Supporting Information

Ramsey and Whiteley 10.1073/pnas.0809533106

SI Materials and Methods

Serum Sensitivity Assays. For all serum sensitivity assays, bacteria were grown in 10% (vol/vol) CO_2. The experiments shown in Fig. 5 were carried out under microaerophilic conditions. Microaerophilic medium was prepared by injecting 10 mL of aerobic brain heart infusion (BHI) broth into rubber-stopper–sealed Balch tubes, which were flushed with N_2 gas. Cultures were allowed to grow overnight and then diluted at least 1:5 into N_2-flushed Balch tubes containing 4 mL of BHI broth. Cultures were grown 2 h to an OD_{600} of 0.2 for *A. actinomycetemcomitans* and 0.4 for *S. gordonii* before treatment. Two hundred microliters of 5000 U/mL catalase (Sigma) or catalase that had been boiled for 10 min at 95 °C (heat inactivated) was added to an N_2-flushed sealed Balch tube. Two milliliters of *S. gordonii* culture or 2 mL of sterile media was then added to each tube. Two milliliters of *A. actinomycetemcomitans* culture was added to each tube and incubated at 37 °C for 15 min. Another 200 μL of heat-inactivated or untreated 5,000 U/mL catalase was added to each culture and allowed to incubate for an additional 15 min. An equal volume of normal human serum (2 mL; Valley Biomedical) or complement-inactivated serum was added to each culture and allowed to incubate for 2 h. One milliliter of culture was removed before serum addition for microscopic analysis, and 1 mL was removed for serial dilution and plate counting.

RT-PCR Primers. Primers *clpX*-for (5′-gcagaaaccatggcgcgtatg-3′) and *clpX*-rev (5′-caattcgcttaatggcgccac-3′) were used for *clpX* amplification, primers *katA*-for (5′-cggcccgttactgttacaagatg-3′) and *katA*-rev (5′-gataaagctgtaagtgtggctgc-3′) were used for *katA* amplification, and primers *apiA*-for (5′-cactggtgcaatttctgtcaatg-3′) and *apiA*-rev (5′-gaatttgctgcaatatcggctctg-3′) were used for *apiA* amplification. For visualization, 5 μL of the resulting PCR was subjected to agarose gel electrophoresis and stained with ethidium bromide.

***luxCDABE* Reporter Fusion Construction.** To study *katA* and *apiA* promoter activity in *A. actinomycetemcomitans*, the vectors pMR310-*katA* and pMR310-*apiA* were constructed. pMR310 incorporates a 5.8-kb region containing the *Photorabdus luminescens luxCDABE* operon from the vector pCGLS1 (1). This region was PCR-amplified with the addition of BamHI and NotI restriction sites at the 5′ end and a SalI site at the 3′ end. Transcriptional TGA stop codons were also added to the 5′ primer in all 3 frames using the following primers: LuxCE-for (5′-atacgggccgctgaatgaatgaggcaaatatgactaaa-3′) and LuxCE-rev (5′-acgcgtcgacatatcaactatcaaacgcttcgg-3′). This promoterless fragment was cloned into the BamHI and SalI restriction sites in the spectinomycin-resistant cloning vector pDMG4 (2), creating the promoterless *luxCDABE* expression vector pMR310. The *A. actinomycetemcomitans katA* promoter region was PCR-amplified with the addition of the BamHI and NotI restriction sites at the 5′ and 3′ ends, respectively, using the primers katApro-5′ (5′-cgggatcctaatcggtcagaaaaaacaccgt-3′) and katA-pro-3′ (5′- ataagaatgcggccgcgctgacataatcgtttccttaat-3′). The *apiA* promoter region was amplified with identical restriction sites using the primers apiApro-5′ (5′-cgggatccctcggcaggtaactattttaacc-3′) and apiApro-3′ (5′-ataagaatgcggccgcgcaacgaggtggtgtttaaataa-3′). Amplified fragments were restriction digested and ligated into pMR310 to create the *katA* reporter vector pMR310-*katA* and the *apiA* reporter vector pMR310-*apiA*. Plasmids were transformed into *E. coli* DH5α cells and then electroporated into *A. actinomycetemcomitans* (3).

Construction and Complementation of an *oxyR*$^-$ Mutant in *A. actinomycetemcomitans*. Construction of the *oxyR*$^-$ mutant was performed as previously described (4) using the primers oxyRKO-for (5′-cggaattcggattcttgtcatgtaagccaac-3′) and oxyRKO-rev (5′-cggaattcgttggacttcaataaaggcttca-3′) to amplify a 413-bp internal fragment of the *oxyR* gene. This fragment was digested with EcoRI and ligated into the suicide plasmid pVT1461 (5) to generate the vector pVT1461-oxyRko. Plasmids were transformed and screened as described (4), and insertion into *oxyR* was confirmed using PCR. Complementation of *oxyR in trans* was performed using the *A. actinomycetemcomitans* streptomycin-resistant expression vector pYGS (6). The 1,068-bp *oxyR*-containing fragment was amplified using the primers oxyRPro-full-for (5′-cccaagcttccatattagacatgatttttctcc-3′) and *oxyR*-full-rev (5′-cggaattcccattaagaagataagatagatttaacc-3′) and cloned into HindIII/EcoRI-digested pYGS to yield the complement vector pYGS-OxyRC, which was introduced into *A. actinomycetemcomitans* by electroporation. Construction of the constitutive *apiA* expression vector was carried out using the primers and methods given by Yue *et al.* (7).

1. Frackman S, Anhalt M, Nealson KH (1990) Cloning, organization, and expression of the bioluminescence genes of *Xenorhabdus luminescens*. *J Bacteriol* 172:5767–5773.
2. Galli DM, Polan-Curtain JL, LeBlanc DJ (1996) Structural and segregational stability of various replicons in *Actinobacillus actinomycetemcomitans*. *Plasmid* 36:42–48.
3. Sreenivasan PK, LeBlanc DJ, Lee LN, Fives-Taylor P (1991) Transformation of *Actinobacillus actinomycetemcomitans* by electroporation, utilizing constructed shuttle plasmids. *Infect Immun* 59:4621–4627.
4. Brown SA, Whiteley M (2007) A novel exclusion mechanism for carbon resource partitioning in *Aggregatibacter actinomycetemcomitans*. *J Bacteriol* 189:6407–6414.
5. Mintz KP, Brissette C, Fives-Taylor PM (2002) A recombinase A-deficient strain of *Actinobacillus actinomycetemcomitans* constructed by insertional mutagenesis using a mobilizable plasmid. *FEMS Microbiol Lett* 206:87–92.
6. Fong KP, Gao L, Demuth DR (2003) *luxS* and *arcB* control aerobic growth of *Actinobacillus actinomycetemcomitans* under iron limitation. *Infect Immun* 71:298–308.
7. Yue G, *et al.* (2007) A second *Aggregatibacter actinomycetemcomitans* autotransporter adhesin exhibits specificity for buccal epithelial cells in humans and Old World primates. *Infect Immun* 75:4440–4448.

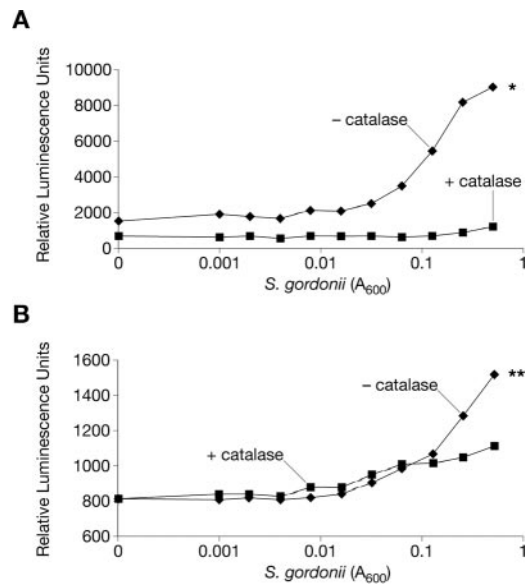

Fig. S1. Light production (relative luminescence units) by *A. actinomycetemcomitans* carrying *katA-luxCDABE* (*A*) or *apiA-luxCDABE* (*B*) during coculture with differing concentrations of *S. gordonii* in the presence of heat-inactivated (− catalase) or active catalase (+ catalase). SEM was <6% for all measurements and was omitted for clarity. $*P < 0.0007$, $**P < 0.01$ via Student's *t* test, $n = 4$. For these experiments, *katA* and *apiA* promoter induction in cocultures was measured by monitoring luminescence using a Thermo Luminoskan Ascent microplate reader (Thermo Electron Co.). *A. actinomycetemcomitans* carrying either the *apiA-luxCDABE* or *katA-luxCDABE* was grown to an A_{600} of 0.8 in BHI containing 50 μg/mL spectinomycin, diluted to an A_{600} of 0.1, and grown to midlogarithmic growth phase (A_{600} of 0.4). Cells were then diluted again in BHI to an A_{600} of 0.1 and 100 μL transferred into a 96-well microtiter dish. One hundred microliters of midlog *S. gordonii* cells (A_{600} of 0.5) was concentrated to an A_{600} of 2.0 by centrifugation and resuspension and added to the first row of wells, then diluted 1:2 across the entire plate, omitting the last row as an untreated control. The microtiter dishes were incubated at 37 °C for 30 min before measuring luminescence in each well.