

Perturbation of the indigenous rat oral microbiome by ciprofloxacin dosing

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SUMMARY

Mucosal surfaces such as the gut, vagina and oral cavity are colonized by microbiota that are an integral component of the healthy ecosystem. Recent molecular techniques make it feasible to correlate antimicrobial dosing levels with changes in microbiome composition. The objective of this study was to characterize the rat oral plaque microbiome composition at doses of ciprofloxacin that were considerably above and below nominal *in vitro* minimal inhibitory concentrations of a variety of gram-positive oral commensal bacteria. We exposed the oral cavities of rats to relatively low ($0.1 \mu\text{g ml}^{-1}$) and high ($20 \mu\text{g ml}^{-1}$) doses of ciprofloxacin in the drinking water over a 3-day period. Plaque microbiota were characterized using 454 pyrosequencing. The rat indigenous community was dominated by the genera *Rothia* (74.4%) and *Streptococcus* (4.7%). Dosing at $0.1 \mu\text{g ml}^{-1}$ was associated with changes in *Rothia* and *Streptococcus* species that were not significant, whereas dosing at $20 \mu\text{g ml}^{-1}$ caused a pronounced (significant) reduction in the relative abundance of the *Streptococcus* genus. Taxonomic independent analysis indicated that the perturbation in the overall community structure attributed to dosing with ciprofloxacin at either the low or high dose was relatively low. The results suggest that it is feasible to use an anti-

microbial dosing regimen to selectively target a specific subset of a mucosal microbiome for elimination with minimal perturbation of the entire community.

INTRODUCTION

Mucosal surfaces such as the gut, vagina and oral cavity are densely colonized by microorganisms that are an integral biological component of healthy humans (Pflughoeft & Versalovic, 2012). Besides the well-established symbiotic relationships in the gut (Sears, 2005), indigenous microbiota that colonize healthy mucosal surfaces compete with putative pathogens (Pultz *et al.*, 2004; Teughels *et al.*, 2007; Ubeda *et al.*, 2010), and contribute to immune homeostasis (Krisanaprakornkit *et al.*, 2000; Neish *et al.*, 2000; Rakoff-Nahoum *et al.*, 2004; Mans *et al.*, 2009). Concerns about the negative consequences of the disturbance of the commensal mucosal community by conventional antimicrobial treatments have led to characterization of the perturbation of ciprofloxacin dosing on the gut microbiome (Dethlefsen *et al.*, 2008; Endt *et al.*, 2012). Ciprofloxacin is used to treat periodontitis in humans (Otero *et al.*, 2009). Fluoroquinolones are generally more efficacious against gram-negative bacteria than gram-positive bacteria (Appelbaum &

Hunter, 2000; Avery, 2004) and our previous results suggested that appropriate dosing with this drug might be used to selectively eliminate a periodontal pathogen in an oral biofilm consortium (Suci & Young, 2011). Rat models are particularly relevant for characterizing the progression of the disease from health to chronic destructive inflammation (Kawai *et al.*, 1998, 2000; Li *et al.*, 2010; Schreiner *et al.*, 2010; Bezerra *et al.*, 2012). Despite evidence that commensal microbes probably play a critical role in determining the outcome of mucosal infections, the rat oral microbiome has not been well characterized using culture-independent methods. An unanticipated result of our study was that the high dose of ciprofloxacin selectively affected a subset of the oral microbiome with minimal disturbance of the overall plaque community structure.

METHODS

Animals

Fifteen, 6-month-old female rats (Sprague-Dawley; Charles River Laboratories, Hollister, CA) were housed individually at 22°C under a 12-h light/12-h dark cycle and fed *ad libitum* with a soft diet (Purina Laboratory Rodent Chow) following the guidelines and regulations for the use and care of animals at the University of Southern California.

Plaque collection and DNA isolation

Supragingival plaque was acquired from buccal and lingual regions of the upper molars of rats using extrafine paper points (Freire *et al.*, 2011). Four plaque samples from each of five rats were pooled for analysis. Samples were pooled with the intention of reducing the effect of between-site and between-animal variability to detect differences in community structure that could be attributed specifically to dosing. Cells were lysed using a previous protocol (Suci & Young, 2011) with an additional bead beating step. DNA was isolated using a GenElute™ Bacterial Genomic DNA kit (Sigma-Aldrich, St Louis, MO).

16S rRNA Tag pyrosequencing

Amplicon libraries were generated using high-performance liquid chromatography-purified fusion primers that consisted of a 454 adaptor (5'-CCTA

TCCCCTGTGTGCCTTGGCAGTCTCAG-3') linked to a 10-nucleotide identification tag followed by a template-specific forward primer (5'-TCACGRCACGAGCTGACGAC-3') or reverse primer (5'-GGATTAGATACCCBRGTAGTC-3') to conserved regions flanking the V5V6 hypervariable region of the 16S rRNA gene (Zaura *et al.*, 2009). There were six identification tags used to discriminate six pooled samples. The polymerase chain reaction (PCR) products were purified using AMPure XP beads (Agencourt®, Beckman Coulter, Brea, CA). Amplicon quality and concentration were analysed by BioAnalyzer (Agilent 2100, Agilent Technologies, Inc, Santa Clara, CA) and Qubit® 2.0 Fluorometer (Life technologies, Grand Island, NY), respectively. Purified amplicons were sequenced at the W.M. Keck Center for Comparative and Functional Genomics at the University of Illinois at Urbana-Champaign, IL.

Ciprofloxacin dosing

There were three groups, each of five rats: (i) a 'no-dose' control group; (ii) a 'low-dose' group (0.1 µg ml⁻¹ ciprofloxacin); and (iii) a 'high-dose' group (20 µg ml⁻¹ ciprofloxacin). These concentrations are, respectively, well below and above nominal *in vitro* minimal inhibitory concentrations (MICs) of a variety of gram-positive oral commensal bacteria (Hoogkamp-Korstanje & Roelofs-Willemse, 2000). The high dose is approximately 5.8 mg kg⁻¹ body weight per day, a dose level that we anticipated the rats would tolerate without reduced water consumption (Xue *et al.*, 2009). Samples were acquired immediately before dosing (day 0) and 72 h later (day 3) yielding six pooled samples. We anticipated that dosing over this time period might affect the microbiome taxa in a manner that was comparable to *in vitro* tests. Rats were dosed with ciprofloxacin (catalog. # 199020; ICN Biomedicals Inc., Irvine, CA) by adding it to the drinking water. In addition, a topical application was made immediately following acquisition of the first plaque sample (day 0) and 24 h after this time-point. For this procedure, animals were anesthetized with xylazine and ketamine, the mouth was retracted and 500 µl of the ciprofloxacin solution was applied to the buccal and lingual regions of the upper molar over a 5-min period. Water consumption was normal (mean, 64 ml day⁻¹ water) and not significantly different between the three groups of rats.

Data analysis

Sequences were filtered and trimmed using the Ribosomal Data Base (RDP) pyrosequencing pipeline. Taxonomic classifications were made using the RDP CLASSIFIER (Wang *et al.*, 2007) (Release 10, Update 30 which consists of 2,578,902 aligned and annotated 16S rRNA sequences) with a bootstrap cutoff of 80%. Taxonomic independent analysis was performed using MOTHUR (Schloss *et al.*, 2009). Unique sequences were aligned to the SILVA database (Pruesse *et al.*, 2007), chimeras were removed and operational taxonomic units (OTUs) were determined by clustering at genetic distances ranging from 0.01 to 0.08 using an average neighbor algorithm. OTUs obtained using a similarity (distance) cutoff of 0.03 (3%) were used for further analysis (Dethlefsen *et al.*, 2008; Zaura *et al.*, 2009). Analysis of molecular variance (Excoffier *et al.*, 1992) as implemented by MOTHUR was used to interpret matrices of Yue & Clayton dissimilarity values (Yue & Clayton, 2005). Phylogenetic trees were constructed with the program MEGA 4 using the neighbor-joining method with Jukes–Cantor distances (Tamura *et al.*, 2007). Phylogenetic trees were analysed to determine similarity of community structure using UNIFRAC (Lozupone *et al.*, 2006).

RESULTS

Sequence data

Of the total of 91,120 reads, 86,513 passed quality control. There were 7234 unique sequences; these included 3454 sequences incorporating chimeras, which were removed. Chimeric sequences were present at relatively low abundance as shown by the moderate reduction in total number of sequences after chimera removal (80,080). The mean length of sequences used for the analysis was 256.52 nucleotides.

Rat plaque community structure

The taxonomic dependent analysis for the four pooled samples from rats not exposed to ciprofloxacin indicated that the population was dominated by Actinobacteria, and to a lesser extent by Firmicutes (Table 1). Of the total of 80,080 sequences that were

Table 1 Taxon relative abundance

Taxon	% relative abundance
Actinobacteria	82.54
<i>Rothia</i>	74.43
<i>Corynebacterium</i>	0.18
<i>Microcella</i>	0.04
<i>Propionibacteria</i>	0.01
<i>Brachybacterium</i>	0.01
<i>Janibacter</i>	0.01
<i>Kocuria</i>	¹
<i>Micrococcus</i>	¹
Firmicutes	7.81
<i>Streptococcus</i>	4.67
<i>Staphylococcus</i>	0.36
<i>Exiguobacterium</i>	0.19
<i>Enterococcus</i>	0.08
<i>Granulicatella</i>	0.02
<i>Anaerobacillus</i>	¹
<i>Gemella</i>	¹
Proteobacteria	1.04
<i>Halomonas</i>	0.55
<i>Shewanella</i>	0.14
<i>Paramoritella</i>	0.13
<i>Urbururuella</i>	0.05
<i>Paracoccus</i>	0.04
<i>Vibrio</i>	0.02
<i>Diaphorobacter</i>	0.02
<i>Neisseria</i>	0.01
<i>Acinetobacter</i>	0.01
<i>Lucibacterium</i>	¹
<i>Sphingomonas</i>	¹
Cyanobacteria	0.09
Bacteroidetes	0.03
Acidobacteria	0.03
Planctomycetes	0.02
<i>Alkaliflexus</i>	0.01
Aquificae	¹
Unclassified	8.42

Phyla are shown in bold type; genera are in italics.

¹Indicates fewer than five sequences.

analysed, 7444 were classified at the genus level and 6303 were unclassified. The genera that contribute by far the most to these phyla are *Rothia* and *Streptococcus*, respectively. We report relative abundance data only for taxa represented by more than five sequences because the confidence limits for relative abundance determinations of taxa represented by less than five sequences are very large (Dethlefsen *et al.*, 2008).

Rank abundance and rarefaction curves obtained from the taxonomic independent analysis exhibit fairly typical shapes (Fig. 1). The richness at a genetic distance of 0.03 was 575 OTUs. Of the 575 total OTUs

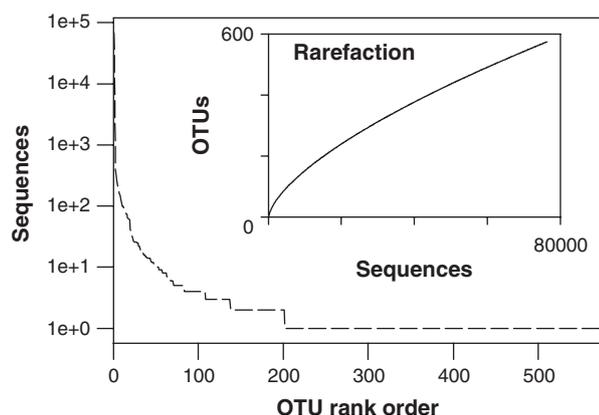


Figure 1 Rank abundance curve and rarefaction curve (insert) for sequence data from all 6 conditions combined. Operational taxonomic units (OTUs) were defined at a distance of 0.03.

there were 490 OTUs (85.2%) represented by fewer than five sequences. These relatively low abundance OTUs contributed < 1% to the total relative abundance. According to the Chao I estimator (Chao, 1984) the projected richness of the rat plaque community based on the rarefaction analysis was 1648 OTUs at a genetic distance of 0.03 with a 95% confidence range of 1355–2050. The estimated community richness of the rat community was considerably less than the oral human supragingival plaque microbiome (reduced by approximately 6.3%) computed using the same parameters (Chao 1 estimator of richness at a genetic distance of 0.3) (Keijsers *et al.*, 2008).

Effect of ciprofloxacin dosing on the indigenous community

Taxonomic classifications (RDP) were used to assess the effect of ciprofloxacin dosing on individual genera or families. A family was included if: (i) it made up

more than 50% of its order and, (ii) other genera listed in that family contributed less than 50% abundance to that family. We first looked for evidence for a significant change in relative abundance of a taxonomic group represented by more than five sequences for a day 0 condition (~ 0.04% relative abundance) (Table 2). A genus or family was excluded from the analysis if it was not present in the no-dose day 3 condition or in all day 0 conditions. Sequences associated with these excluded taxa contributed < 1% to the total relative abundance. There were six taxa for which the relative abundance on day 3 was greater than or equal to one standard deviation unit removed from the mean of the day 0 relative abundance. A single value *t*-test was used to compute *P* values. For the two dominant genera the most significant effect of ciprofloxacin dosing was the reduction in *Streptococcus* at the high dose. *Rothia*, was also perturbed by the high dose more significantly than the low dose. With respect to the low-abundance taxa, the pattern of reduction in the relative abundance of the *Corynebacterium* genus appears to be a consequence of ciprofloxacin dosing, whereas the changes in the relative abundance of the Microbacteriaceae family and the *Uruburuella* genus do not appear to be clearly caused by ciprofloxacin dosing. We also looked for evidence for overgrowth by a genus or family that that was absent or represented by fewer than five sequences for the day 0 low-dose or high-dose conditions. The largest increase between day 0 and day 3 in this category was for the Pasteurellaceae family, which increased from 0.01 to 0.2% relative abundance for the low-dose condition.

Taxonomy independent analysis indicated that the overall perturbation of the indigenous microbiota

Table 2 Ciprofloxacin dosing effect on biofilm genera

	Day 0 ¹	Day 3 ²		
		No dose	Low dose	High dose
<i>Rothia</i> spp.	70.92 ± 9.18	84.98 (1.5)	80.39 (1.0)	93.50 (2.4)*
<i>Streptococcus</i> spp.	4.92 ± 1.90	3.86 (0.6)	7.49 (1.3)	0.22 (2.5)*
Rhodobacteraceae	0.28 ± 0.06	0.10 (2.9)*	0.04 (3.9)*	0.03 (4.0)*
<i>Corynebacterium</i> spp.	0.20 ± 0.07	0.15 (0.7)	0.02 (2.6)*	0.02 (2.6)*
Microbacteriaceae	0.19 ± 0.04	0.05 (3.5)*	0.05 (3.5)*	0.02 (4.4)*
<i>Uruburuella</i> spp.	0.07 ± 0.03	0.01 (2.1)	0.03 (1.4)	0.00 (2.4)*

¹Mean% relative abundance for Day 0 ± standard deviation.

²Mean% relative abundance for Day 3 (difference between Day 0 mean in standard deviation units).

**P* < 0.05.

caused by ciprofloxacin dosing at either the high or low dose was relatively small. UNIFRAC analysis based on phylogenetic trees indicated that community structure was not significantly different between any of the pairs of conditions ($P > 0.015$). Changes in OTU relative abundance over the 3-day period are shown in Fig. 2 for the 50 most abundant OTUs obtained at a distance of 0.03. Most of the sequences were incorporated into shared OTUs. The number of shared OTUs between each pair of day 0/day 3 conditions at the no, low and high dose is shown in parenthesis in the first column of figures (first value in the pair of numbers). Most of the sequences in the community were associated with shared OTUs, as indicated by the second value in the pair of numbers, the percentage of sequences represented by these shared OTUs. The β diversity between pairs of samples acquired on the same day (three pairs each for day 0 or day 3) and between the three pairs of day0/day3

samples at the different dose levels (no, low and high) is relatively low (Fig. 3). The values are all < 0.5 which, by comparison with an extensive study of the human microbiome, suggests modest differences in community structure between each pair of samples (Huttenhower *et al.*, 2012). The β diversity between day 0 and day 3 samples for the low-dose condition is notably lower than the β diversity for other pairs of samples, and the β diversity for the no-dose condition pairs is slightly higher than for the high-dose pairs. This same trend is evident in the Shannon diversity index, which quantifies the within-sample (α) diversity (Fig. 4). These latter data show that in terms of absolute difference in α diversity within each group, the effect of ciprofloxacin was relatively small. Indices of evenness and richness for all six conditions are also shown in Fig. 4. There is a decrease in the means of each of the indices between day 0 and day 3 that is significant at the

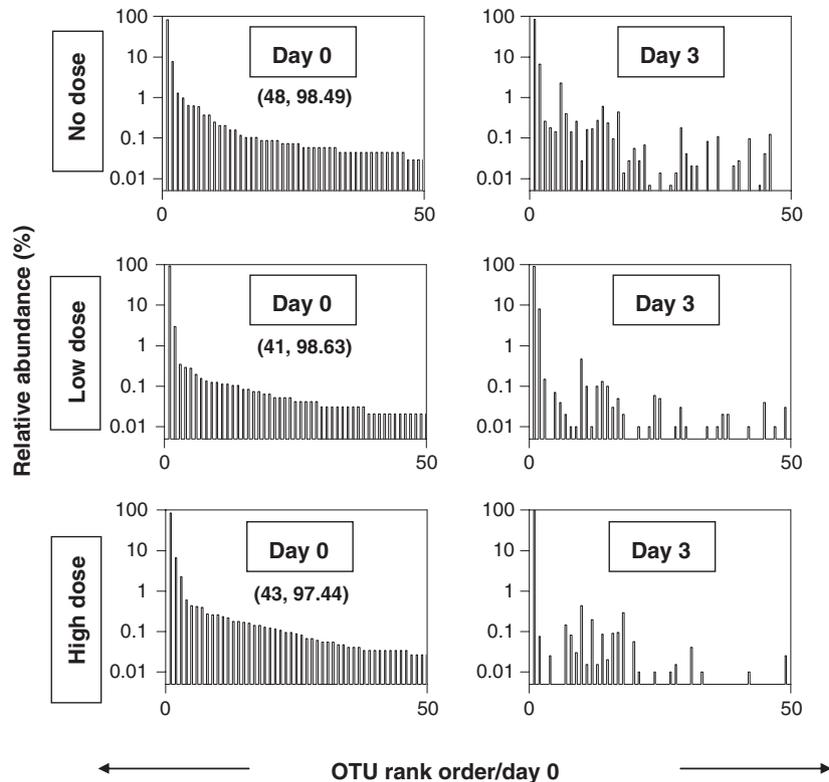


Figure 2 Rank abundance curves of the 50 most abundant operational taxonomic units (OTUs) defined at a distance of 0.03 plotted in terms of per cent relative abundance for each of the six conditions separately. The OTU rank order on the horizontal axes is that obtained from the corresponding day 0 condition for all three pairs of plots. Numbers in parentheses below the day 0 label are, respectively, the number of shared OTUs and the per cent of sequences in these shared OTUs for each of the day0/day3 pairs of sequences at each of the three dose levels.

0.05 level only for the richness index. There is no obvious pattern to indicate that any index presented in Fig. 4 was perturbed by ciprofloxacin dosing.

To determine whether differences in community structure were associated more strongly with temporal changes over the 3-day period, or with the effects of ciprofloxacin dosing, a matrix of Yue & Clayton dissimilarity values was computed. There was a relatively good correlation between the Yu & Clayton values and the UNIFRAC distances ($R = 0.8790$).

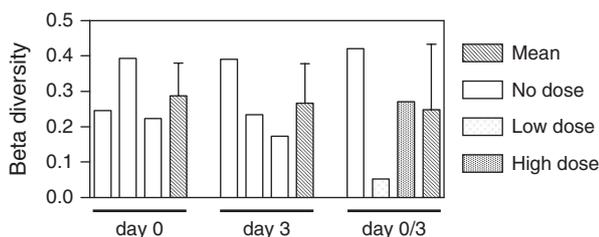


Figure 3 The Bray–Curtis (β) diversity between each of the three pairs of day 0 and day 3 samples and between each pair of day 0/day 3 samples for each dosing condition. Means are presented for each set of three diversity values. Error bars are standard deviations of the mean.

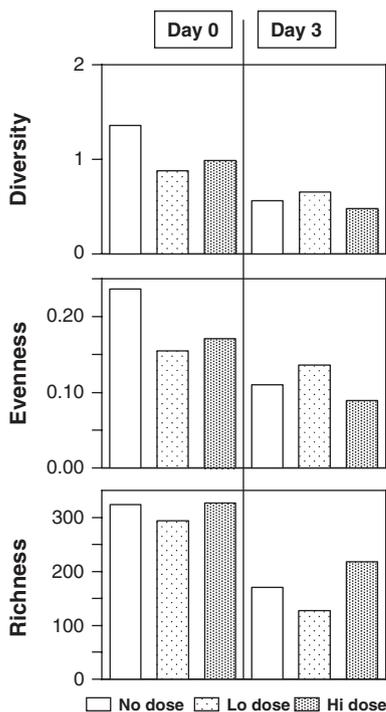


Figure 4 Shannon diversity, Shannon evenness and richness indices for the six conditions. The legend at the bottom applies to all the plots.

Analysis of molecular variance was used to assess the significance of between-group differences. When the matrix of Yu & Clayton values was arranged so that group categories were day 0 and day 3, the difference between groups was more significant ($P = 0.10$) than when the matrix was arranged so that the dose levels were the group categories ($P = 0.76$). This indicates that the perturbation in overall community structure caused by ciprofloxacin dosing was less than the temporal changes over the 3-day period.

In order to determine whether ciprofloxacin dosing affected the community structure within the two dominant genera, sequences classified by RDP as either *Rothia* or *Streptococcus* were analysed separately. Although the *Rothia* genus was more abundant than the *Streptococcus* genus, its diversity according to the Shannon diversity index was significantly less ($P < 0.001$) (Fig. 5). There is no clear pattern in the Shannon diversity index, indicating that ciprofloxacin dosing perturbed the community structure of the *Rothia* genus, whereas there is a clear trend toward increasing diversity from the no-dose to the high-dose condition for the *Streptococcus* population. As above, the community structure was compared between all pairs of the six conditions by computing Yue & Clayton dissimilarity values and using analysis of molecular variance to assess the significance of between-group differences. Similar to the results for the total population, for the *Rothia* genus the difference between day 0 and day 3 groups was

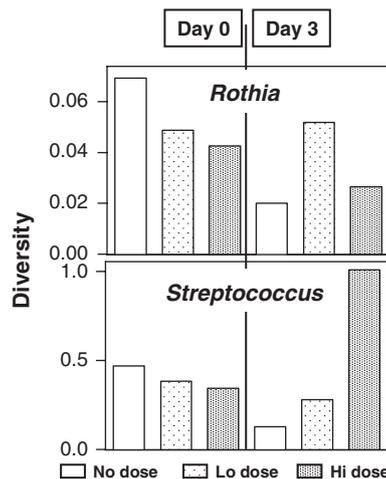


Figure 5 Shannon diversity for sequence data classified as *Rothia* or *Streptococcus* for the six conditions. The legend at the bottom applies to both plots.

more significant than between dose level groups (P values of 0.53 and 0.992, respectively). In contrast, for the *Streptococcus* genus the difference was more significant between dose-level groups than the day 0 and day 3 groups (P values 0.317 and 0.848, respectively). This statistical result is consistent with the dose-dependent pattern exhibited in Fig. 5. This was characterized further using UniFrac distances to perform principle component analysis on this subset of sequences (Fig. 6). Consistent with the trend in the α diversity indices in Fig. 5, the subset of *Streptococcus* sequences belonging to the day 3 high-dose condition is an outlier compared with sequences associated with the five other conditions with respect to the first principle component that describes 97.88% of the variation in the data. Inspection of rank abundance curves suggests that the greater diversity exhibited by the subset of *Streptococcus* sequences belonging to the day 3 high-dose condition (Fig. 5) originates from a relative reduction in the most abundant OTU. This enhanced the uniformity of the distribution of the sequences among the five different OTU in this relatively small subset of sequences and so increases the diversity index (data not shown). The interpretation that the high dose of ciprofloxacin produced selective reduction of a subset of phylogenetically similar sequences in the *Streptococcus* species is supported by a comparison of the phylogenetic trees for the day 0 and day 3 high-dose conditions constructed from unique sequences (Fig. 7). To provide some idea of the possible taxonomic identifications within the *Streptococcus* genus, the trees in Fig. 7 are presented in terms of species level identifications obtained by NCBI BLASTN. Sequences

identified as *Streptococcus plurextorum* represented, respectively, 88.39% and 21.74% of the total sequences in day 0 and day 3 high-dose subsets of sequences. With the acknowledgment of the substantial uncertainty in these species-level identifications, the data in Fig. 7 suggest that ciprofloxacin produced a selective reduction of *S. plurextorum*.

DISCUSSION

The most outstanding effect of ciprofloxacin dosing was a decrease in the relative abundance of the *Streptococcus* genus at the high dose, as indicated by the taxonomic dependent analysis. The decrease in streptococci recovered from the plaque after dosing for 3 days at $20 \mu\text{g ml}^{-1}$ is consistent with the susceptibility of a variety of oral streptococcal species measured *in vitro* for planktonic cells (MIC range of $0.12\text{--}16 \mu\text{g ml}^{-1}$) (Hoogkamp-Korstanje & Roelofs-Willemsse, 2000), and also with the susceptibility of *S. pneumoniae* biofilms to levofloxacin (del Prado *et al.*, 2010). In addition to the decrease in relative abundance of sequences identified by RDP as *Streptococcus*, ciprofloxacin dosing at the high dose appeared to cause a pronounced difference in the community structure associated with this subset of sequences. Specifically, there was a selective reduction in a particular sequence very tentatively classified as *S. plurextorum*. The *S. plurextorum* was recently recognized as a new species (Vela *et al.*, 2009). The absence of *Streptococcus suis* in the day 3 trees presented in Fig. 7B may be related to the reduction in *S. plurextorum* because these two species are closely related. There are some data indicating that

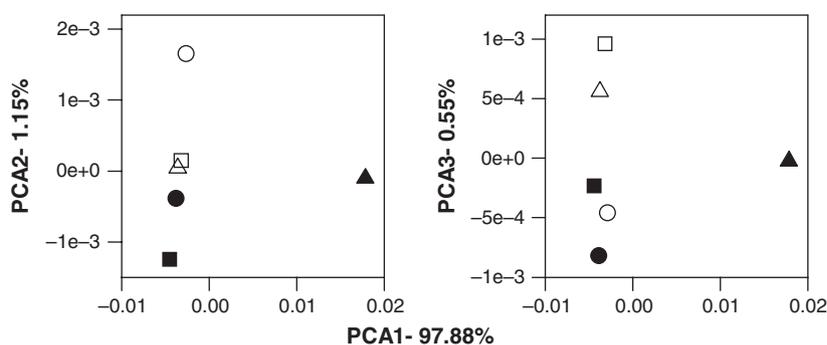


Figure 6 Principle components obtained from UniFrac analyses of the phylogenetic tree structure of subsets of sequences classified as *Streptococcus* by Ribosomal Data Base. Symbols are no-dose condition (circles), low-dose condition (triangles), high-dose condition (squares), open symbols (day 0), filled symbols (day 3). Percent variation accounted for by each component is specified.

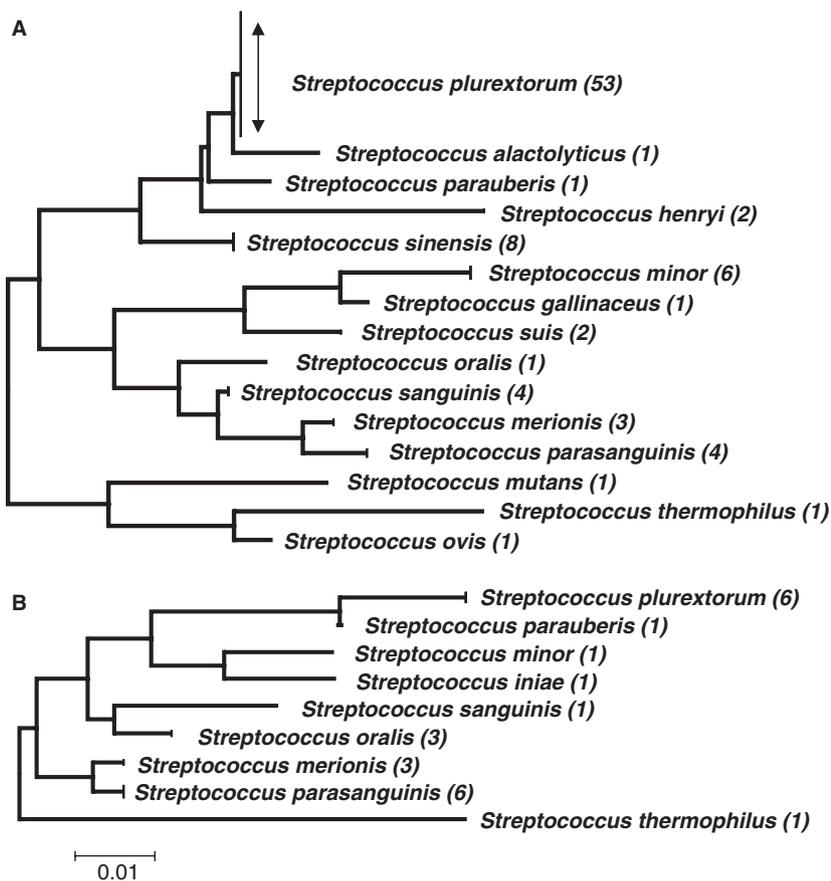


Figure 7 Phylogenetic trees for unique sequences classified as *Streptococcus*. Species identifications were obtained using NCBI BLASTN from unique sequences from the high-dose groups of rats. Numbers in parentheses are the number of unique sequences classified as the corresponding species. (A) Day 0 unique sequences; (B) day 3 unique sequences. The distance scale is the same for each tree.

S. suis biofilms are more susceptible to ciprofloxacin than other antimicrobials (Guo *et al.*, 2012). Pronounced effects on overall community structure that could be readily interpreted as dose dependent were not evident in our data. An interpretation supported by our data analysis is that with respect to common indices of overall community structure, changes in microbiome composition produced by ciprofloxacin dosing were less prominent than relatively modest temporal changes in the microbiome composition that occurred over the 3-day period.

There was a noticeable increase in the relative abundance of streptococci at the low dose and a significant decrease in the relative abundance of the *Corynebacterium* genus. The explanation that *Corynebacterium* species inhibit streptococci is not supported by the literature (Hogan *et al.*, 1987). There is some support for the hypothesis that streptococcal biofilm was reacting to exposure to sublethal doses of

ciprofloxacin by an increase in biomass (Ahmed *et al.*, 2009).

The dominant *Rothia* genus was relatively unaffected by ciprofloxacin dosing. There is no published evidence indicating that *Rothia* species exhibit exceptional resistance to ciprofloxacin (Binder *et al.*, 1997). It is possible that the biofilm form of *Rothia* exhibits more phenotypic resistance to ciprofloxacin than the biofilm form of the *Streptococcus* species. Alternatively, our data are consistent with the interpretation that a portion of the streptococcal community exhibits uncharacteristically enhanced susceptibility to ciprofloxacin, and that only this subpopulation was selectively reduced.

The oral indigenous plaque community structure of the Sprague-Dawley rats used in this study was relatively simple compared with that of humans (Keijser *et al.*, 2008; Zaura *et al.*, 2009) or dogs (Dewhirst *et al.*, 2012). The predominance of the *Rothia* genus

in the plaque community was unexpected. *Rothia* species are isolated from the human oral microbiome with moderate frequency [particularly *Rothia denticariosa* (Pardi *et al.*, 1996) but also *Rothia mucilaginosa* (Zamakhchari *et al.*, 2011)] but are minor components of the community, whereas *Rothia* species were not detected in the (subgingival) plaque of 51 breeds of dogs using clone libraries (Dewhirst *et al.*, 2012). The oral bacterial composition of Wistar Kyoto rats was previously characterized using culture-dependent methods (Isogai *et al.*, 1985). All locations sampled (saliva, tongue, buccal mucosa and gingival crevice) were dominated by *Streptococcus* species, and *Rothia* species were not detected. The taxon classification was made using medium selection under anaerobic conditions, which was supplemented with identification using morphological characteristics. *Rothia* species grow poorly, if at all, under anaerobic conditions (Brown *et al.*, 1969; Zamakhchari *et al.*, 2011) and *Rothia* species have previously been mistaken for streptococci on the basis of cellular morphology (Brown *et al.*, 1969). Supragingival plaque samples collected by us from a different group of Sprague-Dawley rats in the same laboratory at a different time and analysed to obtain preliminary pyrosequencing data were dominated by *Rothia* and *Streptococcus* genera in the same rank order abundance as in Table 1, and the classifications at the species level within these groups were almost identical (data not shown). This suggests that at least within this species of rat the oral plaque community structure is fairly stable with respect to the dominant taxa.

There were a number of genera in the plaque community present at low abundance that were previously associated with periodontal disease—*Exiguobacterium* (Zijngel *et al.*, 2003), *Enterococcus* (Sun *et al.*, 2012), *Granulicatella* (Colombo *et al.*, 2009), *Neisseria* (Uzel *et al.*, 2012), *Acinetobacter* (da Silva *et al.*, 2005) and *Micrococcus* (Anesti *et al.*, 2005). In light of a previous study in Wistar rats, it would be of interest to see if any of these taxa become relatively abundant during ligature-induced periodontitis (Duarte *et al.*, 2010). Some of the genera present at low abundance are found most commonly in marine environments (*Paramoritella*, *Alkaliflexus* and *Janibacter*). These may have been introduced by the feed, which contained fish meal. The presence of *Halomonas* was unexpected

because this genus survives optimally in high-salinity environments (> 3% NaCl) (Stevens *et al.*, 2009) and has not been previously detected in the oral microbiome (to our knowledge).

In summary, our results indicate that it is possible to reduce a phylogenetically defined portion of the microbiome by an antimicrobial dosing regimen without causing a large perturbation in the overall community structure. The idea of selecting antimicrobials on the basis of their ability to target specific periodontal pathogens is not new (Beikler *et al.*, 2004). Our results suggest that it is worth exploring this treatment option more actively as a possible means to kill periodontal pathogens while preserving potentially beneficial commensals. The concentration of the low dose of ciprofloxacin was 10 times the concentration required to selectively kill the periodontal pathogen *Aggregatibacter actinomycetemcomitans* in biofilms composed of *A. actinomycetemcomitans* and *Streptococcus sanguinis* (Suci & Young, 2011). Therefore, it would be of interest to characterize the ability of ciprofloxacin to selectively kill *A. actinomycetemcomitans* during the progression of periodontal disease induced by this microbe in rat models (Li *et al.*, 2010; Schreiner *et al.*, 2010; Freire *et al.*, 2011; Bezerra *et al.*, 2012).

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CONFLICT OF INTEREST

None.

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