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Microbial Diversity in the Early *In Vivo*-Formed Dental Biofilm

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Although the mature dental biofilm composition is well studied, there is very little information on the earliest phase of *in vivo* tooth colonization. Progress in dental biofilm collection methodologies and techniques of large-scale microbial identification have made new studies in this field of oral biology feasible. The aim of this study was to characterize the temporal changes and diversity of the cultivable and noncultivable microbes in the early dental biofilm. Samples of early dental biofilm were collected from 11 healthy subjects at 0, 2, 4, and 6 h after removal of plaque and pellicle from tooth surfaces. With the semiquantitative Human Oral Microbiome Identification Microarray (HOMIM) technique, which is based on 16S rRNA sequence hybridizations, plaque samples were analyzed with the currently available 407 HOMIM microbial probes. This led to the identification of at least 92 species, with streptococci being the most abundant bacteria across all time points in all subjects. High-frequency detection was also made with *Haemophilus parainfluenzae*, *Gemella haemolysans*, *Slackia exigua*, and *Rothia* species. Abundance changes over time were noted for *Streptococcus anginosus* and *Streptococcus intermedius* ($P = 0.02$), *Streptococcus mitis* bv. 2 ($P = 0.0002$), *Streptococcus oralis* ($P = 0.0002$), *Streptococcus* cluster I ($P = 0.003$), *G. haemolysans* ($P = 0.0005$), and *Stenotrophomonas maltophilia* ($P = 0.02$). Among the currently uncultivable microbiota, eight phylotypes were detected in the early stages of biofilm formation, one belonging to the candidate bacterial division TM7, which has attracted attention due to its potential association with periodontal disease.

The microbial diversity in the oral cavity is among the largest so far characterized in the human body (1). Of specific interest is the dental biofilm, which forms first by selective adsorption of bacteria from saliva onto the tooth surface, followed by bacterial growth. It is well known that biofilm microbes interact with each other and thus show characteristics significantly different from those of their planktonic counterparts (2). While the biofilm contains beneficial, as well as harmful, bacteria, their relative proportions have a tendency to change as the dental plaque matures (3). These changes depend on bacterial interactions, as well as host-derived factors, which are all responsible for the ensuing development and biological effects of the structure (4). The very first microbial settlers of tooth surfaces are critical for the maturation process of dental plaque. As such, they are likely to play an unanticipated role in pathological conditions associated with oral biofilm formation, such as caries and periodontal disease. Understanding the earliest but most critical steps in the progression of disease involves identification, timing, and quantitation of the total dental microbiome, an important goal yet to be achieved.

It has been well established that enamel tooth surfaces are immediately covered with a layer of salivary proteins upon exposure to the oral environment. This layer, which is called the acquired enamel pellicle, is several micrometers thick (5–7). It is formed by the selective adsorption of mostly phosphorylated salivary proteins (8–10). The earliest phase of bacterial biofilm formation is the attachment of oral bacteria, via specific molecular interactions, to the acquired enamel pellicle (11–13). This permits the attached bacteria to remain attached to tooth surfaces despite the mechanical forces of salivary flow, tongue movements, and rinsing with water. The first insights into the early biofilm composition, obtained with culture-based techniques, have shown that streptococci, as well as *Neisseria* and *Rothia* species, are the predominant early colonizers. Streptococci express adhesins, specifically α-amylase-binding protein A, antigen I/II, SspA/SspB, and surface lectins, that recognize receptors on proteins in the acquired enamel pellicle (14). As plaque matures, the proportions of facultative and anaerobic filamentous genera, such as *Actinomyces*, *Corynebacterium*, *Fusobacterium*, and *Veillonella*, increase gradually (15–20). Streptococci coaggregate within and between species involving, e.g., receptor polysaccharides and type 2 fimbriae expressed by *Actinomyces* (21–23). The multiple-affinity properties of streptococci confer advantageous characteristics on the genus and explain their dominance as the initial colonizing bacteria of the tooth surface (24, 25).

While valuable insights were obtained with the relatively few biofilm bacteria that could be cultured at that time, inevitably, the true microbial complexity of the biofilm structure could not be fully established. More recently developed molecular techniques have expanded our ability not only to uncover the complexity of colonizing microbial communities, but also to identify the noncultivable species (26, 27). We have previously used the "checkerboard assay" employing whole-genomic probes, limited to detecting cultivable bacteria (24). The successive-adhesion pattern of 40 species was characterized, and the different species contributions were quantitated. *Porphyromonas gingivalis* and *Treponema denti-*

Received 15 December 2015 **Accepted** 6 January 2016

Accepted manuscript posted online 8 January 2016

Citation Heller D, Helmerhorst EJ, Gower AC, Siqueira WL, Paster BJ, Oppenheim FG. 2016. Microbial diversity in the early *in vivo*-formed dental biofilm. Appl Environ Microbiol 82:1881–1888. doi:10.1128/AEM.03984-15.

Editor: A. M. Spormann, Stanford University

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Supplemental material for this article may be found at http://dx.doi.org/10.1128/AEM.03984-15.

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cola were found to be among the early colonizers but were rapidly superseded by streptococci and *Actinomyces* spp. The microbial complexity of oral biofilm has also been studied in plaque formed *in situ* on retrievable enamel chips using a 16S rRNA gene-based pyrosequencing approach, identifying at least 97 different species and some uncultivated phylotypes. Of special note is the fact that noncultivable species were found for the first time in this study, and they were tentatively assigned to *Clostridia* and *Flavobacteria* (26).

The knowledge of mature *in vivo*-formed oral biofilm gained, using cultivation and DNA-based approaches, is considerable (27–29). Much less information, however, is available on the earliest phases of biofilm formation and its noncultivable bacterial fraction. In the present study, we harvested *in vivo* biofilms formed during the very early phases (<6 h) of microbial attachment to tooth surfaces. Bacterial growth during this time interval is limited, and the focus of this study is on the very first bacteria that interact with the pellicle proteins. The Human Oral Microbiome Identification Microarray (HOMIM) was employed, using 407 microbial probes distinguishing over 300 cultivable, as well as noncultivable, species. The data obtained revealed the microbial composition of the very early stage of biofilm formation and provided in-depth information regarding its temporal development.

FIG 1 Dental biofilm collection procedure. For biofilm collection, one folded PVDF membrane presoaked in 0.5 mol/liter sodium bicarbonate, pH 8.4, was used to swab the coronal two-thirds of the buccal dental surfaces of the incisors, canines, premolars, and first molars of both arches while applying mild pressure. For each quadrant, 1 fresh membrane was used.

MATERIALS AND METHODS

Subject population. Early dental biofilms were collected from 11 healthy subjects. All the subjects provided informed consent prior to their participation. The study was conducted according to the principles outlined in the Declaration of Helsinki on experimentation involving human subjects. The study protocol was approved by the Institutional Review Board of Boston University Medical Center. The subjects were screened using oral and systemic health histories. Exclusion criteria were (i) overt signs of gingivitis, periodontal disease, active dental caries, or any other oral condition that could affect oral fluid/biofilm composition; (ii) fewer than 14 teeth; (iii) history of antibiotic use in the past 3 months; (iv) long-term use of anti-inflammatory medication; (v) current smoking; (vi) pregnancy; (vii) presence of systemic diseases that could affect oral health; and (viii) systemic medications and treatments that could affect salivary function. Clinical examinations took place on different days of the same week at the Clinical Research Center at the School of Dental Medicine of Boston University. All clinical examinations were performed once at baseline by the same trained periodontist using the calibrated method. Measurements were taken at 6 sites per tooth (mesiobuccal, buccal, distobuccal, mesiolingual, lingual, and distolingual) for all teeth except third molars and included probing depth (PD) and clinical attachment level (CAL), measured to the nearest millimeter with a periodontal probe (UNC-15; Hu-Friedy, Chicago, IL, USA), and presence or absence of bleeding on probing (BOP), supragingival visible plaque (PL), gingival marginal bleeding (GI), and suppuration. Clinical diagnosis of periodontal health (PH) was established for all the subjects based on the following criteria: ≤10% of sites with BOP and no PD or CAL of >3 mm, although PD or CAL of 4 mm in not more than 5% of the sites without BOP was allowed.

Sample collection and processing. The buccal tooth surfaces in both arches, excluding second and third molars, were thoroughly cleaned to remove the acquired enamel pellicle and dental plaque, using a prophylaxis hand piece with rubber cup and dental pumice containing no additives (Preppies; Whip Mix, Louisville, KY) (30). This was followed by *in vivo* exposure to the oral environment for either 0, 2, 4, or 6 h. At each of these four time points, biofilm was collected. The samples were acquired on two different days within the same week. The 0- and 6-h samples were collected on day 1, and the 2- and 4-h samples were collected on day 2. During the biofilm formation phase, subjects were asked to refrain from eating, drinking (except water), or oral hygiene. For harvesting biofilm, teeth were isolated from the buccal/labial mucosa with cotton rolls to avoid contact between tooth surfaces and the oral mucosa. The collection area was rinsed twice with distilled water and dried with air. Polyvinylidene difluoride (PVDF) membranes (45-μm pore size; 13-mm diameter; Durapore; Millipore, Bedford, MA, USA) soaked in 0.5 mol/liter sodium bicarbonate, pH 8.4, were used to swab the coronal two-thirds of buccal dental surfaces of incisors, canines, premolars, and first molars of both arches while applying mild pressure (Fig. 1). Sodium bicarbonate has previously been shown to be effective in releasing proteinaceous materials adsorbed onto tooth surfaces (24). The pooled membranes (four membranes per time point per subject) were placed into 300 μl of TE buffer (50 mM Tris-HCl, 0.1 mM EDTA, pH 7.6), followed by vortexing for 30 s and sonication for 5 min. DNA isolation was performed using Ready-Lyse Lysozyme Solution and the MasterPure DNA purification kit (both from Epicentre, Madison, WI) following the manufacturers' instructions. The samples were stored at −80°C until analysis.

Microbiological assessment and quantitation. The purified DNA samples were analyzed using HOMIM (31). For microbial identification, a library of 407 probes recognizing the most prevalent oral bacterial species was used. Briefly, 16S rRNA-based reverse-capture oligonucleotide probes (typically 18 to 20 bases) were printed on aldehyde-coated glass slides. The 16S rRNA genes were PCR amplified from DNA extracts using 16S rRNA universal forward and reverse primers and labeled via incorporation of Cy3-dCTP in a second PCR amplification. The labeled 16S rRNA amplicons were hybridized for 16 h with probes on the custom-made arrays. After washing, the microarray slides were scanned using an Axon 4000B scanner, and the raw data were extracted using GenePix Pro software (MDS Analytical Technologies, Sunnyvale, CA). The abundance of each species/phylotype interrogated by the array was then assigned an ordinal, nonlinear HOMIM score from 0 to 5 using an online analysis tool (http://bioinformatics.forsyth.org/homim), where a score of 0 indicates a fluorescent signal that is less than two times the background level and a score of 5 corresponds to the average maximum intensity of a set of universal positive-control probes.

Statistical analyses. Statistical analyses were performed using the R software program for statistical computing (version 2.15.1). The Wilcoxon signed-rank test (as defined in the wilcox.test R function) was used to determine across all subjects whether the abundance of a given species/phylotype was significantly different between two time points. Spearman's correlation coefficient (rho) and its associated P value (as computed using the cor.test R function) were used to identify species whose abundances were significantly associated with specific time points, both

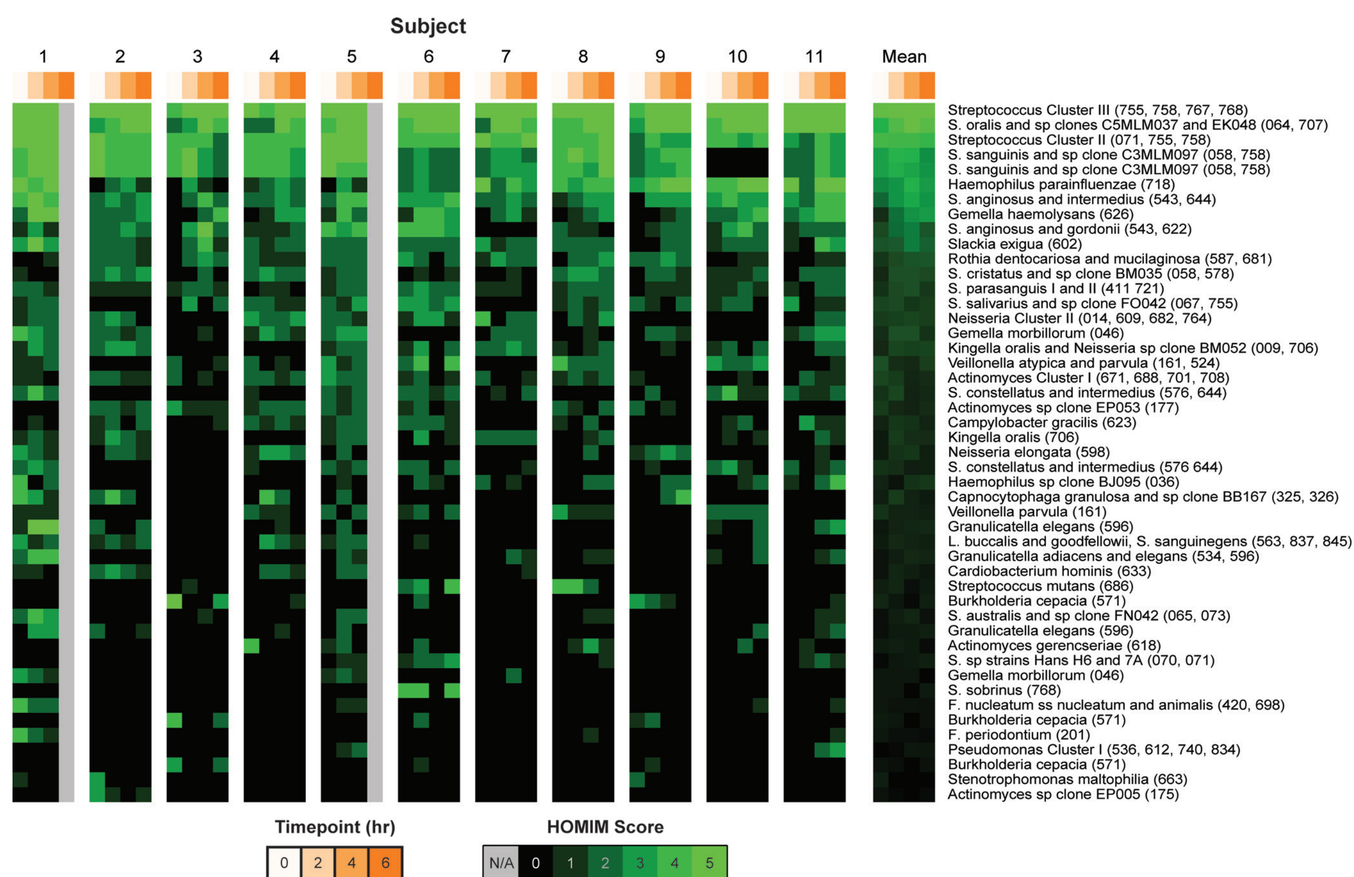

FIG 2 Intensity map of the distribution of bacterial species in each subject and the mean across all subjects after 0, 2, 4, and 6 h of biofilm formation. The image shows the intensities of the 47 probes (rows) with a maximum HOMIM score of >2 in at least one sample. The species are sorted in descending order by mean HOMIM score. The different intensities of green correspond to the signal intensities of the arrays, quantitated by HOMIM scores of 0 to 5. Gray indicates missing data for the 6-h samples in 2 subjects. The probes are labeled with species descriptors, followed by Human Oral Microbiome Database (HOMD) oral taxa in parentheses. *F.*, *Fusobacterium*; *L.*, *Leptotrichia*; *S. sanguinegens*, *Sneathia sanguinegens*; all other *S.*, *Streptococcus*.

within each subject and across all subjects. The rho value measures how well the amount of a given species in a given subject correlates with the amount of time that has elapsed, e.g., a rho value of +1 or −1 means that the HOMIM scores increased or decreased, respectively, at every time point during the 6-h biofilm development time. A species with a rho of 0 (no correlation) would have discrete HOMIM scores that showed high levels of variation over time, whereas a species with an undefined rho would have HOMIM scores that were unchanged over time (e.g., always 0, always 1, etc.). Correction for multiple-hypothesis testing was accomplished using the Benjamini-Hochberg false-discovery rate (FDR). The level of significance for each test was set at 5%.

RESULTS

Demographic and clinical parameters. The demographics and clinical parameters determined for the enrolled subjects are shown in Table S1 in the supplemental material. The periodontal measures obtained, CAL, PD, and BOP, were consistent with periodontal health according to reported criteria (32).

Microbial characterization and prevalence. Early biofilms from the 11 subjects were obtained after 0-, 2-, 4-, and 6-h biofilm maturation times and evaluated by HOMIM. Of the possible 407 target probes, 124 hybridized with PCR products from the DNA samples obtained (HOMIM score, ≥1) (see Table S2 in the supplemental material). In view of the multitargeting by some probes, it was established that the 124 probes represented at least 92 different oral species. Among the 124 probes, 47 showed a HOMIM score of >2 in at least 1 of the 11 subjects (Fig. 2). The identified bacteria primarily belonged to the phylum *Firmicutes* (42.2%), followed by *Protobacteria* (25.6%) and *Actinobacteria* (16.5%). Less prevalent bacteria belonged to the phyla *Bacteroidetes* (8.26%), *Fusobacteria* (4.96%), and *Synergistetes* (0.83%) and the candidate bacterial division TM7 (0.83%). As expected, streptococci (*Streptococcus oralis*, *Streptococcus anginosus/Streptococcus intermedius*, and *Streptococcus mitis*) were the most abundant across all time points in all subjects. Together with the streptococci, the species *Gemella haemolysans*, *Haemophilus parainfluenzae*, *Actinomyces* cluster I (*Actinomyces meyeri*, *Actinomyces viscosus*, *Actinomyces odontolyticus*, and *Actinomyces oricola*), *Rothia dentocariosa/Rothia mucilaginosa*, *Neisseria* cluster II (*Neisseria oralis*, *Neisseria flava*, *Neisseria mucosa*, and *Neisseria sicca*), *Kingella oralis*, *Slackia exigua*, and *Veillonella atypica/Veillonella parvula* were the 16 predominant bacteria identified in dental biofilm formed during the first 6 h (Fig. 2; see Table S2 in the supplemental material).

Species belonging to the "orange complex," (33) comprising *Fusobacterium nucleatum*, *Fusobacterium periodontium*, and *Parvimonas micra*, were also found to be present in the early dental biofilm. This is consistent with previous studies (24), although we

could not confirm the presence of well-established periodontal pathogens, such as *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*. Fastidious and not-yet-cultivated species more recently associated with periodontal disease, *Filifactor alocis*, *Dialister invisus*, and TM7 [G-1] oral taxon (OT) 347 (34, 35), were present in low numbers. The typical cariogenic bacterium *S. mutans* was detected in the early biofilm of 2 of the 11 individuals.

Changes in abundance over time. Temporal changes in bacterial prevalence and proportions during the first 6 h of biofilm formation were assessed by computing the Spearman correlation coefficient (rho), which measures how well the amount of a given species correlates with the time of enamel exposure to the oral environment (see Table S3 in the supplemental material). Among these species, significant increases in rho values over time were noted for *G. haemolysans* ($P = 0.0005$), *S. anginosus* or *S. intermedius* ($P = 0.02$), *S. mitis* bv. 2 ($P = 0.0002$), *S. oralis* ($P = 0.0002$), and *Streptococcus* cluster I ($P = 0.003$). Significant decreases over time were observed for *Stenotrophomonas maltophilia* ($P = 0.02$). When corrected for multiple-hypothesis testing across all probes (see Table S3 in the supplemental material), the rho values for *G. haemolysans* and *S. oralis* remained strongly significant (FDR q [corrected P value] < 0.25), indicating that these species are likely to play important roles during dental biofilm maturation and possibly microbial coaggregation. A heat map of the rho values of the 47 most abundant species is shown in Fig. 3, and the values are shown in Table S3 in the supplemental material. While trends in increases and decreases could clearly be observed for some of the bacteria listed, they were significant for only a few species.

Identification of not-yet-cultivated phylotypes. The HOMIM technology offers the opportunity to detect not only those species that can be cultured, but also those that have escaped identification so far by *in vitro* culturing approaches. Table 1 shows the prevalences of uncultivable species found among the 11 subjects (HOMIM score of ≥ 1). Eight not-yet-cultivated phylotypes were detected, including species belonging to the candidate bacterial division TM7. All noncultivable phylotypes detected exhibited low HOMIM scores (≤ 2), except *Actinomyces* sp. strain OT 177, which showed a HOMIM score of 3 at one time point (see Table S2 in the supplemental material). It was also the most prevalent, since it was detected at all time points in at least half of the subjects (Table 1). The other phylotypes were detected in less than 25% of the subjects at all time points examined. Overall, the data reveal that these noncultivable species constitute a small but integral part of the early biofilm.

DISCUSSION

The results obtained show that the early *in vivo* dental biofilm exhibits considerable bacterial diversity. A total of 124 probes reacted positively, providing evidence for the presence of a minimum of 92 bacterial species belonging to 40 genera and 7 phyla. Furthermore, for the first time, we report on the presence of noncultivable species in this native tooth surface biofilm.

Overall, very good consistency was observed in oral colonization among subjects, whether the species were prevalent or not, as evidenced by the abundancy gradient of the mean values among subjects (Fig. 2, right column). As expected, the streptococci were the most abundant at all time points. Within the bacteria that showed significant increases over time were the well-known early colonizers *S. oralis*, *S. anginosus*, and *S. intermedius* (Fig. 3). For these streptococci, the mechanism of attachment to the acquired enamel pellicle has been related to acidic proline-rich proteins, α-amylase, and various glycoproteins (36–40). Importantly, these streptococci are able to proliferate in the presence of oxygen, a characteristic of only the early biofilm environment.

At the 2-h time point, and at subsequent time points, *G. haemolysans* was among the 10 most prevalent bacteria. What sets this species apart from most of the other prevalent early colonizers is the fact that its rho value for increase over time was highly significant (FDR $q < 0.25$). *G. haemolysans* has previously been identified by classic cultivation methods (19) and is considered to be among the core microbial colonizers of *in situ* dental biofilm of healthy individuals (26). In this study, we identified this species in *in vivo* biofilm and in the very earliest phases of biofilm formation. The potential role that *Gemella* plays in biofilm processes, such as coaggregation and symbiosis, or even in disease promotion, remains to be established.

At the 4-h time point, *H. parainfluenzae* was among the first nonstreptococcal species to appear, with a high mean HOMIM score of 3 (Fig. 2; see Table S2 in the supplemental material). The early appearance of *H. parainfluenzae* can be explained by its adherence characteristics (41, 42), since it displays high affinity for salivary mucin MG1 (43) present in the acquired enamel pellicle (44). Furthermore, *H. parainfluenzae* has been shown to coaggregate with *S. sanguis* and *S. oralis* due to its outer membrane adhesin that recognizes specific receptor polysaccharides (45–48).

The 6-h samples revealed what could be considered the key colonizers of early *in vivo*-formed biofilm. In order of abundance, these colonizers are *S. oralis*, *H. parainfluenzae*, *G. haemolysans*, *S. sanguinis*, *S. anginosus*/*S. intermedius*, *S. exigua*, *Streptococcus gordonii*, and *R. dentocariosa*/*R. mucilaginosa*. Surprisingly, *S. exigua*, which is not well studied, was found to be among the most abundant colonizers of all subjects at all time points, with a mean HOMIM score across time points of 1.9 (see Table S2 in the supplemental material). Typically associated with endodontic (49) and periodontal lesions (50), the species is fastidious and grows poorly (51) and may therefore have been easily overlooked in previous culture-based studies. The key colonizers identified here form the substratum for the developing biofilm architecture. This structure has been shown to form in an orchestrated fashion (48) and is capable of impacting oral health adversely.

It has been estimated that at least 35% of the 700 bacterial species in the oral cavity cannot be grown in culture at this time (52, 53). The identification of both cultivable and not-yet-cultivable bacterial species in biofilm structures is equally important and critical for understanding the complete bacterial "interactome" of dental colonizers. The elegant work of Diaz and coworkers, although using not an *in vivo* but an *in situ* approach, provided evidence for uncultivable species belonging to the phyla *Firmicutes* and *Bacteroidetes* in the early biofilm, with tentative class assignments to *Clostridia* and *Flavobacteria*, respectively (26). In our *in vivo* investigation, we found eight uncultivable phylotypes and were able to make a more definitive genus level assignment (Table 1). They are *Bergeyella* sp., *Haemophilus* sp., *Fretibacterium* sp. (2 strains), TM7 sp., *Actinomyces* sp., *Megasphaera* sp., and *Stomatobaculum* sp.

It is well recognized that there are many qualities of oral biofilm that can lead to the development or suppression of biofilm-induced pathogenicity (54–56). The presence of a few uncultivable species, such as those of the candidate bacterial division TM7, could be highly significant for the manifestation of oral

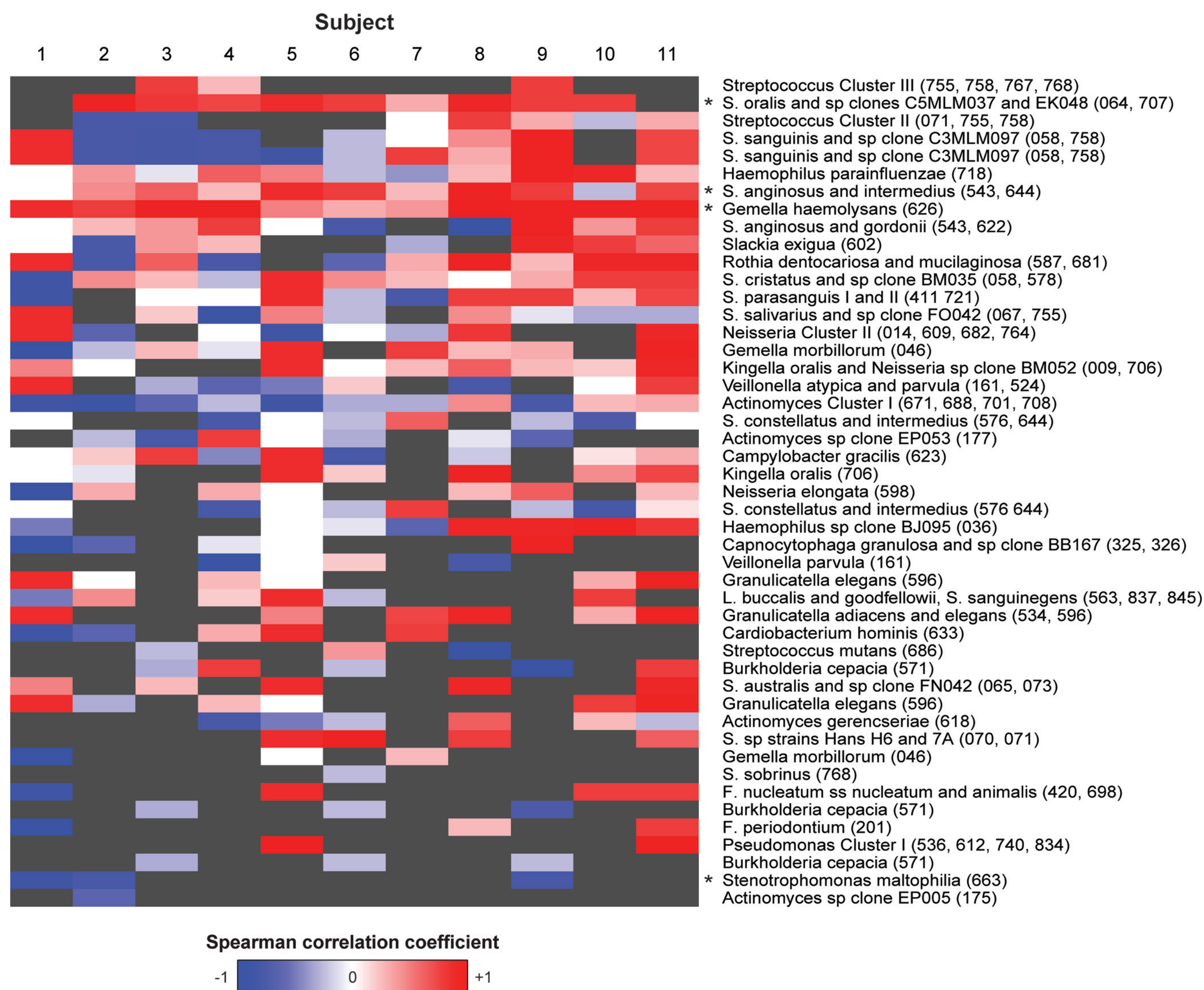

FIG 3 Heat map showing changes in bacterial intensities in the early biofilm over time. Shown are the results for the 47 probes with the highest level of abundance (maximum HOMIM score of >2 across all samples). Each column represents one subject, and each cell indicates Spearman's correlation coefficient (rho) of the HOMIM scores for a given species in a given subject with the four time points of biofilm formation. Red and blue represents rho values of +1 and −1, respectively, indicating that the proportion of a given species continuously increased or decreased, respectively. White represents a rho value of 0, indicating that the scores showed no pattern of increase or decrease over time. Gray indicates that all HOMIM scores for a given species in a given subject were identical and rho is undefined. All of the available time points for each subject (3 or 4) were used to compute the correlation coefficient within that subject. The probes are labeled with species descriptors, followed by HOMD oral taxa in parentheses. *F.*, *Fusobacterium*; *L.*, *Leptotrichia*; *S. sanguinegens*; *Sneathia sanguinegens*; all other *S.*, *Streptococcus*. Spearman's correlation coefficients were also computed for each species with respect to time points across all samples in each row, and probes with across-subject rho values significantly different from zero ($P < 0.05$) are indicated by asterisks.

diseases, particularly periodontal disease (57). In the recently proposed polymicrobial synergy and dysbiosis model of periodontitis, the disease is caused ultimately by a dysbiotic change in the biofilm microbiota that triggers an inflammatory host response (58). This model contends that commensal oral microbes can be "accessory pathogens" (54), since such nonpathogenic species can provide biofilm conditions favoring an increase of periodontal pathogens, leading to disease manifestation. Uncultivable dental biofilm microbes, such as *Actinomyces* sp. OT 177, identified here and shown to be present at all time points, may play hitherto-unappreciated roles in dysbiotic mechanisms.

While the HOMIM approach has significantly expanded our knowledge of the temporal relationships of bacterial attachment, it does not allow us to decipher directly the spatial relationships among the bacterial species investigated. Some insight into the spatial relationships of mature biofilm structures have been obtained using electron microscopy techniques and, more recently, confocal laser scanning microscopy (CLSM), fluorescence *in vitro* hybridization (FISH), and the recent state-of-the-art combinatorial labeling and spectral imaging (CLASI)-FISH methodology (59–61). The use of such techniques will be necessary in order to gain insight into the 3-dimensional architecture of the biofilm.

In summary, this study has provided an in-depth microbial characterization of the *in vivo* dental biofilm formed over the first

TABLE 1 Not-yet-cultivated species identified in early dental biofilm samples[a]

Phylum	Class	Not-yet-cultivated species	Identification at sampling time (h)[b]			
			0	2	4	6
Bacteroidetes	*Flavobacteria*	*Bergeyella* sp. strain OT[c] 322	1/11		1/11	
Proteobacteria	*Gammaproteobacteria*	*Haemophilus* sp. strain OT 035[d]		1/11	1/11	
Synergistetes	*Synergistetes* [C-1]	*Fretibacterium* sp. strain OT 359[d]	1/11	1/11	1/11	
		Fretibacterium sp. strain OT 360[d]	1/11	1/11	1/11	
TM7	TM7 [C-1]	TM7 [G-1] sp. strain OT 347 and TM7 [G-2] sp. strain OT 350	1/11	3/11	2/11	
Actinobacteria	*Actinobacteria*	*Actinomyces* sp. OT 177	8/11	6/11	5/11	5/9
Firmicutes	*Clostridia*	*Megasphaera* sp. strain OT 123[d]	1/11			
Firmicutes	*Clostridia*	*Stomatobaculum* sp. strain OT 097	1/11			

[a] Uncultivability as reported by Dewhirst et al. (53) and at www.homd.org.
[b] Identification of phylotypes among the subjects is depicted as follows: number of subjects/total number of subjects.
[c] OT, oral taxon.
[d] Recent cultivability reported by Thompson et al. (64).

6 h of development. It has yielded the first microbial identifications at the genus level of not-yet-cultivable microbes and has provided semiquantitative insights into their abundance in the incipient oral biofilm. It is obvious that the functional properties of these microorganisms in the oral biofilm environment are of high interest from the clinical and disease prevention perspectives. While one strategy would be to prevent growth conditions favoring detrimental bacteria, other pursuits will require a more detailed knowledge pertaining to the cultivation and cocultivation of all biofilm inhabitants (62–65). Such insights will be helpful in designing target-specific approaches for the prevention of and/or intervention in diseases exhibiting an oral-biofilm-based etiology.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grants DE05672 (F.G.O.), DE07652 (F.G.O.), AI087803 (E.J.H.), AI101067 (E.J.H.), and DE021565 (B.J.P.); CTSA award U54-TR001012 (A.C.G.); Natural Sciences and Engineering Research Council of Canada grant 371813 (W.L.S.); Canadian Institutes of Health Research grants 106657 and 97577 (W.L.S.); and CIHR New Investigator Award grant 113166 (W.L.S.).

We declare no conflicts of interest.

FUNDING INFORMATION

HHS | National Institutes of Health (NIH) provided funding to Frank G. Oppenheim under grant numbers DE05672 and DE07652. HHS | National Institutes of Health (NIH) provided funding to Eva J. Helmerhorst under grant numbers AI087803 and AI101067. HHS | National Institutes of Health (NIH) provided funding to Bruce J. Paster under grant number DE021565. HHS | NIH | National Center for Advancing Translational Sciences (NCATS) provided funding to Adam C. Gower under grant number U54-TR001012. Gouvernement du Canada | Natural Sciences and Engineering Research Council of Canada (NSERC) provided funding to Walter L. Siqueira under grant number 371813. Gouvernement du Canada | Canadian Institutes of Health Research (CIHR) provided funding to Walter L. Siqueira under grant numbers 106657, 97577, and 113166.

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