

Aggregatibacter actinomycetemcomitans mediates protection of *Porphyromonas gingivalis* from *Streptococcus sanguinis* hydrogen peroxide production in multi-species biofilms

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Supporting information

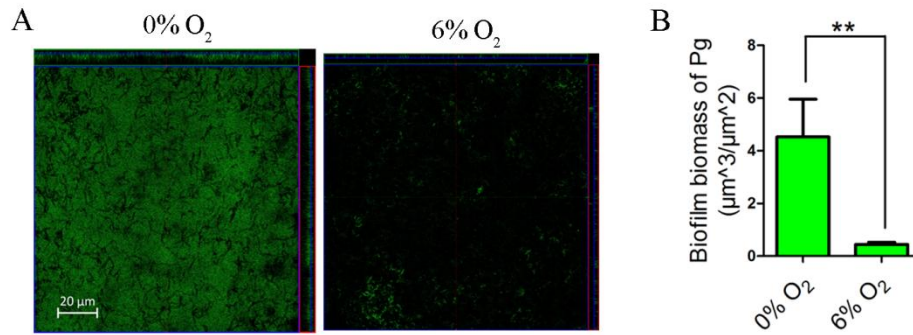


Fig. S1 CLSM analysis of *Pg* biomass in FISH stained 4 day-old biofilms grown under anaerobic and micro-aerobic conditions. **(A)** Orthogonal images of *Pg* biofilm grown in 0% O₂ (left) and 6% O₂ (right). Scale bar shown on left image = 20 μm. **(B)** The fluorescent signal from images in Fig. S1A was quantified by COMSTAT analysis. **P ≤ 0.01, Student's *t*-test. Means and standard deviations from triplicate experiments are shown.

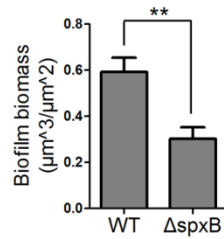


Fig. S2 CLSM analysis of *Ss* biomass in 4-day old FISH stained biofilms under micro-aerobic conditions. The biomass of *Ss* in figure 2B was quantified by COMSTAT and shown as a bar chart. Scale bars were indicated on the corresponding images. **P ≤ 0.01, Student's *t*-test. Means and standard deviations from triplicate experiments are shown.

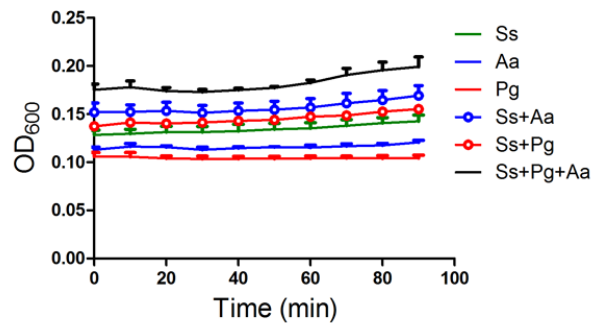


Fig. S3 Cell density measurements recorded during H₂O₂ concentrations time course experiment shown in Fig. 3B. Cell densities were recorded at intervals of 10 minutes using a Synergy H1 Hybrid Reader (OD₆₀₀).

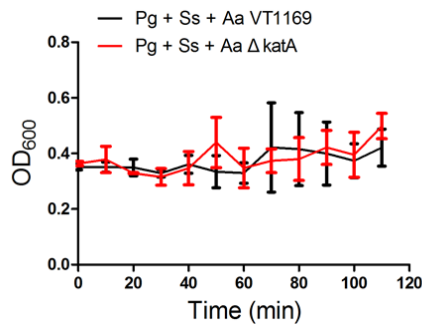


Fig. S4 Cell density measurements recorded during H₂O₂ concentrations time course experiment shown in Fig. 4A. Cell densities were recorded at intervals of 10 minutes using the Synergy H1 Hybrid Reader (OD₆₀₀).

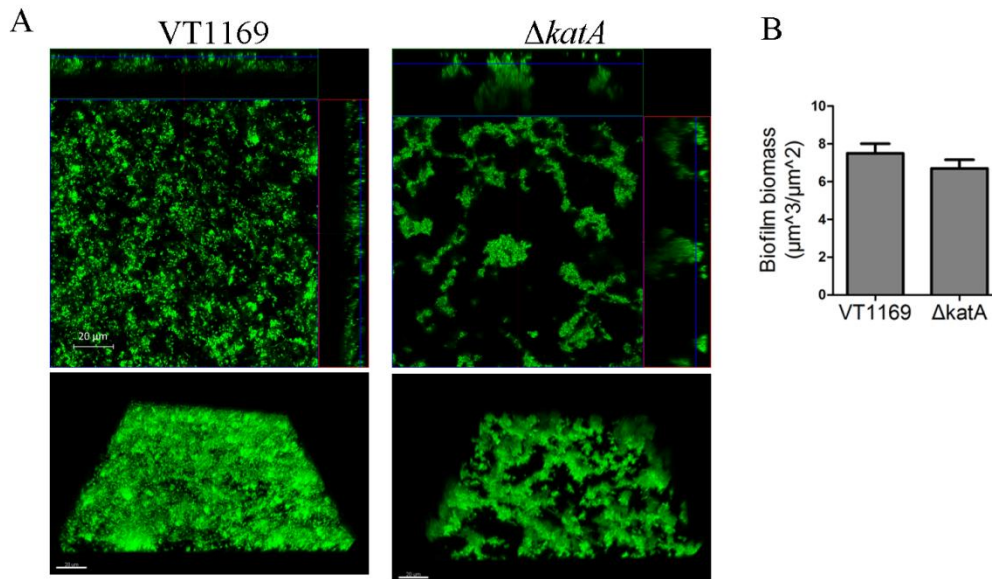


Fig. S5 The biofilm formation of *Aa* ΔkatA. *Aa* VT1169 WT and ΔkatA single species biofilms were cultured in CDM for 4 days, stained by STYO 9 and imaged by CLSM. The orthogonal images at the top and the 3D images at the bottom were shown (A) and biofilm biomass was quantified by COMSTAT (B). Student's *t*-test. Means and standard deviations from triplicate experiments are shown.

Table S1 Strains used in this study.

Strain	Genotype and/or relevant characteristics	Source or reference
<i>S. sanguinis</i> SK36	Wild type, Human plaque isolate	1
<i>P. gingivalis</i> ATCC 33277	Wild type	ATCC
<i>A. actinomycetemcomitans</i> 652	Wild type	2
<i>A. actinomycetemcomitans</i> VT1169	Wild type	3
<i>A. actinomycetemcomitans</i> VT1169 ΔkatA	VT1169, katA deletion mutant, Spc ^R	3

Table S2 FISH probes used in the study

Name	Sequence (5'-3')	Fluorescent dye	Application
FISH-Ss	AGAGCAAGCTCCTCTCTTCAGCGTTC TA/3' atto 488/	ATTO 488	FISH staining of <i>S. sanguinis</i>
FISH-Aa	AGTACAAGTACTTACCTGCTACCGTC CGA/3' atto 590/	ATTO 590	FISH staining of <i>A. actinomycetemcomitans</i>
FISH-Pg	ACCTTAGCAAGCTAAGATCATGCTG CCCCT/3' atto 647N/	ATTO 647N	FISH staining of <i>P. gingivalis</i>

- 1 Kilian, M. & Holmgren, K. Ecology and nature of immunoglobulin A1 protease-producing streptococci in the human oral cavity and pharynx. *Infection and immunity* **31**, 868-873 (1981).
- 2 Brogan, J. M., Lally, E. T., Poulsen, K., Kilian, M. & Demuth, D. R. Regulation of *Actinobacillus actinomycetemcomitans* leukotoxin expression: analysis of the promoter regions of leukotoxic and minimally leukotoxic strains. *Infection and immunity* **62**, 501-508 (1994).
- 3 Ramsey, M. M. & Whiteley, M. Polymicrobial interactions stimulate resistance to host innate immunity through metabolite perception. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 1578-1583, doi:10.1073/pnas.0809533106 (2009).