Supporting Information

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SI Materials and Methods

Bacterial Strains and Growth Conditions. Bacterial strains, their cultivation medium, and growth conditions are listed in Table S1. Bacteria were cultivated under either aerobic or anaerobic conditions (nitrogen, 90%; carbon dioxide, 5%; and hydrogen, 5%).

Peptide Synthesis and Purification. C16G2 was synthesized using 9-fluorenylmethoxy carbonyl solid-phase chemistry (431A Peptide Synthesizer; Applied Biosciences) as previously described (1) and purified to >95% by HPLCy (Waters) as described previously (2). Peptide mass was determined by electrospray ionization MS (3100 Mass Detector; Waters). C16G2 stock solution was prepared by dissolving in sterile distilled water to make a final stock solution of 1.25 mM and stored frozen before use.

Determination of IC_{50}. Bacterial species were grown with the media and conditions indicated in Table 1. Organisms were grown in liquid culture overnight before dilution for testing. Strains were stored as frozen glycerol stocks for long-term storage.

IC_{50} values were determined by examining killing after limited exposure of bacteria to C16G2 at various concentrations after a 5-min treatment period and then, calculating the concentration required to eliminate 50% of cfu per 1 mL from control cultures. Bacteria were grown overnight and diluted to reach ~10^7 cfu/mL; 100 μL these cells were then added to wells containing twofold serial dilutions of C16G2. The final concentration of C16G2 in each sample varied from 6.1 to 784.5 μg/mL. After exposure to peptide for 5 min at 37 °C, bacteria were rescued by dilution and plated on the appropriate growth medium for enumeration of cfu per 1 mL. IC_{50} values were determined as the concentration at which the best-fit trend lines crossed 50% of surviving cfu per 1 mL from control cultures treated with PBS.

Preparation of Frozen Stock of the Saliva-Derived Multispecies Bacterial Community. Saliva samples were collected from four healthy subjects ages 25–35 years old under University of California, Los Angeles Institutional Review Board number 09–08-068–02A. Subjects had not taken any prescription or nonprescription medications and were not being treated for any systemic disease. Subjects were asked to refrain from any food or drink 2 h before donating saliva; 2 mL saliva was obtained from each person by having them expectorate directly into the saliva collection tube. Each sample was centrifuged at 2,600 × *g* for 10 min to spin down large debris and eukaryotic cells; 1 mL supernatant from each sample was pooled together and referred to as pooled saliva, and 1 mL pooled saliva was seeded into 5 mL SHI medium, which contains 5% (vol/vol) sheep blood, has been previously described to be the optimal medium for culturing oral bacteria, and is able to sustain the growth of an in vitro microbial community with high diversity and similar microbial profiles to original salivary microflora (3). The cultures were incubated under anaerobic conditions (nitrogen, 90%; carbon dioxide, 5%; and hydrogen, 5%) at 37 °C for 24 h. Bacteria were collected by centrifugation at 14,000 × *g* for 3 min and resuspended in fresh SHI medium with 20% glycerol to make a frozen stock of the saliva-derived multispecies bacterial community. The stock was kept at −80 °C and used throughout this study.

C16G2 Treatment of Saliva-Derived Multispecies Planktonic Culture Spiked with *Streptococcus mutans*.

Preparation of the saliva-derived multispecies bacterial community from frozen stock. Frozen stock of the saliva-derived multispecies bacterial community (20 μL) was inoculated into 10 mL fresh prereduced SHI medium and incubated under anaerobic conditions at 37 °C for 24 h. Bacteria were collected by centrifugation at 14,000 × *g* for 3 min and resuspended in fresh SHI medium to a final OD_{600} of 0.5.

Preparation of* S. mutans *JM11 culture. *S. mutans* JM11 (UA140::pldh-luc), an *S. mutans* UA140 derivative that carries a spectinomycin resistance cassette (4), was used; 5 μL JM11 frozen stock was inoculated into 2 mL reduced SHI medium and incubated under anaerobic conditions at 37 °C for 24 h. JM11 cells were resuspended in fresh reduced SHI medium to a final OD_{600} of 0.5.

***Preparation of the saliva-derived multispecies bacterial mixture spiked with* S. mutans.** *S. mutans* JM11 culture was added to the resuspended saliva-derived multispecies bacterial community in a 1:10 volume ratio.

***C16G2 treatment of the saliva-derived multispecies community spiked with* S. mutans.** A 1.5-mL bacterial mixture spiked with *S. mutans* was transferred into 2-mL microfuge tubes. Based on different treatments, tubes with cultures were divided into two groups with three tubes in each group: group 1, Carrier solution treatment as negative control; and group 2, C16G2 treatment. Cells in tubes were pelleted followed by the different treatments. For group 1, cells were resuspened in 1.5 mL Carrier solution (SH09 vehicle); in group 2, cells were resuspended in 1.5 mL Carrier solution with 50 μM C16G2. Tubes were incubated at 37 °C under anaerobic conditions for 30 min. The bacterial mixture in each group was collected for (*i*) viability counting assays, (*ii*) DNA extraction and PCR-DGGE analysis, and (*iii*) regrowth.

Regrowth of communities subjected to different treatment. Bacterial mixtures in each treatment group were collected by centrifugation at 14,000 × *g* for 3 min. Bacterial cell pellets were washed three times with prereduced PBS and resuspended in an equal volume of SHI medium. Cell cultures were incubated at 37 °C under anaerobic conditions for 24 h. Bacterial cells were then harvested for DNA extraction and 454 pyrosequencing.

Viability Assay. A 100-μL bacterial mixture from each treatment group was taken, washed two times with PBS, subjected to serial dilution, and seeded onto nonselective and selective SHI medium plates (spectinomycin at 800 μg/mL). Plates were incubated at 37 °C under anaerobic conditions for 2 d. Colonies were counted, and viability was calculated.

Pull-Down Assay. Overnight cultures (OD_{600} of 1) of *S. mutans* UA140 were diluted 1:100 in Todd-Hewitt broth medium containing 0.5% (wt/vol) sucrose; 2 mL bacterial suspension was added to the well of a six-well flat-bottom polystyrene microtiter plate (Corning) followed by a 16-h static incubation at 37 °C in an atmosphere of 5% CO_2 in air to allow *S. mutans* biofilm formation. Then, the biofilms were rinsed with PBS three times to remove planktonic and loosely bound cells.

Frozen stock of saliva samples (10 μL) was inoculated into 5 mL prereduced SHI medium, and the cultures were incubated at 37 °C under anaerobic conditions for 16 h until the exponential growth phase was reached.

The overnight saliva-derived microbial flora was added to *S. mutans* biofilms and incubated in an anaerobic jar with shaking at 50 rpm for 3 h. The unbound cells were removed by washing biofilms three times with PBS. Then, the biofilms were detached immediately or kept growing in SHI medium for another 16 h under anaerobic conditions. The cells were collected, and genomic DNA was prepared using the MasterPure DNA Purification Kit (EPICENTRE) and subjected to PCR-DGGE analysis.

Ethidium Monoazide Bromide Cross-Linking. To prevent amplification of DNA from dead bacterial cells and limit DNA-based PCR-DGGE community analysis to the viable fraction, the collected bacterial samples were treated with ethidium monoazide bromide (EMA) before DNA extraction. EMA cross-linking was performed as described previously (5). Briefly, EMA (Biotium) was dissolved in water to a stock concentration of 5 mg/mL and stored at −20 °C in the dark. EMA was added to the culture samples to a final concentration of 100 μg/mL, and samples were incubated in the dark for 5 min with occasional mixing before samples were incubated on ice and light-exposed for 1 min using a 650-W halogen light source placed about 20 cm from the samples. After photo-induced cross-linking, bacterial cells were collected by centrifugation at 5,000 × *g* for 5 min followed by total genomic DNA isolation.

PCR-DGGE Analysis. Total genomic DNA of bacterial samples was isolated using the MasterPure DNA Purification Kit (Epicentre). DNA quality and quantity were determined by a Nanodrop 2000 Spectrophotometer. Amplification of bacterial 16S rRNA genes by PCR was carried out as described previously (6). Briefly, the universal primer set, Bac1 (5′-CGCCCGCCGCGCCCCGCG-CCCGTCCCGCCGCCCCCGCCCGACTACGTGCCAGCAG-CC-3′) and Bac2 (5′-GGACTACCAGGGTATCTAATCC-3′), was used to amply an ∼300-bp internal fragment of the 16S rRNA gene. Each 50-μL PCR contained 100 ng purified genomic DNA, 40 pmol each primer, 200 μM each dNTP, 4.0 mM $MgCl_2$, 5 μL 10× PCR buffer, and 2.5 units Taq DNA Polymerase (Invitrogen). Cycling conditions were 94 °C for 3 min followed by 30 cycles of 94 °C for 1 min, 56 °C for 1 min, and 72 °C for 30 s, with a final extension period of 5 min at 72 °C. The resulting PCR products were evaluated by electrophoresis in 1.0% agarose gels.

Polyacrylamide gels at an 8% concentration were prepared with a denaturing urea/formamide gradient between 40% [containing 2.8 M urea and 16% (vol/vol) formamide] and 70% [containing 4.9 M urea and 28% (vol/vol) formamide]; ∼300 ng PCR product was applied per lane. The gels were submerged in 1× Tris-acetate-EDTA (TAE) buffer (40 mM Tris base, 40 mM glacial acid acetic, 1 mM EDTA), and the PCR products were separated by electrophoresis for 17 h at 58 °C using a fixed voltage of 60 V in the Bio-Rad DCode System (Bio-Rad Laboratories, Inc.). After electrophoresis, the gels were rinsed and stained for 15 min in 1× TAE buffer containing 0.5 μg/mL ethidium bromide followed by 10 min of destaining in 1× TAE buffer. DGGE profile images were digitally recorded using the Molecular Imager Gel Documentation System (Bio-Rad Laboratories, Inc.). Diversity Database Software (Bio-Rad Laboratories, Inc.) was used to assess the change in the relative intensity of bands corresponding to bacterial species of interest.

Identification of Bacterial Species in the Pull-Down Assay. Bands of interest were excised from the DGGE gels and transferred to a 1.5-mL microfuge tube containing 10 μL sterile double-distilled H_2O. Tubes were incubated at 4 °C overnight before the recovered DNA samples were reamplified with the universal primer set [Bac1 (5′-CGCCCGCCGCGCCCCGCGCCCGTCCCGCCGCCCCC-GCCCGCCTACGGG-AGGCAGCAG-3′) and Bac2 (5′-GGAC-TACCAGGGTATCTAATCC-3′)]. The PCR products were purified using the QIAquick PCR Purification Kit (Qiagen Inc.) and sequenced at the University of California, Los Angeles Sequencing and Genotyping Core Facility. The obtained partial 16S rRNA gene sequences (about 300 bp) were used to BLAST search against the Human Oral Microbiome Database (HOMD; homd.org) (7) and the National Center for Biotechnology Information (ncbi.nlm.nih.gov) databases. Sequences with 98–100% identity to those deposited in the public domain databases were considered to be positive identification of taxa.

DNA Extraction and Processing of Pyrosequencing Data. DNA was isolated as described in ref. 8 by using the DNeasy Blood and Tissue Kit (Qiagen Inc.) and eluted in a final volume of 200 μL water. After genomic DNA extraction and quantification, all samples were prepared for 16S amplification and titanium-based 454 pyrosequencing at the J. Craig Venter Institute Joint Technology Center. Genomic DNA sample concentrations were normalized to 2–6 ng/μL; 16S rRNA gene sequencing samples were amplified and sequenced as described previously using the Roche-454 FLX Titanium Platform according to the HMP 16S Protocol (nature.com/nature/journal/v486/n7402/extref/nature11209-s1.pdf) (9).

Amplification primers were designed with FLX Titanium Adapters (A adapter sequence, 5′-CCATCTCATCCCTGCGT-GTCTCCGACTCAG-3′; B adapter sequence, 5′-CCTATCCC-CTGTGTGCCTTGGCAGTCTCAG-3′) and a sample barcode sequence, where applicable. Forward primers contained the B adapter, and the reverse primers contained the A adapter. The 16S primer sequences used are listed as follows: 27F (V1 primer), 5′-AGAGTTTGATCCTGGCTCAG-3′; and 534R (V3 primer), 5′-ATTACCGCGGCTGCTGG-3′. Using each sample's individual barcodes, the 454 sequence data were deconvolved into the respective samples. After trimming the bar codes, low-quality and short sequences (<100 bp) were removed by using the J. Craig Venter Institute 16S rRNA Analysis Pipeline. Subsequently, the remaining filtered reads were aligned against the SILVA database of 16S to verify that the reads were, indeed, 16S. The Chimera Slayer tool was used to filter out potentially chimeric reads (3). To identify previously defined oral bacterial taxa in the samples, blastn (10) was used with the sequence reads from the samples aligned to the HOMD reference sequences (7). Matches at 97% sequence identity cutoff and 95% sequence coverage were considered as described previously (11). The HOMD 16S RefSeq Extended Version 1.1 was used, and it contains 16S rRNA gene sequences representing all currently named and unnamed oral taxa as well as taxa that have not yet been assigned with a taxon identity. After processing 16S rRNA as described above, STAMP version 1 (12) was used to carry out additional statistical analyses.

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Table S1. Bacteria used and growth conditions

Bacterium	Growth medium	Temperature, °C	Anaerobic
S. mutans UA140	TH	37	Yes
Streptococcus crista #6	TH	37	Yes
Streptococcus gordonii ATCC 10558	TH	37	Yes
Streptococcus mitis JM3	TH	37	Yes
Streptococcus pyogenes ATCC 10096	TH	37	Yes
Streptococcus salivarius K12	TH	37	Yes
Streptococcus sanguinis ATCC 10556	TH	37	Yes
Streptococcus oralis ATCC 35057	TH	37	Yes
Actinomyces naeslundii ATCC 12104	*Actinomyces* broth	37	Yes
Fusobacterium nucleatum ATCC 23726	Columbia	37	Yes
Lactobacillus casei ATCC 4646	MRS	37	Yes
Bacteroides fragilis 145091	Chopped meat	37	Yes
Bacteroides fragilis 90917	Chopped meat	37	Yes
Corynebacterium striatum ATCC 43751	BHI	30	No
Escherichia coli 49979	Nutrient broth	37	No
Klebsiella pneumoniae Kay 2026	Nutrient broth	37	No
Salmonella typhimurium ssp. *enterica* ATCC 29629	Nutrient broth	37	No
Pseudomonas aeruginosa PAO1	Mueller Hinton	37	No
Micrococcus luteus MS2	Nutrient broth	30	No
Yersinia enterolitica ATCC 23715	TSB	37	No

BHI, brain-heart infusion; MRS, deMan, Rogosa, and Sharpe *Lactobacillus* medium; TH, Todd-Hewitt broth; TSB, tryptic soy broth.