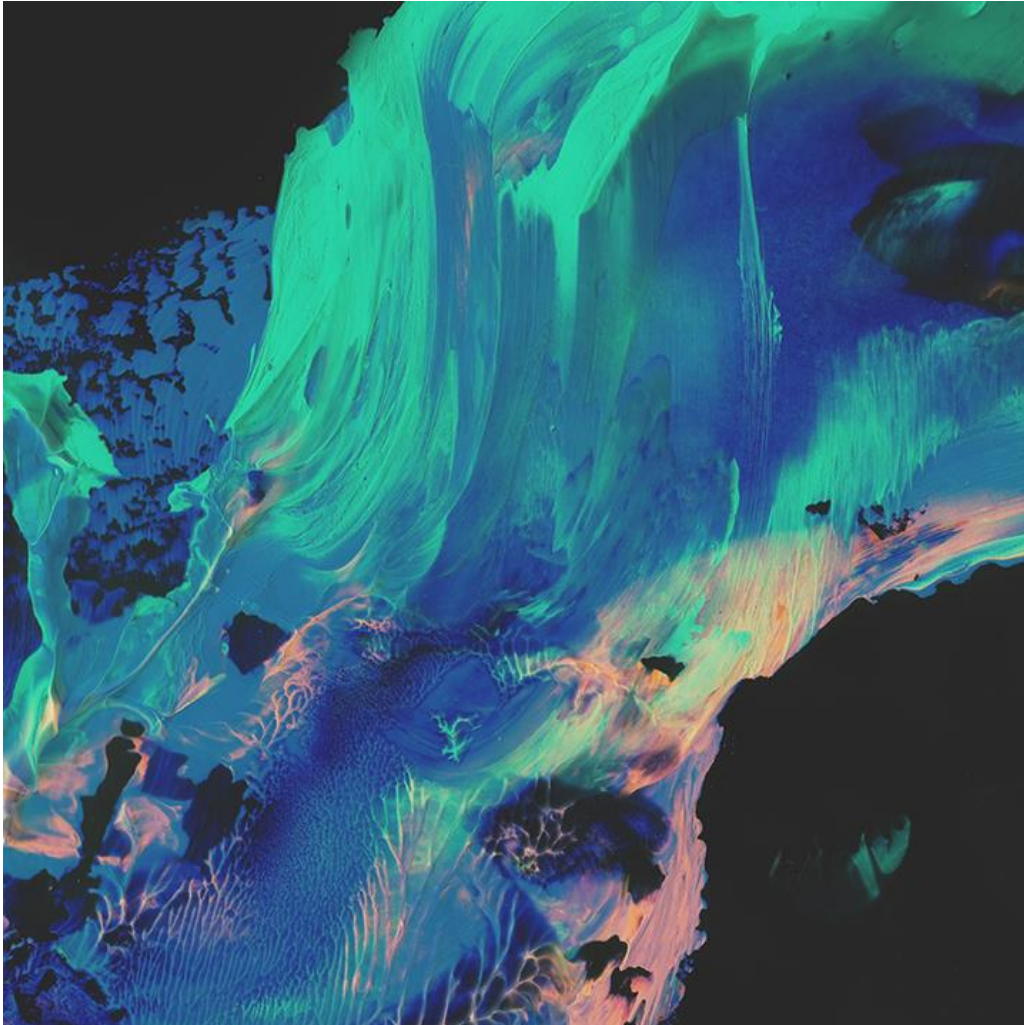




UMEÅ UNIVERSITET

# **Regional differences or similarities in human tooth biofilm microbiota: a pilot study**



Hanna Albertsson, Melina Isik

Tutor: Anders Esberg

Word in the abstract: 235 words

Word in text: 3560 words

Number of tables: 3

Number of figures: 1

Number of references: 26



## ABSTRACT

**Background:** More than 700 oral bacterial species have been found and together they make out the oral microbiota. Specific species have shown to correlate with various oral diseases such as caries and periodontitis, but also systemic diseases. Most studies have looked at the whole microbiota but the knowledge about tooth site-specific variation within supragingival plaque after lack of oral hygiene in healthy participants is limited.

**Aim:** This pilot study aimed to characterize variations in the supragingival plaque with regards to the; anterior (incisors and canines) and posterior (molars and premolars) teeth, upper and lower jaw, and left versus right tooth arches.

**Method:** After three days of accumulating plaque, supragingival tooth biofilm was collected from 16 different tooth sites, from six healthy participants. Bacterial DNA was extracted, and 16s rRNA gene (V3-V4) was amplified by PCR and sequenced on an Illumina MiSeq platform. Sequences were blasted and taxonomically allocated using the Human Oral Microbiome Database.

**Result:** In summary, 50 species showed a difference between the anterior and the posterior region, 30 species differed between the upper and lower jaw, and three species differed between the left and right sides.

**Conclusion:** This study indicates a difference in oral microbiota composition in supragingival plaque on different tooth regions. These findings emphasize the choice of method when analyzing the oral microbiota—also highlighting the importance of further understanding the dynamic forces driving local enrichment and reduction of specific species.



## BACKGROUND

The oral cavity consists of over 700 different bacteria species, and together they make out the oral microbiota (Dewhirst *et al.*, 2010). Due to its vast range of soft and hard tissue and its exposure to a multitude of environmental conditions, the oral cavity provides multiple surfaces for bacteria to adhere to. Unlike soft tissue, teeth offer a hard surface that does not naturally shred. The hard surface is covered by a thin layer called the pellicle. The pellicle consists of proteins from the host saliva, creating a surface to which bacteria can adhere to. Bacteria have adhesins and receptors, making it possible to adhere to each other and the pellicle, making a biofilm referred to as plaque (Kolenbrander *et al.*, 2000). Depending on many complex factors the bacteria is more or less likely to adhere and survive in the oral cavity. Factors such as: host pellicle genetics, the property of the bacteria, environmental changes, to mention a few (Kolenbrander *et al.*, 2000; Lamont *et al.*, 2018).

Oral microbiota have been shown to correlate with both oral- and systemic health and disease. Oral bacteria and their interaction with the host is a balance important to maintain health, an imbalance on the other hand could lead to a dysbiotic microbiota which in turn could cause disease (Sampaio-Maia *et al.*, 2016). Today it is well known that oral diseases such as caries and periodontitis are associated with specific complexes of bacteria (Lamont *et al.*, 2018). To understand oral bacteria and its correlation to diseases, it is essential to understand the oral microbiota in healthy individuals, i.e., no caries in dentin and no systemic disease.

Studies have explored variations in oral microbiota inter-individually using saliva or tooth scrapings and its maturation in the first years of life (Lif Holgersson *et al.*, 2020). In the past, most such studies relied on culturing and DNA probes in chip or blotting-based methods (Tanner *et al.*, 1997). In recent decades several multiplex sequencing methods that allow characterization of the microbial communities, i.e., microbiota, have become accessible. Studies have shown a difference between the microbiota composition on different soft tissue sites in the oral cavity in healthy individuals, showing a diverse microbiota that is site-specific (Mager *et al.*, 2003; Aas *et al.*, 2005). It is known that supra- and subgingival plaque consists of different microbiota compositions due to these sites' environmental disparity (Welch *et al.*, 2016; Lamont *et al.*, 2018). A few studies have compared site-specific microbiota in caries-free and caries-diseased children (Gross *et al.*, 2012; Richards *et al.*, 2017; Pang *et al.*, 2021). However, studies who have evaluated tooth regional variations in the oral cavity after lack of oral hygiene in healthy participants using a multiplex technique are limited (Simón-Soro *et al.*, 2013; Welch *et al.*, 2016). Welch *et al.* (2016) looked at the



structural organization within dental plaque in healthy subjects. Simón-Soro et al. (2013) reported tooth site-specific variation in two healthy subjects and indicated differences between different regions of the tooth arches, but the limited number of participants restrained the estimation of population variation. The knowledge about tooth site-specific variation within supragingival plaque is still limited (Simón-Soro *et al.*, 2013).

This pilot study aimed to characterize variations in the oral microbiota concerning collection sites. Supragingival plaque from 16 different tooth sites in six healthy participants were collected. 16S rRNA gene sequencing was employed to analyze the differences or similarities in the oral microbiota composition in different tooth regions: anterior (incisors and canines) and posterior (molars and premolars) teeth, upper and lower jaw, and left versus right tooth arches (Figure 1).



## **METHODS**

### **Study participants**

Six dental students (two men and four women born between 1981-1998) in the seventh semester of the Dentistry program at Umeå University, Umeå, Sweden, volunteered and gave their consent to participate in this study. Inclusion criteria were being able to speak Swedish or English, having no chronic disease, no active caries in dentin, not taking antibiotics three months prior to sampling and taking no probiotics one week before sampling. All participants fulfilled the requirements and were considered healthy.

### **Pre-sampling rules and documentations**

For this study, the participants accumulated plaque for three days. Oral hygiene, including tooth brushing, interproximal cleaning, fluoride, mouth wash, chlorhexidine rinsing, chewing gum, and alcohol consumption, was prohibited during the accumulation time. Products containing xylitol and probiotic bacteria were omitted one week prior to the accumulation period. The participants were informed to keep their daily eating, sleeping, and habitual routines during the accumulation period. Smoking and snuff were allowed if it was an already habitual routine by the participant and would therefore be kept identical during the whole accumulation period. All participants kept to the instructions and had no medication treatment, flu-like symptoms including stomach flu, fever, and changes in dietary during the accumulation period.

### **Oral examination**

Prior to sample collection, each participant's oral status was thoroughly examined by a dental student and then controlled by one general dentist at the department of cariology at Umeå university. The examination included radiographic examinations (bitewings) taken within a year together with a clinical inspection. Findings of interest were caries in dentin.

### **Sample collection**

Tooth biofilm sampling was performed at Norrland's Hospital University in Umeå, Sweden, spring of 2021. The participants were refrained from eating and drinking anything (apart from water) three hours before sample collection on the sampling day. Tooth biofilm was gathered by gently scratching the supragingival tooth surface (buccal, lingual and approximal sites) using sterile curettes. One curette per sampling



## UMEÅ UNIVERSITET

site, the samples were as follows: first and second molar, first and second premolar, canines, central and lateral incisors, from each quadrant, respectively, i.e., 16 curettes per individual. Collected tooth biofilm was swirled for 10 s in 200  $\mu$ L sterile ice-chilled 10mM Tris-EDTA (1xTE) solution in labelled Eppendorf tubes. Eppendorf tubes were kept on ice and stored in a -80°C freezer until used. The participants were recoded with a random number before analysis, ensuring that the samples could not be traced to participants, i.e., blinded.

### **DNA extraction**

DNA was extracted using the GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich, St. Louis, MO, USA) as previously described in Esberg *et al.*, 2021. Shortly, bacterial cells were collected by centrifugation (5 minutes at 13,000 rpm), resuspended and lysed in a buffer with lysozyme and mutanolysin, and treated with RNase A and Proteinase K. Lastly, the DNA was bound, washed, and eluted in 1xTE according to Sigma's Aldrich instruction. DNA Quality was evaluated by NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific, Uppsala, Sweden) and quantity with Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

### **Microbiota analysis**

From the extracted DNA, a specific region of the 16S rRNA gene (V3-V4) was amplified by PCR using KAPA hot start ready mix (KAPA HiFi HotStart ReadyMix (2x), United States Wilmington, MA, USA) and the 341F (ACGGGAGGCAGCAG) forward and 806R (GGACTACHVGGGTWTCTAAT) reverse primers (Caporaso *et al.*, 2012). The size of the PCR products was checked on a 0.8% agarose gel to verify the amplification of the target region. An equal amount of amplified products were pooled and sequenced using Illumina MiSeq (2x300bp). Obtained raw sequences were bioinformatically processed to remove primers and low-quality sequences (Callahan *et al.*, 2016; Bolyen *et al.*, 2019). Sterile water (Milli-Q Ultrapure Water) was used as negative control and a mix of known bacteria as a positive control. For each run, two negative and three positive controls were used. Washed sequences were matched against the Human Oral Microbiome Database, and identified species with  $\geq 2$  reads were kept and evaluated as a proportion of the bacterial composition (Chen *et al.*, 2010). In total, 96 samples were sequenced (16 samples x 6 individuals). The DNA extraction and microbiota analysis was done by expert personnel.



### **Data management and statistical analyses**

Data management and statistical analyses were done using the program IBM SPSS Statistics version 27 and Microsoft Office Excel 2010. The Mann-Whitney U test was used for group comparison, and the significance level was set to  $p < 0,05$ . For comparison of the anterior and the posterior; anterior region consists of canines and incisors, while the posterior region consists of the first and second molar as well as the first and second premolar.

### **Literature**

Literature used in this study was found by MeSH-terms and free-text searching on the international medical database PubMed. The tutor also provided literature. Searching terms used were; “oral microbiota”, “oral microbiome”, “biofilm”, “bacteria 16s rRNA v3-v4”, “16s rRNA gene”, “oral cavity”, “site-specific”, “dental plaque”, “oral health”, “oral diseases”, “dental pellicle”. Only articles available in full text, written in English were used.

### **Ethical reflection**

The voluntary participants in question are dental students. To avoid participant pressure, it was clarified that no special treatment or influence on the studies would occur regardless of participation. Information about how the samples and data were handled was communicated in writing to each participant well before the collection began. Sample collection only took place with the individual's consent. There was a minimal risk that the participants would be harmed during sampling. The participants could, at any time during the sampling period, with no further explanation, interrupt their consent without any effect on future treatment in dentistry or in studies. Gathered samples are anonymous and cannot be traced to any individual. To keep each participant's integrity and because of the low number of participants, no study group characteristics are being presented in this article. The result from this study can be used for future studies targeting the oral microbiota. Therefore, the benefit of this study outweighs the risk. The study, which was reviewed and approved by the Ethical Board for student scientific studies at the Department of Odontology, Umeå University, followed the four basic principles of healthcare studies, i.e., autonomy, make no harm, make good, and adhere to equality and justice. (Dnr 2017/450-31 with an addendum Dnr 2018-199-32).





## RESULTS

This pilot study included six healthy individuals in the range of 23-40 years old, of which four were women. No participant had active caries in dentin and were able to participate in the study. All participants were assigned strict regulations for three days before and during the plaque accumulation period. Regulations included not brushing their teeth and not using gum or any dental products that may affect the plaque. They were also told not to consume alcohol during this period and not to have taken antibiotics within the previous three months, nor probiotics the previous week. In total, 96 tooth biofilm samples were evaluated for their microbiota composition. After bioinformatic processing of the raw sequences, including removing low-quality- and primer sequences, the forward and reverse reads were paired and taxonomically classified against the Human Oral Microbiome Database (<http://www.homd.org>.) Taxonomically classified species with  $\geq 2$  reads were retained and their proportion evaluated. In total, 294 species were detected and evaluated for differences between (i) the anterior and posterior regions, (ii) the upper- versus lower jaw, and (iii) the left and right side of the mouth. Figure 1.

### Microbiota comparison of the anterior and posterior regions

Initially, the microbiota composition of the mouth's anterior and posterior region was compared. In total, 50 species showed a significant difference between the regions, and the top 20 species are presented in Table 1. Of the top 20, nine species had a higher abundance anteriorly than posteriorly, with the highest fold difference observed for *Porphyromonas gingivalis* and *Prevotella* sp. HMT 475 ( $>10$ -fold), but also including species like *Streptococcus sanguinis* (1.4-fold). Eleven species had a significantly higher abundance posteriorly than anteriorly, with the most prominent fold difference observed for *Capnocytophaga*, *Stomatobactum* and *Atopobium* species ( $>10$ -fold), but also observed for species like *Streptococcus gordonii* (3.7-fold).

### Comparison of the upper- versus lower jaw microbiota composition

Next, we compared the microbiota composition of the upper and lower jaw. In total, 30 species showed to be significant, and the top 20 species are presented in Table 2. Of the top 20, twelve species showed an increased abundance in the upper jaw e.g., *Alloprevotella* sp. HMT 473 and *Oribacterium sinus* ( $>10$ -fold), but also including *Streptococcus gordonii* (2.9-fold). Eight species had a significantly higher abundance in the lower jaw with the most observed change in the species *Bacteroidales* [G-2]





bacterium HMT 274, *Aggregatibacter aphrophilus*, and *Campylobacter rectus* (>10-fold), but also shown for e.g., *Streptococcus sanguinis* (2.3-fold).

### **Microbiota of the left and right side of the mouth**

Finally, the microbiota composition of the left versus right side of the mouth was compared. Three species displayed significant differences, presented in Table 3.

*Granulicatella elegans* showed significantly higher abundance on the left side, whereas *Leptotrichia* sp. HMT 223 and *Capnocytophaga* sp. HMT 864 on the right side.



## DISCUSSION

**Summary** - This pilot study aimed to evaluate bacterial composition in supragingival plaque relative to tooth- or oral regions. More specifically, the anterior (canines and incisors) versus posterior (molars and premolars), upper versus lower jaw, and left versus right side, were compared (Figure 1). The abundance of each bacterial specie in each sample was compared using the non-parametric Mann-Whitney U test. In summary, 50 species showed a difference between the anterior and the posterior region, 30 species differed between the upper and lower jaw and three species differed between the left and right sides. This highlights a likely global change in the microbiota composition with respect to the anterior versus the posterior part of the oral cavity, and to a lesser extent, the upper versus lower jaw. The limited differences between the left versus right side suggest no global change, instead temporary or sporadic alterations.

**Compared to other studies** - Sreenivasan *et al.*, (2017) discovered site-specific bacterial compositions comparing anterior and posterior teeth surfaces. Concluding that posterior parts of both arch and/or the entire tooth, as well as approximal vestibular surfaces, obtained more plaque compared to anterior teeth and mid-vestibular regions. Additionally, their study indicated that molar and lingual regions adhere more plaque than anteriorly. However, current study analyzed the proportion of bacteria and thereby evaluated the composition of the plaque. These two studies together suggest that both the amount and composition differ between the anteriorly and posteriorly regions.

Another study (Simón-Soro *et al.*, 2013), looked at bacterial composition in samples from supragingival plaque and from the gingival sulci in two individuals. They found surface-specific differences in bacterial composition e.g., a more similar composition was found in quadrant three and four than in one and two. They also found that composition varied between the upper and lower jaw. This study supports site-specific bacterial differences in the mouth within the individual tooth and between teeth in supragingival plaque. However, due to the limited number of participants the study did not allow comparison of individual bacterial species.

Sreenivasan *et al.*, (2010) also suggested regional differences for plaque composition between anterior versus posterior regions in the mouth. Although, this study does not use multiplex sequencing or specify microbiota composition, rather measured viable bacteria under anaerobic conditions in 37 degrees. Overall, this suggested anterior/posterior differences; here we have confirmed on a species level



resolution an anterior and posterior difference. Altogether, rather few studies have studied regional and tooth specific differences.

### **What could be the driving force of change?**

*Chewing cycles and patterns, tongue, lips, and speech* - The anterior incisors are used to bite off food, and the premolars and molars are used to grind the food, making the posterior region the most active part of the masticatory pattern (Okeson, 2013, ch.2). Lips help the food stay in the mouth. Bucca and tongue help direct the food to the occlusal surfaces to grind it during the chewing stroke. The tongue wipes clear the teeth of access to food after swallowing (Okeson, 2013, ch.2). The knowing movement during mastication of lips, tongue, and bucca, together with the chewing pattern, might be a factor in the differences between the anterior and the posterior region and the upper and lower jaw. Believing the movements to affect the shredding of plaque and its composition, however, no studies to prove this was found.

*Oxygen* - Oxygen reaches mainly the outmost part of the supragingival plaque. Bacteria are often divided into aerobes, facultative aerobes, and anaerobes, depending on how well they survive oxidative stress. Therefore, access to oxygen metabolism is directly related to the biofilm's characteristics (Re, 1995). Knowing this, the anterior teeth might have an increased access to oxygen by being closer to the outside environment than the posterior teeth. However, this study does not characterize individual species ability to cope with oxygen.

*Saliva and salivary glands* - Saliva plays an important role in maintaining oral health. To mention a few functions of saliva; neutralizing pH, remineralize tooth surface and clear the oral cavity of acid and sugar. It also clears bacteria both mechanically and via antimicrobial agents such as proteins, peptides, and enzymes (Scannapieco, 1994). There are three major salivary glands: Parotid, Sublingual and Submandibular. The Parotid gland discharges close to the buccal side of the secondary maxillary molar and secretes serous (protein rich and low viscous) saliva, and is the primary gland to produce amylase- enzyme which decomposes starch and inhibits several bacterial species to grow. The sublingual gland discharges from the lingual sulcus and secretes a more mucus (higher viscous) saliva. In contrast, the submandibular gland discharges at the side of the lingual frenulum, creating a combination of serous and mucous saliva (Scannapieco, 1994). Studies have shown that when stimulated, saliva has a higher velocity and thereby a greater clearance of acid and sugar on the lingual sites of the teeth and lowest on the buccal sites, except for the buccal sites of the maxillary molars, where parotid glands discharge (Dawes, 2008).



Saliva could be one explanation to the differences found between the anterior and posterior region and the upper and lower jaw.

*Anatomy of individual teeth* - Bacterial colonization are commonly found in places difficult to brush or interproximal cleaning including pits, fissures, crowding/contact points, in/under filling joints (dental work), retainers and other artifacts. Pits and fissures occur on molars and premolars, whilst crowding is more common between incisors and canines. These areas have a likely reduced flow or access of saliva and oxygen, potentially enriching anaerobic and acid-tolerant bacterial species (Re, 1995). Mineralization defects, fluorosis, root surface or dentine exposure have a higher sensitivity to e.g., acid-producing bacteria, therefore also caries (Abou *et al.*, 2016). Tooth surfaces substituted with restorative materials (e.g., composite fillings) are more likely to adhere and accumulate plaque compared to enamel due to surface quality differences (Litonjua *et al.*, 2012). Food may also be more frequently trapped in specific regions (anatomic features) impacting growth and microbiota composition. It would be interesting to look further into local differences between the participants and its potential correlation to microbiota composition. But due to limitations of this study we have not looked closely into this.

**Strengths and limitations** - This study involved six participants which is a limitation; however, this allowed for collection of multiple sites per participant and in total this study involves 96 tooth biofilm samples. Due to the limited number of subjects used here, our study analysis was performed on group level and not the individual level; thus, species carried by a limited number of participants may be influenced. The limited number of participants could also risk the potential of one individual driving the result. The participants were all students from a dental school which could affect, e.g., oral health, lifestyle and food selection (maybe preferably non-cariogenic food); however, the impact on the results is unclear. Given the participant's dental knowledge about what can affect plaque accumulation, instructions given were likely to be followed more precisely compared to a potential participant who may not have had this knowledge.

In this study, three days of plaque accumulation were chosen, as this gives the biofilm more time to adapt to local, oral conditions and bacterial partners. However, plaque accumulation time may influence the results as species proportions may fluctuate during biofilm maturation.



## UMEÅ UNIVERSITET

Another limitation could be that periodontal pockets were not registered with the argument that only supragingival plaque was collected; however, pocket depths and gingivitis cannot be excluded in affecting supragingival plaque formation.

This study analyzed proportion of bacterial species rather than absolute abundance to reduce the risk and influence of the amount of plaque collected, potentially providing a more comparable result.

**Conclusions** - The present pilot study supports overall regional microbiota differences in supragingival plaque but also highlights specific species. This study illustrates in some cases larger differences between regions than the authors expected, however these findings need to be repeated in a larger independent sample. This study may also shed light on the importance of the method used for collecting tooth biofilm samples linked to a research aim. For example, caries or periodontal studies may favor specific sites instead of pooling tooth surfaces. The target species of interest may therefore in some cases be missed because the main part of the sample would represent healthy sites. For clinical and individual use this study shows that some tooth sites may require greater focus to notice, examine, and clean, compared to other regions that are relatively freely accessible. To further understand the oral cavity dynamic features on the microbiota composition and selection/exclusion may potentially contribute to further treatment tools/plans. For example, to further understand why *P. gingivalis*, a known periodontal associated bacterium, is observed >10-fold more in the anterior part of the mouth is interesting, but to do this more research is required.

## ACKNOWLEDGMENTS

Linda Johansson for assisting clinical sampling, Agneta Rönnlund for DNA extractions, Anders Esberg our mentor and for performing sequencing and bioinformatics, and Ingegerd Johansson for mentoring/discussions.



## REFERENCES

- Aas, J. A., Paster, B. J., Stokes, L. N., Olsen, I., & Dewhirst, F. E. (2005) "Defining the normal bacterial flora of the oral cavity," *J. Clin. Microbiol.* 43(11), pp. 5721–5732. doi:10.1128/JCM.43.11.5721-5732.2005.
- About Neel, E. A., Aljabo, A., Strange, A., Ibrahim, S., Coathup, M., Young, A. M., Bozec, L., & Mudera, V. (2016). "Demineralization-remineralization dynamics in teeth and bone," *Int. J. Nanomed.* 11, 4743–4763. doi.org:10.2147/IJN.S107624.
- Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., Alexander, H., Alm, E. J. (2019) "Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2," *Nat. Biotechnol.* pp. 852–857. doi:10.1038/s41587-019-0209-9.
- Callahan, B.J. Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J., & Holmes, S. P. (2016) "DADA2: High-resolution sample inference from Illumina amplicon data," *Nat. Methods*, 13(7), pp. 581–583. doi:10.1038/nmeth.3869.
- Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Huntley, J., Fierer, N., Owens, S. M., Betley, J., Fraser, L., Bauer, M., Gormley, N., Gilbert, J. A., Smith, G., & Knight, R. (2012) "Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms," *ISME Journal*, 6(8), pp. 1621–1624. doi:10.1038/ismej.2012.8.
- Chen, T., Yu, W. H., Izard, J., Baranova, O. V., Lakshmanan, A., & Dewhirst, F. E. (2010) "The Human Oral Microbiome Database: a web accessible resource for investigating oral microbe taxonomic and genomic information.," *Database: j. biol. databases curation*, 2010. doi:10.1093/database/baq013.
- Dawes C. (2008) "Salivary flow patterns and the health of hard and soft oral tissues," *JADA*, 139, pp. 18S-24S. PMID: 18460676
- Dewhirst, F. E., Chen, T., Izard, J., Paster, B. J., Tanner, A. C., Yu, W. H., Lakshmanan, A., & Wade, W. G. (2010) "The human oral microbiome," *J. Bacteriol.* 192(19), pp. 5002–5017. doi:10.1128/JB.00542-10.
- Esberg, A., Eriksson, L., Hasslöf, P., Haworth, S., Lif Holgersson, P., & Johansson, I. (2021) "Using oral microbiota data to design a short sucrose intake index," *Nutrients*, 13(5). doi:10.3390/nu13051400.
- Gross, E. L., Beall, C. J., Kutsch, S. R., Firestone, N. D., Leys, E. J., & Griffen, A. L. (2012) "Beyond Streptococcus mutans: Dental Caries Onset Linked to Multiple Species by 16S rRNA Community Analysis," *PLoS ONE*, 7(10). doi:10.1371/journal.pone.0047722.



- Kolenbrander, P. E., Palmer, R. J., Jr, Rickard, A. H., Jakubovics, N. S., Chalmers, N. I., & Diaz, P. I. (2000) "Bacterial interactions and successions during plaque development," *Blackwell Munksgaard PERIODONTOLOGY*. doi: 10.1111/j.1600-0757.2006.00187.x. PMID: 16930306.
- Lamont, R.J., Koo, H. and Hajishengallis, G. (2018) "The oral microbiota: dynamic communities and host interactions," *Nat. Rev. Microbiol.* pp. 745–759. doi:10.1038/s41579-018-0089-x.
- Lif Holgersson, P., Esberg, A., Sjödin, A., West, C. E., & Johansson, I. (2020) "A longitudinal study of the development of the saliva microbiome in infants 2 days to 5 years compared to the microbiome in adolescents," *Sci. Rep.*, 10(1), p. 9629. doi:10.1038/s41598-020-66658-7.
- Litonjua, L. A., Cabanilla, L. L., & Abbott, L. J. (2012). "Plaque formation and marginal gingivitis associated with restorative materials," *Compend. Contin. Educ. Dent. (Jamesburg, N.J. : 1995)*, 33(1), e6–e10. PMID: 23268568.
- Mager, D. L., Ximenez-Fyvie, L. A., Haffajee, A. D., & Socransky, S. S. (2003) "Distribution of selected bacterial species on intraoral surfaces," *J Clin Periodontol.* doi: 10.1034/j.1600-051x.2003.00376.x.
- Mark Welch, J. L., Rossetti, B. J., Rieken, C. W., Dewhirst, F. E., & Borisy, G. G. (2016) "Biogeography of a human oral microbiome at the micron scale," *PNAS*, 113(6), pp. E791–E800. doi:10.1073/pnas.1522149113.
- Marquis R. E. (1995) *Oxygen metabolism, oxidative stress and acid-base physiology of dental plaque biofilms*, *Journal of Industrial Microbiology*. doi:10.1007/BF01569826
- Okesson, Jefferey, P. (2013) *Management of temporomandibular disorders and occlusion*. 7th ed. Missouri: Elsevier.
- Pang, L., Wang, Y., Ye, Y., Zhou, Y., Zhi, Q., & Lin, H. (2021) "Metagenomic Analysis of Dental Plaque on Pit and Fissure Sites With and Without Caries Among Adolescents," *Front. Cell. Infect. Microbiol.*, 11. doi:10.3389/fcimb.2021.740981.
- Richards, V. P., Alvarez, A. J., Luce, A. R., Bedenbaugh, M., Mitchell, M. L., Burne, R. A., & Nascimento, M. M. (2017) "Microbiomes of sitespecific dental plaques from children with different caries status," *Infect. Immun.* 85(8). doi:10.1128/IAI.00106-17.
- Sampaio-Maia, B., Caldas, I. M., Pereira, M. L., Pérez-Mongiovi, D., & Araujo, R. (2016) "The Oral Microbiome in Health and Its Implication in Oral and Systemic Diseases," *Adv. Appl. Microbiol.* 97, pp. 171–210. doi:10.1016/bs.aambs.2016.08.002.





UMEÅ UNIVERSITET

- Scannapieco, F.A. (1994) "Saliva-Bacterium Interactions in Oral Microbial Ecology," *Crit. Rev. oral biol. Med.* doi: 10.1177/10454411940050030201
- Simón-Soro, A., Tomás, I., Cabrera-Rubio, R., Catalan, M. D., Nyvad, B., & Mira, A. (2013) "Microbial geography of the oral cavity," *J. Dent. Res.* 92(7), pp. 616–621. doi:10.1177/0022034513488119.
- Sreenivasan, P. K. DeVizio, W., Prasad, K. V., Patil, S., Chhabra, K. G., Rajesh, G., Javali, S. B., & Kulkarni, R. D. (2010). "Regional differences within the dentition for plaque, gingivitis, and anaerobic bacteria," *J. Clin. Dent.* 21(1), 13–19.
- Sreenivasan, P. K., & Prasad, K. (2017). "Distribution of dental plaque and gingivitis within the dental arches," *Int. J. Med. Res.* 45(5), 1585–1596. doi.org:10.1177/0300060517705476.
- Tanner, A. Maiden, M.F., Lee, K., Shulman, L. B., Weber, H. P. (1997) "Dental implant infections". *Clin Infect Dis.* sep;25 Suppl 2:S213-7. doi: 10.1086/516243.



## TABLES

**Table 1.** Bacterial abundance differences between anterior and posterior region of the mouth. Presented are mean and 95% confidence interval (95%CI) of the top 20 bacteria with the lowest p-value. P-value was set to <0.05. Bacterial abundance was compared using Mann-Whitney U test. Within the top 20 species, nine had a higher abundance in the anterior region, see bold in left section, and eleven in the posterior region, see bold in right section. Species in each section is presented from lowest p-value to highest.

Species	Anterior mean (95%CI)	Posterior mean (95%CI)	P-value
<b>Species increased in the anterior part</b>			
<i>Aggregatibacter paraphrophilus</i>	<b>3.86E-03 (1.70E-03 - 6.03E-03)</b>	1.98E-03 (-1.46E-04 - 4.10E-03)	1.9E-04
<i>Selenomonas</i> sp. HMT 892	<b>5.11E-04 (2.18E-04 - 8.04E-04)</b>	1.27E-04 (-6.74E-05 - 3.20E-04)	0.001
<i>Saccharibacteria</i> (TM7) [G-1] <i>bacterium</i> HMT 952	<b>2.02E-03 (9.78E-04 - 3.07E-03)</b>	5.27E-04 (3.90E-05 - 1.02E-03)	0.005
<i>Porphyromonas</i> sp. HMT 930	<b>3.60E-04 (1.45E-04 - 5.75E-04)</b>	5.93E-05 (-8.98E-06 - 1.28E-04)	0.006
<i>Prevotella</i> sp. HMT 475	<b>1.33E-04 (1.53E-05 - 2.50E-04)</b>	6.66E-06 (-6.74E-06 - 2.01E-05)	0.008
<i>Streptococcus sanguinis</i>	<b>6.23E-02 (5.23E-02 - 7.23E-02)</b>	4.59E-02 (3.85E-02 - 5.34E-02)	0.009
<i>Alloprevotella</i> sp. HMT 473	<b>1.73E-03 (4.88E-04 - 2.98E-03)</b>	1.85E-04 (3.27E-05 - 3.37E-04)	0.011
<i>Porphyromonas gingivalis</i>	<b>3.09E-05 (5.87E-06 - 5.60E-05)</b>	0.00E+00 (0.00E+00 - 0.00E+00)	0.012
<i>Capnocytophaga leadbetteri</i>	<b>3.44E-02 (2.55E-02 - 4.34E-02)</b>	2.02E-02 (1.26E-02 - 2.79E-02)	0.013
<b>Species increased in the posterior part</b>			
<i>Streptococcus gordonii</i>	1.16E-02 (7.70E-03 - 1.55E-02)	<b>4.27E-02 (2.96E-02 - 5.58E-02)</b>	3.5E-07
<i>Veillonella parvula</i>	2.51E-02 (2.03E-02 - 2.99E-02)	<b>3.97E-02 (3.48E-02 - 4.46E-02)</b>	6.5E-05
<i>Leptotrichia hongkongensis</i>	2.75E-03 (1.62E-03 - 3.88E-03)	<b>1.34E-02 (-7.74E-03 - 1.90E-02)</b>	1.1E-04
<i>Leptotrichia</i> sp. HMT 215	3.78E-03 (2.89E-03 - 4.67E-03)	<b>7.14E-03 (5.68E-03 - 8.61E-03)</b>	2.4E-04
<i>Campylobacter gracilis</i>	5.87E-04 (1.55E-04 - 1.02E-03)	<b>1.53E-03 (9.19E-04 - 2.13E-03)</b>	0.001
<i>Peptidiphaga</i> sp. HMT 183	5.57E-05 (7.73E-06 - 1.04E-04)	<b>3.10E-04 (1.63E-04 - 4.58E-04)</b>	0.001
<i>Fusobacterium nucleatum subsp. animalis</i>	2.19E-04 (-2.22E-04 - 6.60E-04)	<b>1.46E-03 (-1.02E-04 - 3.02E-03)</b>	0.003
<i>Stomatobaculum</i> sp. HMT 097	9.34E-05 (3.32E-05 - 1.54E-04)	<b>1.09E-03 (4.91E-04 - 1.69E-03)</b>	0.003
<i>Capnocytophaga haemolytica</i>	0.00E+00 (0.00E+00 - 0.00E+00)	<b>1.44E-04 (-6.32E-05 - 3.51E-04)</b>	0.003
<i>Atopobium parvulum</i>	2.40E-05 (-3.61E-06 - 5.16E-05)	<b>2.73E-04 (5.82E-05 - 4.87E-04)</b>	0.006
<i>Selenomonas noxia</i>	7.10E-04 (6.20E-05 - 1.36E-03)	<b>1.20E-03 (4.96E-04 - 1.91E-03)</b>	0.012



**Table 2.** Bacterial differences between upper and lower jaw. Presented are mean and 95% confidence interval (95%CI) of the top 20 bacteria with the lowest p-value. P-value was set to <0.05. Bacterial abundance was compared using Mann-Whitney U test. Within the top 20 species, twelve had a higher abundance in the upper jaw, see bold in left section, and eight in the lower jaw, see bold in right section. Species in each section is presented from lowest p-value to highest.

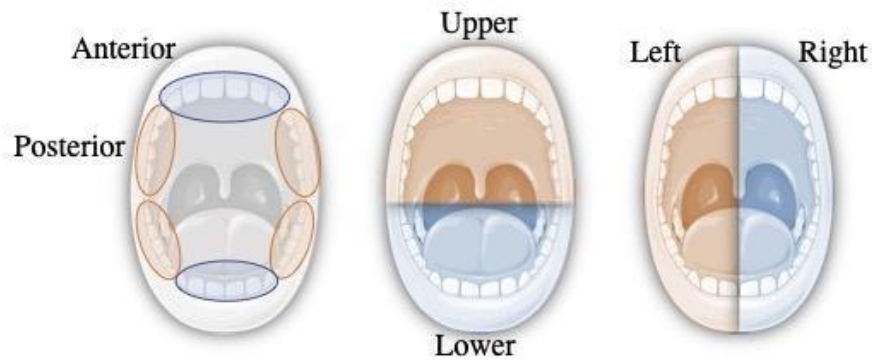
Species	Upper jaw mean (95%CI)	Lower jaw mean (95%CI)	P-value
<b>Species increased in the upper jaw</b>			
<i>Streptococcus gordonii</i>	<b>4.01E-02 (2.80E-02 - 5.22E-02)</b>	1.42E-02 (6.95E-03 - 2.15E-02)	1.5E-08
<i>Fusobacterium periodonticum</i>	<b>9.98E-03 (7.64E-03 - 1.23E-02)</b>	6.35E-03 (3.93E-03 - 8.77E-03)	5.1E-04
<i>Leptotrichia</i> sp. HMT 215	<b>6.42E-03 (5.27E-03 - 7.57E-03)</b>	4.50E-03 (3.10E-03 - 5.90E-03)	0.002
<i>Alloprevotella</i> sp. HMT 473	<b>1.79E-03 (5.47E-04 - 3.03E-03)</b>	1.30E-04 (6.61E-06 - 2.53E-04)	0.003
<i>Stomatobaculum</i> sp. HMT 097	<b>8.31E-04 (3.31E-04 - 1.33E-03)</b>	3.52E-04 (-2.76E-05 - 7.32E-04)	0.004
<i>Veillonella parvula</i>	<b>3.71E-02 (3.24E-02 - 4.18E-02)</b>	2.77E-02 (2.22E-02 - 3.32E-02)	0.005
<i>Veillonella rogosae</i>	<b>9.09E-04 (3.36E-04 - 1.48E-03)</b>	1.34E-04 (-1.20E-05 - 2.80E-04)	0.007
<i>Gemella sanguinis</i>	<b>1.04E-03 (4.07E-04 - 1.67E-03)</b>	3.00E-04 (4.13E-05 - 5.58E-04)	0.009
<i>Leptotrichia</i> sp. HMT 221	<b>9.16E-04 (3.79E-04 - 1.45E-03)</b>	1.65E-04 (1.32E-05 - 3.16E-04)	0.012
<i>Neisseria flavescens</i>	<b>3.46E-04 (2.85E-05 - 6.63E-04)</b>	0.00E+00 (0.00E+00 - 0.00E+00)	0.012
<i>Oribacterium sinus</i>	<b>5.85E-05 (5.75E-06 - 1.11E-04)</b>	3.08E-06 (-3.11E-06 - 9.27E-06)	0.014
<i>Capnocytophaga gingivalis</i>	<b>4.39E-02 (3.33E-02 - 5.45E-02)</b>	3.21E-02 (2.26E-02 - 4.17E-02)	0.037
<b>Species increased in the lower jaw</b>			
<i>Bergeyella</i> sp. HMT 322	6.96E-03 (6.09E-03 - 7.82E-03)	<b>1.59E-02 (1.32E-02 - 1.86E-02)</b>	2.0E-09
<i>Streptococcus sanguinis</i>	4.24E-02 (3.56E-02 - 4.91E-02)	<b>6.59E-02 (5.60E-02 - 7.57E-02)</b>	7.4E-05
<i>Bacteroidales</i> [G-2] <i>bacterium</i> HMT 274	5.21E-05 (-1.70E-05 - 1.21E-04)	<b>5.73E-04 (1.14E-04 - 1.03E-03)</b>	5.1E-04
<i>Aggregatibacter aphrophilus</i>	8.42E-05 (-6.87E-06 - 1.75E-04)	<b>4.43E-03 (-8.48E-04 - 9.70E-03)</b>	7.2E-04
<i>Campylobacter rectus</i>	1.40E-03 (8.32E-04 - 1.97E-03)	<b>2.92E-03 (2.08E-03 - 3.77E-03)</b>	0.009
<i>Leptotrichia</i> sp. HMT 225	1.56E-03 (7.18E-04 - 2.41E-03)	<b>5.65E-03 (2.16E-03 - 9.15E-03)</b>	0.016
<i>Leptotrichia</i> sp. HMT 212	9.82E-03 (7.19E-03 - 1.25E-02)	<b>1.62E-02 (1.25E-02 - 1.99E-02)</b>	0.018
<i>Prevotella saccharolytica</i>	1.78E-04 (-7.20E-05 - 4.28E-04)	<b>3.67E-04 (1.81E-04 - 5.54E-04)</b>	0.019



**Table 3.** Comparison of bacterial abundance between the left and right side of the mouth. Presented are mean and 95% confidence interval (95%CI) of the species with a p-value <0.05. Bacterial abundance was compared using Mann-Whitney U test. Three species showed a significant difference, one had a higher abundance in the left side, see bold in left section, and two in the right side, see bold in right section. Species in each section is presented from lowest p-value to highest.

<b>Species</b>	<b>Left mean (95%CI)</b>	<b>Right mean (95%CI)</b>	<b>P-value</b>
<b>Species increased in the left side of the mouth</b>			
<i>Granulicatella elegans</i>	<b>4.24E-05 (-9.76E-06 - 9.47E-05)</b>	2.09E-04 (3.53E-05 - 3.82E-04)	0.007
<i>Leptotrichia</i> sp. HMT 223	<b>2.51E-04 (1.31E-05 - 4.90E-04)</b>	1.10E-05 (-1.11E-05 - 3.31E-05)	0.046
<b>Species increased in the right side of the mouth</b>			
<i>Capnocytophaga</i> sp. HMT 864	1.26E-03 (4.91E-04 - 2.04E-03)	<b>3.48E-04 (1.04E-04 - 5.93E-04)</b>	0.049



**FIGURE WITH LEGENDS****Figure 1. Study design and aims.**

The three aims of this study. Left: aim 1, anterior vs. posterior region. Anterior includes incisive and canines and posterior include premolars together with first and second molar, in both upper and lower jaw. Middle: aim 2, upper vs. lower jaw. Right: aim 3, left vs. right side, dividing the oral cavity into left and right side with the cutoff between the central incisors.