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The Severity of Human Peri-Implantitis Lesions Correlates with the Level of Submucosal Microbial Dysbiosis

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Abstract

Aim: To cross-sectionally analyze the submucosal microbiome of peri-implantitis (PI) lesions at different severity levels.
Materials and Methods: Microbial signatures of 45 submucosal plaque samples from untreated peri-implantitis lesions obtained from 30 non-smoking, systemically healthy subjects were assessed by 16s sequencing. Linear mixed models were used to identify taxa with differential abundance by probing depth, after correction for age, gender, and multiple samples per subject. Network analyses were performed to identify groups of taxa with mutual occurrence or exclusion. Subsequently, the effects of peri-implant probing depth on submucosal microbial dysbiosis was calculated using the microbial dysbiosis index.
Results: In total, we identified 337 different taxa in the submucosal microbiome of peri-implantitis. Total abundance of 12 taxa correlated significantly with increasing probing depth; a significant relationship with lower probing depth was found for 16 taxa. Network analysis identified two mutually exclusive complexes associated with shallow pockets and deeper pockets, respectively. Deeper peri-implant pockets were associated with significantly increased dysbiosis.
Conclusion: Increases in peri-implant pocket depth are associated with substantial changes in the submucosal microbiome and increasing levels of dysbiosis.

Clinical Relevance

Scientific rationale for the study: Peri-implantitis is increasingly prevalent and poses a significant clinical challenge. Thus far, the microbial signatures of peri-implantitis sites of different severity have not been characterized. Specifically, it is unknown whether deeper peri-implant pockets feature an increased level of dysbiosis.
Principle findings: The microbiome of peri-implantitis lesions consists of both common periodontal bacteria and novel species. Peri-implant probing depth correlates with distinct microbial profiles, and deeper pockets show higher levels of dysbiosis.
Practical implications: Peri-implant pockets contain disease-associated biofilms that become increasingly dysbiotic with increasing probing depth.

Introduction

Peri-implantitis is an increasingly prevalent chronic inflammatory disease, with up to 22 % of patients being affected (Derks and Tomasi, 2015). Similar to periodontitis, the chronic inflammatory condition affecting teeth and caused by dysbiotic biofilms in susceptible hosts. Principal clinical signs of peri-implantitis include pocketing, bleeding-on-probing and/or suppuration. Both diseases lead to destruction of supporting tissues and, in terminal stages, to the loss of the implant or the tooth (Lindhe et al., 2008). Both diseases share common risk factors, including environmental exposures, such as smoking and poor oral hygiene, and systemic predispositions such as diabetes. Interestingly, a history of periodontitis has been shown to increase the risk for subsequent peri-implantitis, suggesting commonalities in their etiology (Sousa et al., 2016, Stacchi et al., 2016, Renvert and Quirynen, 2015). Still, peri-implantitis differs from periodontitis with respect to anatomic features and cellular composition of the lesion (Carcuac and Berglundh, 2014, Berglundh et al., 2011) and, most notably, to disease progression rate which is significantly higher in peri-implantitis than in periodontitis (Salvi et al., 2017).

Thus far, the pathophysiological underpinnings accounting for the faster progression rate in peri-implantitis are poorly understood. Distinctions in key features of the lesion-associated microbiomes of the two diseases can conceivably play a role in this respect. Specifically, peri-implantitis lesions of increasing clinical severity may be associated with specific microbial signatures that may result in accelerated progression.

Thus far, no studies have investigated the relationship between peri-implantitis severity and the associated submucosal microbial patterns. Previous work has mainly focused on identifying microbial differences between healthy and diseased peri-implant sites (Al-Ahmad et al., 2018, Apatzidou et al., 2017, Sanz-Martin et al., 2017, Shiba et al., 2016, Zheng et al., 2015, Maruyama et al., 2014, Tamura et al., 2013, Kumar et al., 2012, Koyanagi et al., 2010). Importantly, confounders including general health status, smoking, age, gender, and prior treatment have generally not been accounted for in the existing literature, hampering inferences on the microbial features of peri-implantitis at different levels of disease severity.

In this work, we therefore sought to characterize the submucosal microbiome of peri-implantitis (PI) lesions at different severity levels. To do so, we used 16s rRNA sequencing to analyze of previously untreated peri-implantitis lesions from systemically healthy, non-smoking subjects. We identified taxa with significant associations with deeper or shallower peri-implant pockets and demonstrate a link between deeper probing depths and increasing levels of microbial dysbiosis.

Material and Methods

Study population and clinical procedures

The protocol for the study was approved by the institutional review boards / ethics committees at the Universities of Bonn, Würzburg, Tübingen and Düsseldorf, Germany, and the regional ethics committee of the Jönköping district, Sweden. The study was conducted in accordance with the guidelines of the World Medical Association Declaration of Helsinki. Each participant was informed of potential risks and benefits of this study and gave written informed consent.

Study participants were recruited at the dental clinics of the universities of Bonn, Würzburg, Tübingen, Düsseldorf and the Dental Hospital Jönköping. Participants had to be at least 18 years old, non-diabetic, non-smokers or former smokers (> 6 months) who did not receive any antibiotics or anti-inflammatory drugs within the past six months prior to the examination. Subjects that qualified for the diagnosis of periodontitis as a manifestation of systemic diseases, and subjects that were pregnant or lactating were excluded. All participants had at least one dental implant with peri-implantitis that had not received any therapeutic intervention in the preceding 6 months, except for occasional supragingival prophylaxis. Sites had to qualify for Cumulative Interceptive Supportive Therapy C.I.S.T., (Lang et al., 2000) scheme grade D, i.e., probing depth (PD) ≥ 5mm, presence of bleeding on probing (BoP), and bone loss ≥ 3mm. All participants underwent a full-mouth periodontal examination, each implant was probed at six sites and the maximum PD (PDMax). BoP/suppuration, implant/surface type, reconstruction method and the width of keratinized mucosa were recorded.

Sample harvesting and sequencing preparation

Submucosal plaque samples were obtained from each peri-implantitis affected implant before surgical intervention, using one sterile paper points at each of the mesial, distal, buccal and lingual aspects of the implant, and were subsequently pooled (Jervoe-Storm et al., 2007). Sample tubes were stored immediately at -20°C or -80°C until further processing. DNA was extracted and purified using spin columns (Sigma-Aldrich GenElute Bacterial Genomic DNA Kit, Sigma-Aldrich, Munich, Germany). Quality and quantity of DNA was assessed spectrophotometrically using a Nanodrop spectrophotometer.

Sequence Processing

Sequencing data of the V3-V4 hypervariable region of the 16s rRNA gene were obtained using the Illumina MiSeq platform (Illumina, San Diego, California, United States) and a paired-end approach with 2x300 bp reads (Caporaso et al., 2011) providing substantial depth and breadth of coverage (Frey et al., 2014, Nelson et al., 2014). Library preparation was performed following the Illumina 16S rRNA Sequencing library preparation guide (Illumina, 2013) using the Nextera XT Index Kit (Illumina, San Diego, California, United States), PhiX Control Kit (Illumina, San Diego, California, United States) and MiSeq V3 reagent kit (Illumina, San Diego, California, United States). Initial purity and quantity was measured on a Nanodrop spectrophotometer. Subsequently, a PCR amplification was performed using the Kapa Hifi Hotstart ReadyMix (KAPABIOSYSTEMS, Boston, Massachusetts, United States) and the primer pair 5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and 5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC (Klindworth et al., 2013). Sequencing of the obtained libraries followed the manufacturer´s instructions. The mean paired-end reads/sample were 109,945 ± 35,169.

Quality control of the obtained reads was performed using FastQC (Andrews, 2010). Data processing, including demultiplexing, quality filtering to a minimum Phred score of 20 and joining of paired end reads, was carried out using QIIME 1.9.1 (Caporaso et al., 2010b). In the next step, the open reference picking strategy was selected. Sequences were clustered with a similarity threshold of 97 % via UCLUST (Edgar, 2010) to operating taxonomic units (OTUs). Assignment of representative sequences, picked by QIIME, was done by the RDP classifier v2.2 (Wang et al., 2007), and alignment

to the reference database, the Human Oral Microbiome Database v15.1 (Chen et al., 2010) was conducted via PyNAST (Caporaso et al., 2010a). OTU data were normalized using the DESeq2 approach in QIIME (Love et al., 2014, McMurdie and Holmes, 2014). α-diversity through whole tree PD (Moritz and Faith, 1998) - a measure of abundance-based richness, was assessed using QIIME. The same platform was used to assess β-diversity by means of unweighted UniFrac (Lozupone and Knight, 2005).

Statistical Analysis

The relationship of the deepest PD per implant (PDMax) with the abundance of each OTU was assessed through regression analysis using linear mixed models in R 3.3.3 (R Development Core Team, 2008), utilizing the lme4 1.1-15 package (Bates et al., 2015) in conjunction with lmerTest 2.0-36 to test for significance (Kuznetsova et al., 2017). The higher within-subject correlation was taken into account by modeling the subjects as a random effect. Furthermore, gender and age distribution were added to the model as fixed factors. Results were corrected for multiple testing through FDR-adjustment (Benjamini et al., 2001).

Taxa significantly associated with probing depth were considered in the network analysis. Taxa co-occurrence and/or co-exclusion were determined via renormalization and permutation with the R package ccrepe ('Compositionality Corrected by Renormalization and Permutation') 1.10.0, designed to detect correlations in microbial data using the nc.score as the similarity measure.

To assess the extent of dysbiosis of the biofilm associated with each implant site, we utilized the microbial dysbiosis index (MD-index) (Gevers et al., 2014), calculated as follows:

$$MDI = \log\left(\frac{[\text{total abundance of OTUs positively associated with PDmax}]}{[\text{total abundance of OTUs negatively associated with PDmax}]}\right)$$

Regression analysis was run with mixed effects linear models correcting for gender, age and within subject correlation to detect possible associations of MDI and PD, as outlined above.

Analysis of microbiome characteristics of deep and shallow pocket groups was conducted via Chi-Square-Test (Chernoff and Lehmann, 1954).

Results

Study population

A total of 45 implants with peri-implantitis, obtained from 30 subjects, were included in the present analyses. As shown in Table 1, 70.0% of the participants were female with a mean age of 64.7±11.5 years [range 32-82 years]. On average, participants had 15.85 ± 8.04 teeth and 3.46 ± 1.61 dental implants. Mean PDmax was 8.00±2.04mm [range 5-11mm]. All implants showed BoP, and suppuration was observed at 73% of the sites.

Structure and Diversity of the Bacterial Community

All samples provided sufficient DNA quality and quantity for analysis. OTUs were classified in 14 phyla, 169 genera and 337 species-level phylotypes. A complete list of observed taxonomic units can be viewed in the supplement material (Supplemental Table S1).

Dysbiosis

A highly significant ($p = 1.32 \times 10^{-7}$) positive association of the MD-index with increasing PDmax was observed (Figure 1).

Characteristics of the Microbiome in Relation to Disease Severity

To characterize the microbial shifts in relation to PDmax leading to the observed increase in dysbiosis, we performed linear mixed model analyses of the normalized relative abundance of observed phylotypes on the species-level in relation to PDmax. We identified 28 significantly differentially abundant taxa after correction for age and gender (Table 2). Abundance of the taxa *Eubacteriaceae [XV], Fretibacterium sp. HMT 362, Fretibacterium fastidiosum, Peptostreptococcaceae [XI][G-6], Alloprevotella sp. HMT 473, Fastidiosipila sanguinis, Filifactor alocis, Peptostreptococcaceae [XI][G-4], Bacteriodetes [G-3] bacterium HMT 365, Treponema parvum, Clostridiales [F-1][G-1] bacterium HMT 093, and Orobacterium* was significantly (FDR-corrected p<0.05) higher with increasing PDmax; whereas the taxa *Selemonas sp. HMT 136, Granulicatella elegans, Rothia aeria, Corynebacterium durum, Veillonella dispar, Acinetobacter, Streptococcus oralis subsp. dentisani clade 398, Veillonella parvula, Bifidobacterium, Bergeyella, Veillonella rogosae, Neisseria, Veillonella atypica, Rhodobacteriaceae, Lautropia mirabilis, Oribacterium asaccacrolyticum* were significantly (FDR-corrected $p < 0.05$) decreased in samples with increasing PDmax. Virtually identical results were obtained when using alternative methodology. Results on different taxonomic levels can be found in the Supplemental Material (Table S2).

Taxa associated with increasing PDmax were exclusively anaerobes, whilst taxa associated with lower PDmax showed significantly different ($p < 0.03$), more variable oxygen requirements (Table 3). α-diversity was significantly ($p < 0.033$) decreased in groups with deeper pockets (> 7 mm) (Figure 2) demonstrating higher species richness in samples from shallower pockets (≤ 7 mm) and vice versa. Collectively, the principle coordinate analysis plots PCoA (Figure 3) suggest that the second principle coordinate (PC2) layers the samples by PDmax (A), which is positively associated with MD-index (C), representing similar species diversity of samples with similar probing depths. α-diversity, on the other hand, tends to be distributed on PC1. The scatter density plot of the samples of whole tree PD versus MD-index suggests that samples with higher MD-index (> 0.1) are characterized by markedly reduced species richness, as compared to the samples with lower MD-index.

Correlation Network

Correlation analysis using the ccrepe package showed a clear division into two groups of cooperation units. *Lautropia mirabilis, Neisseria, Granulicatella elegans, Bergeyella, Rothia aeria,*

*Corynebacterium durum, Oribacterium asaccharolyticum, Selemonas sp. HMT 136, Veillonella rogosae, Veillonella parvula, Veillonella atypica, Veillonella dispar, Streptococcus oralis subsp. dentisani clade 398 and Bifidobacteriu*m formed one complex with strong co-occurrence of taxa that negatively associated with PDmax. A second, smaller complex negatively associated with PDmax was formed by the taxa *Acinetobacter* and *Rhodobacteraceae*. These two complexes showed mutual exclusion with the three complexes comprised of taxa positively associated with PDmax: (i) *Filifactor alocis, Bacteroidetes bacterium HMT 365* and *Clostridiales [F-1][G-1] bacterium HMT 093*; (ii) *Peptostreptococcaceae [XI][G-6], Peptostreptococcaceae [XI][G-4], Fretibacterium sp. HMT 362 and Fretibacterium fastidiosum; and (iii) Eubacteriaceae [XV]* . Figure 4 shows a visualization of the network, detailed results are captured in table 4. Additionally, findings from genus-level analysis are presented in the Supplemental Material (Supplemental Table S3, Supplemental Figure SF1).

Discussion

Our findings reveal significant associations of specific features of the submucosal microbiome from peri-implantitis lesions with peri-implantitis severity, expressed through the depth of the peri-implant pocket. Our analyses cover multiple levels of the submucosa bacterial ecology and aspects of co-occurrence and co-exclusion. To the best of our knowledge, this is the first study investigating these associations on a whole-microbiome level, and the first study that included microbial samples from a well-defined patient cohort specifically enrolled to minimize bias introduced by subject-related factors (including smoking, diabetes, or medications) and local factors (such as prior therapeutic interventions at the site). Possible remaining confounders were addressed using multi-variate analyses.

In analyses examining the effects of ecological shifts on disease severity, we observed that the MD-index, a measure of submucosal bacterial dysbiosis, is positively associated with increasing peri-implant pocket depths, suggesting that potential pathogens outcompete health-associated taxa (Marsh, 2015), similar to earlier observations derived from several other human diseases – including diabetes, autism, obesity, and periodontitis (Boulange et al., 2016, Buffington et al., 2016, Carding et al., 2015). To the best of our knowledge, our study is the first to show such an association in the context of human peri-implantitis.

When analyzing the microbial profiles underlying the observed dysbiosis, our findings indicate that peri-implantitis lesions are primarily inhabited by anaerobic gram-negative taxa, corroborating previous studies by other investigators (Sanz-Martin et al., 2017, Koyanagi et al., 2010, Tamura et al., 2013, Al-Ahmad et al., 2018, Apatzidou et al., 2017, Kumar et al., 2012). All aforementioned studies compared peri-implant health and peri-implantitis, whereas our study focused on peri-implantitis lesions with differing disease severity. Interestingly, the taxa identified in our analyses as associated with shallower peri-implant pocket depth do overlap with those identified as associated with health in case-control studies, e.g., *Streptococcus* and V*eillonella*.
In addition, we identified several taxa not previously reported to occur at peri-implantitis sites, including *Oribacterium asaccharolyticum*, *Veillonella dispar, Granulicatella elegans* and *Corynebacterium durum*, i.e., natural inhabitants of the human aerodigestive tract (Chen et al., 2018, Kumar et al., 2006, Sizova et al., 2014, Ohara-Nemoto et al., 2005).
A number of the taxa identified to be associated with deeper peri-implant pockets have been associated with severe periodontal disease, e.g., *Filifactor alocis* (Aruni et al., 2015, Aruni et al., 2014), *Fretibacterium fastidiosum* (Vartoukian et al., 2013, Correa et al., 2017, Moon et al., 2015), *Alloprevotella, Clostridiales* (Chen et al., 2018) or *Treponema* (Socransky et al., 1998) – especially *Treponema parvum*, which was also found in sites originating from acute necrotizing ulcerative gingivitis lesions (Wyss et al., 2001). In addition, we also identified significant positive associations with taxa not previously linked to peri-implantitis or periodontitis, e.g., *Fastidiosipila sanguinis,* a species recently discovered in human blood samples (Falsen et al., 2005) but hitherto not related to human nosology.

In addition, the dysbiotic shift observed in the transition from shallower to deeper pockets is characterized by decreasing species richness: The group consisting of exclusively anaerobic, mostly gram negative taxa, some of which are known periodontal pathogens, outcompeted the health-associated taxa that were prevalent in samples with shallower probing depths.

Network analysis facilitated the study of inter-related taxa and further identified two groups of mutually exclusive complexes. Interestingly, we found the taxa in the first, larger group to be associated with shallow pockets, whilst the taxa in the second group were significantly more numerous in deeper pockets. These findings indicate that peri-implantitis is clearly not a mono-infection by a specific pathogen, but rather a multifactorial, dysbiosis-associated process. Specifically,

within the limits of a study only including ‘diseased’ implants, our data point to the importance of a balanced peri-implant ecosystem, with an equilibrium between health-associated bacteria and pathogens (Socransky and Haffajee, 2005).

Strengths of our study include stringent inclusion criteria, a sizeable patient sample, and use multivariate analyses to account for possible residual confounders. Limitations include the cross-sectional nature of the study and the absence of a control group of implants without peri-implantitis that obviously preclude inferences regarding the temporality of the observed associations and cannot prove a cause and effect relationship between submucosal microbial dysbiosis and peri-implantitis. Longitudinal studies are needed to establish such relationships, but their implementation is challenging for both logistical and ethical reasons. Although our findings partly corroborate a number of earlier reports, discrepancies can conceivably be explained by differing analytic and data processing approaches, differences in sequencing platforms, length and sensitivities, and different 16s target regions (Kozich et al., 2013).

Taken together, this is the first study to demonstrate an association of both previously recognized and novel taxa with the severity of human peri-implantitis. Within the peri-implantitis microbiome, we identified groups with significant co-occurrence and co-exclusion, suggesting that alterations of ecological scale, rather than single taxa, underlie the progression of a lesion. Furthermore, we demonstrated a significant positive association between the level of microbial dysbiosis and the probing depth of the peri-implantitis lesion. Our findings provide a basis for further mechanistic work of the role of microbial ecological shifts and host responses in dysbiosis-associated peri-implant tissue destruction.

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Figure Legends

Figure 1

Mean Dysbiosis Index (MDI) of each sample plotted against probing depth (PD [mm]). The slope of the line is statistically significant (p = 1.32 x 10^{-7}). MDI increases with growing PD, indicating gain of taxa abundance associated with disease severity, whilst taxa with opposite association show decreased abundance.

Figure 2

Results of α-diversity-analysis: (A) Rarefaction plot: samples with shallow pockets (blue) show larger whole tree PD measures. Samples of deeper pockets (PDmax > 7 mm) on the other hand demonstrate lower species richness. (B) Boxplot diagram of whole tree PD measure of shallower and deeper pocket sample groups. α-diversity is significantly (p < 0.033) decreased in latter group.

Figure 3

Principle coordinate plots, whereas each axis exemplifies one of the three top principle coordinates seizing the major part of the diversity. Shown in percent, the fraction of diversity is described. (A) shows the samples colored by grouping of deeper (PDmax > 7 mm) and shallower pockets (PDmax ≤ 7 mm), (B) by the scope of whole tree PD and (C) reflecting the gradient of MDI. The scatter plot in (D) of alpha diversity versus MDI: samples with increased MDI (> 0.1) show a sudden reduction of species richness.

Figure 4

Network of taxa significantly associated with disease severity. Red lines indicate co-exclusion while green lines indicate co-occurrence. The results were filtered for better visualization: Correlations with fdr-corrected p-values <0.1. Weights vary between -0.424 and 0.673. The width of the lines mirrors the weight strengths. Taxa with positive association with pocket depth appear above the broken line without co-occurring taxa associated with shallower pockets which cluster below the broken line. Results can be viewed in Table 2 (unfiltered version in Supplement Material Table S3).

Tables

Table 1

List of selected study characteristics.

Study Characteristics	
Population-specific	30 patients
Female	70% (21 individuals)
Male	30% (9 individuals)
Mean age	64.7±11.5 years (range: 32y-82y)
Mean number of teeth	15.85 ± 8.04 (range: 0-27 teeth)
Mean number of dental implants	3.46 ± 1.6 (range: 1-≥8 implants)
Site-specific	45 implants with peri-implantitis Inclusion criteria: • Implant sites qualified for CIST (Lang et al., 2000) therapy scheme D • PD ≥ 5mm • BoP + • Bone loss ≥ 3mm
Mean PDmax	8.00±2.04 mm (range: 5mm-11mm)
Suppuration	Observed in 73% of sites

Table 2

Abundance of taxa associated with PDmax. Positive estimates stand for positive correlation and vice versa. Only taxa with an FDR-corrected p-value < 0.05 are shown. Residual taxa and analyses on other taxa-level can be viewed in supplement material (Table S2).

Taxon	Estimate	Std.-Error	p-value	FDR-corr.
Eubacteriaceae [XV]	0.7598	0.1414	0.0000	0.0014
Fretibacterium sp. HMT 362	0.4660	0.0938	0.0001	0.0088
Selenomonas sp. HMT 136	-0.7589	0.1695	0.0001	0.0095
Granulicatella elegans	-0.6547	0.1450	0.0002	0.0135
Fretibacterium fastidiosum	0.4069	0.0916	0.0002	0.0135
Rothia aeria	-0.7634	0.1916	0.0003	0.0167
Corynebacterium durum	-0.3884	0.1046	0.0006	0.0206
Peptostreptococcaceae [XI][G-6]	0.5954	0.1561	0.0005	0.0206
Veillonella dispar	-0.5955	0.1539	0.0005	0.0206
Acinetobacter	-0 .6184	0.1526	0.0006	0.0206
Streptococcus oralis subsp. dentisani clade 398	-0.4994	0.1313	0.0009	0.0267
Alloprevotella sp. HMT 473	0.4511	0.1222	0.0010	0.0286
Veillonella parvula	-0.4540	0.1233	0.0012	0.0310
Fastidiosipila sanguinis	0.3670	0.1065	0.0014	0.0326
Bifidobacterium	-0.5306	0.1563	0.0019	0.0332
Bergeyella	-0.4549	0.1326	0.0018	0.0332
Filifactor alocis	0.4915	0.1312	0.0017	0.0332
Veillonella rogosae	-0.4570	0.1367	0.0018	0.0332
Neisseria	-0.6571	0.1906	0.0016	0.0332
Veillonella atypica	-0.6161	0.1904	0.0025	0.0414
Rhodobacteraceae	-0.4294	0.1328	0.0026	0.0416
Peptostreptococcaceae [XI][G-4]	0.4873	0.1424	0.0028	0.0424
Bacteroidetes [G-3] bacterium HMT 365	0.4473	0.1322	0.0034	0.0468
Lautropia mirabilis	-0.5693	0.1793	0.0032	0.0468
Treponema parvum	0.5249	0.1628	0.0035	0.0468
Clostridiales [F-1][G-1] bacterium HMT 093	0.4993	0.1516	0.0037	0.0473
Oribacterium	0.4296	0.1377	0.0041	0.0496
Oribacterium asaccharolyticum	-0.3888	0.1272	0.0040	0.0496

Table 3

Characteristics of the taxa significantly associated with PDmax

* Information were compiled from the American Type Culture Collection database

** Information were compiled from *BacDive* (Sohngen et al., 2016)

Taxa	Gram +/-	Anaerobic/ aerobic
positive association with PDmax		
*Eubacteriaceae [XV]****	+	NA
*Fretibacterium sp. HMT 362 **	-	anaerobic
*Fretibacterium fastidiosum **	-	anaerobic
*Peptostreptococcaceae [XI][G-6] **	+	anaerobic
*Bacteroidetes [G-3] bacterium HMT 365 **	NA	anaerobic
*Peptostreptococcaceae [XI][G-4] **	+	anaerobic
*Oribacterium**	NA	anaerobic
*Treponema parvum **	-	anaerobic
Filifactor alocis (Jalava and Eerola, 1999)	+	anaerobic
*Alloprevotella sp. HMT 473 **	NA	anaerobic
*Fastidiosipila sanguinis**	+	anaerobic
Clostridiales [F-1][G-1] bacterium HMT 093 (Yutin and Galperin, 2013)	+	anaerobic
negative association with PDmax		
Bifidobacterium (Schell et al., 2002)	+	anaerobic
*Selenomonas sp. HMT 136**	-	anaerobic
*Granulicatella elegans**	+	anaerobic
*Rothia aeria **	+	aerobic
Acinetobacter (Bitrian et al., 2013)	-	aerobic
*Streptococcus oralis subsp. dentisani clade 398 **	+	anaerobic
*Neisseria **	-	aerobic
*Veillonella parvula **	-	anaerobic
Rhodobacteraceae (Simon et al., 2017)	-	NA
Bergeyella (Shukla et al., 2004)	-	aerobic
*Veillonella rogosae **	-	anaerobic
*Lautropia mirabilis **	-	anaerobic
*Veillonella atypica **	-	anaerobic
*Veillonella dispar **	-	anaerobic
*Oribacterium asaccharolyticum **	+	anaerobic
*Corynebacterium durum **	+	aerobic

From	To	Weight	FDR-corr.	Interaction Type

Table 4

Edgelist of the network analysis. It displays interaction of two taxa, whereas each line represents one single interaction. Negative values in the weight column mean co-exclusion, whilst positive values indicate co-occurrences. Only edges with significant FDR-corrected p-values <0.1. The complete list can be displayed in the supplement material (Table S3).

Selemonas sp. HMT 136	*Bifidobacterium*	0.6610	0.0000	co-occurrence
Fretibacterium sp. HMT 362	*Fretibacterium fastidiosum*	0.5888	0.0000	co-occurrence
Veillonella rogosae	*Veillonella dispar*	0.6734	0.0000	co-occurrence
Veillonella dispar	*Selemonas sp. HMT 136*	0.5609	0.0000	co-occurrence
Neisseria	*Bergeyella*	0.5491	0.0000	co-occurrence
Veillonella parvula	*Veillonella dispar*	0.5849	0.0000	co-occurrence
Veillonella dispar	*Bifidobacterium*	0.5262	0.0001	co-occurrence
Acinetobacter	*Rhodobacteraceae*	0.4874	0.0002	co-occurrence
Neisseria	*Rothia aeria*	0.4863	0.0003	co-occurrence
Neisseria	*Lautropia mirabilis*	0.4999	0.0003	co-occurrence
Veillonella rogosae	*Veillonella parvula*	0.5269	0.0014	co-occurrence
Veillonella dispar	*Streptococcus oralis subsp. dentisani clade 398*	0.4422	0.0036	co-occurrence
Neisseria	*Granulicatella elegans*	0.4429	0.0037	co-occurrence
Selemonas sp. HMT 136	*Oribacterium asaccharolyticum*	0.4953	0.0038	co-occurrence
Corynebacterium durum	*Rothia aeria*	0.4692	0.0047	co-occurrence
Veillonella parvula	*Selemonas sp. HMT 136*	0.4817	0.0055	co-occurrence
Veillonella rogosae	*Selemonas sp. HMT 136*	0.5026	0.0059	co-occurrence
Veillonella dispar	*Veillonella atypica*	0.4132	0.0065	co-occurrence
Filifactor alocis	*Bacteroidetes bacterium HMT 365*	0.4879	0.0073	co-occurrence
Eubacteriaceae [XV]	*Rothia aeria*	-0.4064	0.0082	co-exclusion
Veillonella rogosae	*Streptococcus oralis subsp. dentisani clade 398*	0.4195	0.0106	co-occurrence
Filifactor alocis	*Clostridiales [F-1][G-1] bacterium HMT 093*	0.4911	0.0110	co-occurrence
Bergeyella	*Rothia aeria*	0.4292	0.0114	co-occurrence
Peptostreptococcaceae [XI][G-4]	*Oribacterium asaccharolyticum*	-0.4226	0.0208	co-exclusion
Lautropia mirabilis	*Bergeyella*	0.3834	0.0423	co-occurrence
Fretibacterium fastidiosum	*Peptostreptococcaceae [XI][G-6]*	0.4005	0.0435	co-occurrence
Clostridiales [F-1][G-1] bacterium HMT 093	*Bacteroidetes bacterium HMT 365*	0.4320	0.0450	co-occurrence
Veillonella parvula	*Veillonella atypica*	0.4449	0.0567	co-occurrence
Veillonella rogosae	*Bifidobacterium*	0.4224	0.0656	co-occurrence
Acinetobacter	*Clostridiales [F-1][G-1] bacterium HMT 093*	-0.4243	0.0673	co-exclusion
Fretibacterium fastidiosum	*Peptostreptococcaceae [XI][G-4]*	0.3254	0.0749	co-occurrence
Veillonella parvula	*Streptococcus oralis subsp. dentisani clade 398*	0.3807	0.0940	co-occurrence
Selemonas sp. HMT 136	*Corynebacterium durum*	0.4102	0.0943	co-occurrence

Supplemental Material

Table S1

Overview of all relative abundances of observed taxa split up into taxa level phylum, genus and species. Data are normalized and display the mean of each PD-group.

Table S2

Complete regression analysis results of phylum-, class-, order-, family-, genus- and species-level.

Table S3

Complete edgelist of the network analysis on species-level.

Table S4

Complete edgelist of the network analysis on genus-level.

Supplemental Figure SF1

Network of taxa significantly associated with disease severity on genus level. Red lines indicate co-exclusion while green lines indicate co-occurrence. The results were filtered for better visualization: Correlations with FDR-corrected p-values <0.1. The width of the lines mirrors the weight strengths. Taxa with positive association with pocket depth appear above the broken line without co-occurring taxa associated with shallower pockets which cluster below the line.