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Levels of *Selenomonas* species in generalized aggressive periodontitis

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Abstract

Aim—To compare the levels of *Selenomonas sputigena* and uncultivated/unrecognized *Selenomonas* species in subgingival biofilms from generalized aggressive periodontitis (GAgP) and periodontaly healthy (PH) subjects.

Material and Methods—GAgP (n=15) and PH (n=15) subjects were recruited and their clinical periodontal parameters were evaluated. Subgingival plaque samples were collected (9 samples/subject) and analyzed for the levels of 10 bacterial taxa, including cultivated and uncultivated/unrecognized microorganisms using the RNA-oligonucleotide quantification technique (ROQT). Differences in the levels of the test taxa between groups were sought using the Mann-Whitney test.

Results—GAgP subjects showed significantly higher mean counts of *Porphyromonas gingivalis, Selenomonas sputigena* and *Selenomonas* oral clone CS002 (Human Oral Microbial Database (HOMD) Oral Taxon 131), while *Actinomyces gerencseriae* and *Streptococcus sanguinis* were found in higher mean counts in PH subjects ($p<0.01$). *Selenomonas* EW084 (HOMD OT 146) was only detected in the GAgP group. In the GAgP group, levels of *P. gingivalis* and *S. sputigena* were higher in sites with probing depth (PD) ≥5mm than in shallow sites (PD ≤3mm) ($p<0.01$). Furthermore, sites with PD≤3mm in GAgP subjects harbored higher levels of these two species than sites in PH subjects. There were positive correlations between PD and levels of *P. gingivalis* ($r=0.77$; $p<0.01$), *S. sputigena* ($r=0.60$; $p<0.01$) and *Selenomonas sp. EW076 (OT 139)* ($r=042$, $p<0.05$).

Conclusion—*S. sputigena, Selenomonas* sp. oral CS002 (OT 131) and *Selenomonas s*p. oral clone EW084 (OT 146) may be associated with the pathogenesis of GAgP, and their role in the onset and progression of this infection should be further investigated.

Keywords

Selenomonas sputigena; molecular biology; *16S rRNA*; generalized aggressive periodontitis; not-yet-cultivated species

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INTRODUCTION

The role of the oral microbiota in the etiology of periodontal diseases has been well established, and specificity may exist among certain bacterial species or groups and the various forms of periodontal disease (1-5). The complexity and diversity of the periodontal microbiota has been confirmed by numerous studies (6-8). However, only a few species have been recognized as periodontal pathogens, namely *Aggregatibacter actinomycetemcomitans, Porphyromonas gingivalis* and *Tannerella forsythia* (9).

Aggressive periodontitis is characterized by a rapid rate of tissue destruction and progression, early age of onset, specific patterns of periodontal breakdown and familial aggregation. These features are quite distinct from those observed in chronic periodontitis (10). The microbiota of the localized (LAgP) and generalized forms of aggressive periodontitis (GAgP) present higher proportions of *Aggregatibacter actinomycetemcomitans*, in comparison with that of chronic periodontitis (ChP); whereas the proportion of red complex pathogens (5) did not differ between these periodontal conditions, despite their clinical differences (11). These observations suggest that further analyses of the microbiota are necessary in order to explain differences in clinical outcomes.

The use of open-ended culture-independent approaches has expanded the breadth of knowledge of the oral microbiota. The oral cavity may harbor more than 700 bacterial species (6,12), of which 35% remains uncultivated (13,14). Even though those taxa have remained undetected in most studies, it is possible that specific uncultivated/unrecognized taxa may play a role in the etiology of oral diseases, or alternatively, they may represent beneficial bacterial species.

Using *16S rRNA* sequencing analysis, Faveri *et al.* (11) *observed* that *Selenomonas* species dominated the diseased sites of subjects with GAgP. *Selenomonas sputigena w*as the most frequently detected bacterial species whereas other species of *Selenomonas* were often present in high levels, including *Selenomonas noxia, Selenomonas* oral clone EW076, *Selenomonas* oral clone EW084 and *Selenomonas* oral clone CS002. High prevalence of *Selenomonas* species has also been observed in subgingival samples from ChP subjects (6, 16-18). However, none of these studies were able to quantify the levels of multiple uncultivated/unrecognized taxa in large number of individual biofilm samples simultaneously. Information on the absolute numbers and proportions of organisms in samples is important in distinguishing species associated with periodontal health or disease and to evaluate the effects of periodontal therapy (5, 19).

The RNA-oligonucleotide quantification technique (ROQT) allows the quantification of uncultivated/unrecognized and cultivated taxa in multiple subgingival biofilm samples in parallel (20). In addition, the method does not require sample pooling, amplification or dilution, which represent procedures that may introduce biases in the composition of the microbial communities under study (16,19,21). Thus, the purpose of the present study was to evaluate the levels of *Selenomonas* species, as well as their relationship with *P.gingivalis* and some host-compatible species (*Actinomyces gerensceriae, Streptococcus anginosus/ gordonii* and *Streptococcus sanguinis*) in subgingival biofilm samples from GAgP patients and periodontally healthy subjects controls using ROQT.

MATERIAL AND METHODS

Subject population

Fifteen periodontally healthy subjects and 15 individuals presenting GAgP were included in this investigation. All study participants were systemically healthy and were recruited at the

Guarulhos University School of Dentistry (Guarulhos, SP, Brazil). The medical and dental histories were obtained and a full-mouth periodontal examination was performed. Based on these data, the periodontal diagnosis was made, and subjects who fulfilled the inclusion/exclusion criteria were invited to participate in the study. The study protocol was explained to each subject, and a signed Informed Consent was obtained. This study protocol was approved by Guarulhos University's Ethics Committee in Clinical Research.

Clinical examination

One trained and calibrated examiner performed the clinical examination in all subjects. Visible plaque (0/1), gingival bleeding (0/1), bleeding on probing (BOP, 0/1), suppuration (0/1), probing depth (PD, mm) and clinical attachment level (CAL, mm) were measured at six sites per tooth (mesiobuccal, buccal, distobuccal, distolingual, lingual and mesiolingual) in all teeth excluding third molars at the baseline visit. PD and CAL measurements were recorded to the nearest millimeter using a North Carolina periodontal probe (Hu-Friedy, Chicago, IL, USA).

Inclusion criteria

GAgP and PH were diagnosed based on the periodontal classification of the American Academy of Periodontology (18). Subjects had at least 20 teeth and needed to meet the following criteria in order to be included in this study:

Generalized aggressive periodontitis (GAgP): ≤ 35 years of age; minimum of six permanent incisors and/or first molars with at least one site each with PD and CAL ≥ 5 mm; minimum of six teeth other than first molars and incisors with at least one site each with PD and CAL ≥ 5 mm; familial aggregation (at least one other member of the family presenting or with history of periodontal disease) (11).

Periodontal health (PH): ≤ 35 years of age; No sites with PD and CAL measurements > 3 mm and < 10% of sites exhibiting bleeding on probing.

Exclusion criteria

Exclusion criteria were pregnancy, lactation, smoking, subgingival periodontal therapy in the past 12 months, any systemic condition that could affect the progression of periodontal disease (e.g. diabetes and immunological disorders), long-term administration of anti-inflammatory medication, and antibiotic therapy in the previous 6 months.

Microbiological examination

Sample collection—Individual subgingival plaque samples were collected from nine non-contiguous interproximal sites per subject. For the GAgP group, three sites with PD ≤ 3mm and six sites with PD ≥ 5mm were selected. Nine sites with PD ≤ 3mm were collected from the PH group. The selected sites were randomized in different quadrants. After the clinical parameters had been recorded, the supragingival plaque was removed and the samples were taken with individual sterile Gracey curettes and immediately placed in separate polypropylene tubes containing 100 μl RNAse-free TE (10mM Tris-HCl, 1 mM EDTA, pH 7.6) and kept at -80 °C until extraction of total nucleic acids (TNA).

RNA-oligonucleotide quantification technique

Counts of the 10 bacterial species/phylotypes (Table 1) were determined in each sample, using the RNA-oligonucleotide quantification technique (ROQT) (20). The microbiological analysis was performed at the Laboratory of Microbiology of Guarulhos University.

Extraction of total nucleic acids

Extraction of TNA from all samples was performed using a Masterpure RNA & DNA purification kit (Epicentre, Madison, WI, USA). Cells were pelleted by centrifugation and resuspended in 25 μl TE. One microliter of proteinase K (50 μg μl^{-1}) and 300 μl of tissue and cell lysis buffer were added followed by incubation at 65 °C for 15 min. After 5 min in ice, 175 μl MCP protein precipitation reagent (Epicentre) was added to each sample. After centrifugation, 500 μl isopropanol added to the supernatant and TNA was precipitated by centrifugation. The pellets were rinsed twice with 70% ethanol, air dried and ressupended. TNA samples were kept at -80 °C until analysis.

Probe and standards preparation

Oligonucleotide probes targeting the 16S ribosomal DNA (*rRNA*) gene of 7 cultivated and 3 uncultivated/unrecognized bacterial taxa were synthesized (Table 1). The probe panel also included a universal (eubacterial) probe, based on a conserved region of the bacterial *16S rRNA* gene. The universal probe is only a positive control in the test and the levels were not considered. Probes sequences were 18-22 nucleotides in length and had minimal secondary structure. The sequences employed in ROQT were those routinely used in the Human Oral Microbial Identification Microarray (HOMIM) and are published elsewhere (21). One hundred picomolar (pM) of each oligonucleotide probe were labeled using a digoxigenin 3'end labeling kit (Roche, Indianapolis, IN, USA). The standards for detection of the oligonucleotide probes were a mixture of sequences complementary to each oligonucleotide probe (Table 1). The final mixtures of standards had 0.004 and 0.04 pM of each sequence, corresponding to 10^5 and 10^6 bacterial cells, respectively. The oligonucleotide probes and their 'complementary' sequences were synthesized by Invitrogen (São Paulo, SP, Brazil).

Hybridization

Ninety microliters of 2% glutaraldehyde (Ted Pella, Redding, CA, USA) and 910 μl 6 × SSC (1× SSC = 150mM NaCl, 15 mM sodium citrate, pH 7.0) were added to each TNA sample and standards. The final solutions were deposited in individual lanes of a Minislot (Immunetics, Cambridge, MA) concentrated onto a nylon membrane (Boehringer Mannheim, Indianapolis, IN, USA) and fixed to the membrane. The membranes were pre-hybridized into a plastic hybridization bag at 42°C for at least 90 min in 35ml of a pre-hybridization solution containing 50% formamide, 5 × SSC, 1% casein (Vetec, Rio de Janeiro, RJ, Brazil), 5x Denhardt's reagent (Sigma, St. Louis, MO, USA), 25mM sodium phosphate (pH6.5) and 0.5mg ml^{-1} yeast RNA (Boehringer Mannheim). Then, the membranes were placed in a Miniblotter 45 (Immunetics), with the "lanes" of TNA at 90° to the channels of the device. The digoxigenin-labeled oligonucleotide probes were diluted in hybridization buffer (Ultrahyb Oligo buffer; Ambion, Austin, TX, USA), and added to individual lanes of the Miniblotter 45 at final concentrations ranging from 2 to 40 pM. The membranes were hybridized at 42°C for 80 min and then washed with 2 × SSC, 0.5% sodium dodecyl sulfate at 37°C for 60 min. After blocking with maleic acid buffer containing 10% casein, hybrids were detected by incubation with 1:2,500 dilution of anti-digoxigenin antibody conjugated with alkaline phosphate (Roche) for 30 min. The membranes were washed with maleic acid buffer thrice for 15 min and then with 'buffer 3' [0.04% $MgCl_2$ and 2.1 diethanolamine (pH 9.5), equal volumes] for 5 min. Finally, 1 ml of a chemiluminescent substrate (CDP Star; Tropix, Bedford, MA, USA) diluted in 'buffer 3' was deposited onto the membrane surface, which was then exposed to an X-ray film (IBF-Medix, AGFA, Rio de Janeiro, RJ, Brazil). The films were scanned and signal intensities of the samples and the standards were measured using the TotalLab software (NonLinear USA, Durham, NC). Signals were converted to absolute "counts" by comparison with standards on the membrane. Absence of signal detection was recorded as zero.

Statistical analysis

The mean age and the mean percentage of sites with visible plaque, gingival bleeding, BOP and suppuration, as well as mean PD and CAL were computed for each subject and then averaged across subjects in each group separately. Mean estimated 'counts' ($\times 10^5$) of individual bacterial species were computed for each site individually, averaged within each subject and then across subjects in each group separately. Prevalence of the test taxa was computed by determining the percentage of sites per subject colonized by $\geq 10^5$ cells of each species. The mean percentage of "positive" sites was calculated for each subject and then averaged across subjects in each group. The significance of differences between the two groups for age and the clinical and microbiological parameters was sought using the Mann–Whitney U-test. The Wilcoxon test was used to detect statistically significant differences within PD categories in GAgP group. Chi-square test was employed to compare the differences in the frequency of gender and prevalence of subjects colonized by bacterial species. Spearman correlation was used to assess possible associations between PD and mean 'counts' of bacterial species.

RESULTS

Clinical findings

The demographic characteristics and clinical parameters of the studied population are presented in Table 2. No statistically significant differences were observed between groups for age and gender. The GAgP group displayed significantly higher mean PD, mean CAL, % BOP as well as levels of plaque and number of moderate and deep periodontal pockets ($p < 0.05$) in comparison with the periodontally healthy group.

Microbiological findings

The mean estimated 'counts' ($\times 10^5$) of the 10 species evaluated in the subgingival plaque samples from the PH and GAgP groups are shown in Table 3. Subjects with GAgP showed significantly higher mean 'counts' of *P. gingivalis* ($p<0.001$), *S. sputigena* ($p<0.001$) and *Selenomonas* sp. CS002 (OT 131) ($p<0.01$). *Selenomonas* sp. EW084 (OT 146) was detected only in the GAgP group (n= 4 subjects). Throughout this manuscript, OT or oral taxon designations for uncultivated/unrecognized taxa are provided in accord with the Human Oral Microbiome Database (13, HOMD; www.homd.org) when available. GenBank assession numbers can also be found in the HOMD website.

Levels of *A. actinomycetemcomitans* were more elevated in GAgP subjects than in PH, however the difference between the groups did not reach statistical significance ($p>0.05$). Mean 'counts' of *Actinomyces gerencseriae* and *Streptococcus sanguinis* were significantly higher in subjects with PH ($p<0.01$).

The mean estimated 'counts' of the bacterial species detected in shallow and deep periodontal pockets (PD ≤3mm and ≥5mm) in subjects with GAgP are shown in Table 4. *P. gingivalis* and *S. sputigena* were detected in significantly higher mean 'counts' in deep pockets than in shallow sites. In addition, both shallow and deep sites in subjects with GAgP harbored higher mean 'counts' of these two pathogens, compared with the shallow sites of PH subjects (Figure 1).

The percentage of sites colonized by the 10 species/phylotypes evaluated in subgingival plaque samples taken from subjects with GAgP and PH are presented in Table 5. Subjects with GAgP showed higher mean percentage of sites colonized by *S. sputigena* ($p<0.005$), *Selenomonas* sp. CS002 (OT 131) ($p<0.041$) and *P. gingivalis* ($p<0.001$) in comparison with those with PH. No differences in the prevalence of subjects colonized by all the species

analyzed were observed between groups, except for *P. gingivalis*, which was more prevalent in subjects with GAgP (data not show, p<0.05). Spearman correlations revealed positive correlations between PD and mean 'counts' of *P. gingivalis* (r=0.77, *p*=0.0001), *S. sputigena* (r=0.60, p< 0.009) and *Selenomonas* sp. EW076 (OT 139) (r= 0.42, p=0.031).

DISCUSSION

Previous investigations using the *16S rRNA* cloning and sequencing strategy had shown that species of *Selenomonas* are present in greater proportions in the subgingival biofilm samples of subjects with chronic (6, 16, 18, 22, 23) and aggressive periodontitis (Faveri *et al*, 2008). Recent studies have reported contrasting findings regarding the potential role of *Selenomonas* species in periodontitis. Whilst a high levels of *Selenomonas noxia*, in association with other bacterial species, was observed in a subset of young periodontitis patients (23), the use of a specific oligonucleotide probe targeting the majority of all oral *Selenomonas spp*. indicated that members of this genus were ubiquitous both in periodontitis resistant and diseased patients (24). Therefore, the association between species of *Selenomonas* and periodontal disease remains unclear. Thus, the main goal of the present study was to determine the levels of selected *Selenomonas* species, including uncultivated/ unrecognized phylotypes in subgingival biofilm samples from subjects with GAgP and PH individuals.

The present study used the RNA-oligonucleotide quantification technique (ROQT) (20) to determine the levels of the test taxa in subgingival biofilm samples from GAgP and PH subjects. The use of ROQT can overcome some of the limitations presented by other molecular biology techniques, including checkerboard DNA-DNA hybridization, real time PCR and *16S rRNA* cloning analysis. None of these techniques has the ability to quantify the levels of multiple uncultivated species in large numbers of individual biofilm samples simultaneously. ROQT is a high-throughput method for bacterial enumeration in clinical biofilm samples. In addition, it does not require sample pooling, amplification or dilution, because an entire individual sample is laid onto the membrane. Therefore, introduction of biases in the microbial profiles of the ecosystem under study due to PCR-associated procedures can be eliminated (16, 25, 26).

In agreement with previous studies that used other microbiological techniques such as culture techniques (4, 27, 28, 29), PCR (30,31), checkerboard DNA-DNA hybridization (5, 7, 11, 32) the data from the present study by ROQT support the notion that *P. gingivalis* plays an important role in the etiology of GAgP, as well as, *A. gerensceriae* and *S. sanguinis* were elevated in the healthy in comparison with periodontally diseased subjects.

The results of the present study reinforce the association of *S. sputigena* with the etiology of GAgP. This species was found in significantly higher levels (Table 3) and prevalence (% of sites colonized) in subjects with GAgP than PH group (Table 5; p<0.01), as well as in higher levels in deep sites compared with shallow sites in GAgP subjects (Table 4). *S. sputigena* is a Gram-negative, multiflagellated, motile, anaerobic rod (33). An early study showed that periodontitis patients showed high antibodies titers against *S. sputigena* (34). Later, investigations demonstrated that this species was also associated with necrotizing ulcerative periodontitis (35), aggressive periodontitis (27), active periodontitis lesions (36, 37) and GAgP (15). Studies, using cloning and sequencing, as well as culture, also identified this species as a highly prevalent (38) and dominant member of the periodontal pocket microbiota of ChP subjects (28, 29, 39, 40).

Despite its possible association with disease, the components and products of *S. sputigena* involved in the colonization of the oral cavity and the mechanisms of induce tissue

destruction are unknown. This species presented a bone resorption activity in experimentally colonized rats (41). It is noteworthy that Lipopolysaccharide/lipid A of *S. sputigena* differs from that generally found in other Gram-negative bacteria, which consisted mainly of fatty acids such as undecanoic, trideanoic, tridecenoic, 3-hydroxytridecanoic and 3-hydroxytetradecanoic acid. In addition, Lipid A from *S. sputigena* is able to induce IL-1α and IL-6 in murine macrophages (42), suggesting its role in inducing inflammation. However, there is no evidence that Lipid A from *S. sputigena* leads to a different inflammatory phenotype compared with other Gram-negative bacterial species.

Members of the genus *Selenomonas* were frequently detected as part of the not-yet-cultivated species of the oral cavity (6, 17, 22, 43). The present analysis of uncultivated/unrecognized *Selenomonas* phylotypes revealed that these taxa are frequently part of the subgingival microbiota of GAgP patients. *Selenomonas* sp. EW084 (OT 146) was only detected in GAgP subjects, *Selenomonas* sp. CS002 (OT 131) was more prevalent and present in higher levels in GAgP than in periodontal healthy subjects. This species has also been associated with other anaerobic sites of the oral cavity such as deep dentin caries (44). In addition, the levels of *Selenomonas* sp. EW076 were positively correlated with the increase of PD, which is in accord with the findings reported by Kumar et al (17). Drescher et al. (24) analyzed the topography of the subgingival biofilm by fluorescence in situ hybridization (FISH) and electron microscopy in subjects with GAgP, ChP and periodontitis-resistant subject and revealed that *Selenomonas* spp. appeared in large numbers in all parts of the collected biofilms and seemed to make a relevant contribution to their structural organization. The authors reported that it is hard to imagine that a group of organisms constituting an important part of the biomass does not contribute to the pathogenetic process of periodontal disease.

As a whole, the data indicated that the evaluated *Selenomonas* species/phylotypes are heterogeneous in their ability to colonize oral sites, and cannot be seen as a single taxonomic unit. Although their role in inducing periodontal destruction cannot be drawn unless longitudinal studies are performed and their phenotypic traits are evaluated, the data indicated that *S. sputigena* and *Selenomonas* sp. CS002 (OT 131) are part of the subgingival microbiota associated with aggressive periodontitis and may play a role in the disease onset and progression.

CONCLUSION

S. sputigena and Selenomonas sp. CS002 (OT 131) may be associated with the pathogenesis of GAgP, and therefore, their role in the onset and progression of this infection merits further investigation.

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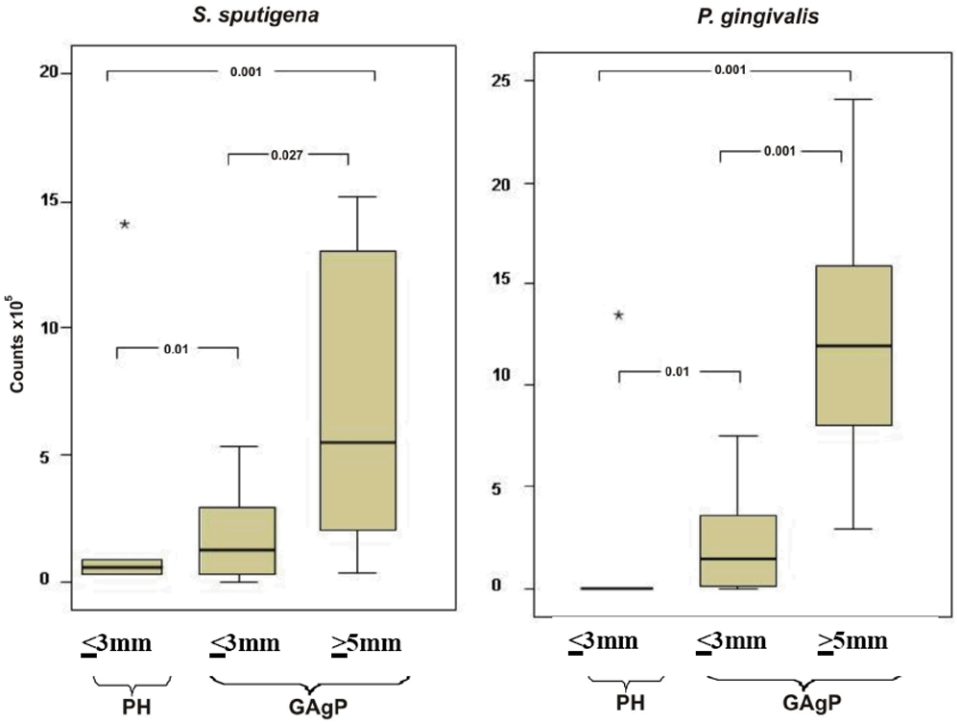

Figure 1.

Mean estimated 'counts' ($\times 10^5$) of *S. sputigena* and *P.gingivalis* in the PH subjects and in sites with PD ≤3mm and PD ≥4mm in the GAgP group. Boxplots show the median, minimum, and maximum values. The significance of differeces between groups was assessed using Mann-Whitney and Wilcoxon test. The star (*) represent extreme outliers. PH, periodontal health; GAgP, generalized aggressive periodontitis;

Table 1

Sequences for oligonucleotide probes and for the standards for quantification of the species/phylotypes evaluated.

Species/phylotypes	Sequence (5'-3')	Control (3'-5')
Selenomonas sputigena	CCGTCACCCAAACTCAAT	GGCAGTGGGTTTGAGTTA
Selenomonas noxia	CTATTCGCATTAGGCACG	GATAAGCGTAATCCGTGC
Selenomonas sp. EW084 (OT 146)	GGACTCATCTCTGAGTCT	CCTGAGTAGAGACTCAGA
Selenomonas sp. EW076 (OT 139)	CTCTGCATGCTTCAGTCA	GAGACGTACGAAGTCAGT
Selenomonas sp. CS002 (OT 131)	GGCGCAACATTCGGTATT	CCGCGTTGTAAGCCATAA
A.actinomycetemcomitans	TTAAAGGTCCGCCTACGT	AATTTCCAGGCGGATGCA
Porphyromonas gingivalis	GTGGAAGCTTGACGGTAT	CACCTTCGAACTGCCATA
Actinomyces gerensceriae	ACCCCAGAAGCCCGTT	TGGGGTCTTCGGGCAA
S. anginosus/gordonii	CAACTCACAGTCTATGGTGTAG	GTTGAGTGTCAGATACCACATC
Streptococcus sanguinis	CAATAATCAATTTTATGCGGT	GTTATTAGTTAAAATACGCCA
16S rRNA Universal	CTGCTGCCTCCCGTAGG	GACGACGGAGGCATCC

A subset of the probes employed were 'combination probes', in that they could not distinguish species/phylotypes. This was the case for *S. anginosus/gordonii.*

OT : oral taxon ; oral taxon designations for uncultivated/unrecognized taxa are provided in accord with the Human Oral Microbiome Database, (www.homd.org), when available; GenBank assession numbers can also be found in the HOMD website.

Table 2

Demographic characteristics and mean (± SD) full-mouth clinical parameters in both groups.

Clinical variables	PH (n=15)	GAgP (n=15)
Age[NS]	26.6 ± 3.6	27.4 ± 4.3
Gender (M/F)[NS]	9/6	8/7
Probing depth (mm) [*]	1.96 ± 0.6	4.62 ± 0.81
Clinical Attachment level (mm) [*]	1.04 ± 0.5	4.53 ± 1.23
Percentage of sites with		
Plaque accumulation [*]	34.5 ± 9.9	74.1 ± 10.4
Gingival bleeding [*]	4.5 ± 2.9	24.2 ± 10.9
Bleeding on probing [*]	6.1 ± 3.2	74.1 ± 15.7
Suppuration [*]	0.0 ± 0.0	4.5 ± 6.2
PD≤3 mm [*]	100	37.3 ± 20.1
PD 4-6 mm [*]	0	39.9 ± 28.2
PD≥7 mm [*]	0	22.8 ± 15.7

PH, periodontal health; GAgP, generalized aggressive periodontitis;

[*] $p<0.05$;

Mann-Whitney test; NS, not significant ($p>0.05$); PD, probing depth; SD, standard deviation.

Table 3

Mean estimated 'counts' ($\times 10^5 \pm$ SD) of 10 bacterial taxa in subgingival biofilm samples obtained from subjects with generalized aggressive periodontitis and subjects with periodontal health.

Species/phylotypes	Experimental groups		
	PH (n=15)	GAgP (n=15)	*p-value
S. sputigena	0.3 ± 0.5	4.5 ± 2.9	0.001
S. noxia	0.2 ± 0.4	1.2 ± 2.0	NS
Selenomonas sp. EW084	0	0.1 ± 0.4	NS
Selenomonas sp. EW076	0.05 ± 0.9	0.5 ± 1,0	NS
Selenomonas sp. CS002	0.01 ±0.02	0.12 ± 0.09	0.01
A. actinomycetemcomitans	0.06 ± 0.01	0.2 ± 0.4	NS
P. gingivalis	0.1 ± 0.3	7.8 ± 3.9	0.001
A. gerencseriae	1.7 ± 0.6	0.3 ± 0.4	0.001
S. anginosus/gordonii	1.3 ± 1,0	1.4 ± 1.8	NS
S. sanguinis	1.3 ± 1.5	0.3 ± 0.01	0.01

*$p<0.05$;

Mann-Whitney test; PH, periodontal health; GAgP, generalized aggressive periodontitis; NS, not significant; PD, probing depth; SD, standard deviation.

Table 4

Mean estimated 'counts' ($\times 10^5 \pm$ SD) of 10 bacterial taxa in subgingival biofilm samples obtained baseline PD ≤3mm and PD ≥5mm at subjects with generalized aggressive periodontitis.

	GAgP		
Species/phylotypes	**PD ≤3mm**	**PD ≥5mm**	***p-value**
S. sputigena	1.7 ± 1.7	6.7 ± 5.5	0.027
S. noxia	0.6 ± 0.9	1.3 ± 1.1	NS
S. oral clone EW084	0.01 ± 0.01	0.3 ± 0.8	NS
S. oral clone EW076	0.04 ± 0.09	1.2 ± 2.9	NS
S.oral clone CS002	0.08 ± 0.01	0.02 ± 0.05	NS
A. actinomycetemcomitans	0.01 ± 0.04	0.3 ± 0.8	NS
P. gingivalis	2.4 ± 2.7	12.2 ± 6.1	0.001
A. gerencseriae	0.2 ± 0.5	0.5 ± 0.8	NS
S. anginosus/gordonii	1.4 ± 2.8	1.4 ± 1.8	NS
S. sanguinis	0.5 ± 0.09	0.1±0.02	NS

*$p<0.05$;

Wilcoxon test; GAgP, generalized aggressive periodontitis; NS, not significant; PD, probing depth; SD, standard deviation.

Table 5

Percentage of sites colonized by 10 bacterial taxa at levels ≥ 10^5 in subgingival plaque samples taken from subjects with generalized aggressive periodontitis and subjects with periodontal health.

Species/phylotypes	Experimental groups		
	PH (n=15)	GAgP (n=15)	*p-value
S. sputigena	15.4 ± 18.9	51.4± 32.7	0.005
S. noxia	12.4± 19.4	27.8 ± 28.4	NS
Selenomonas sp. EW084	0	7.3 ± 10.7	NS
Selenomonas sp. EW076	2.9 ± 8.0	15.4 ± 13.7	NS
Selenomonas sp. CS002	6.1 ± 5.7	22.1 ± 11.9	0.041
A. actinomycetemcomitans	3.6 ± 6.7	13.9 ± 16.8	NS
P. gingivalis	4.4 ± 8.1	65.5 ± 30.9	0.001
A. gerencseriae	38.2 ± 29.6	22.9 ± 15.0	NS
S. anginosus/gordonii	41.8 ± 30.4	27.8 ± 28.4	NS
S. sanguinis	27.9 ± 29.2	24.9 ± 26.1	NS

* $p<0.05$;

Mann-Whitney test; PH, periodontal health; GAgP, generalized aggressive periodontitis; NS, not significant; PD, probing depth; SD, standard deviation.