Streptococcus gordonii pheromone s.g.cAM373 may influence the reservoir of antibiotic resistance determinants _of Enterococcus faecalis_ origin in the oral metagenome

by

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Supplemental Material

Table S1. Bacterial strains used in this study.

Bacterial strain*	Relevant phenotype†	Source or reference
S. gordonii CH1	strain Challis parent; ComCDE-positive, s.g.cAM373-positive	1
S. gordonii CH1-S	spontaneous derivative of CH1 Sm^R;	2
S. gordonii CH1811	s.g.cAM373-negative	3
S. gordonii CH1811-S	s.g.cAM373-negative, Sm^R	This study
S. gordonii CH9278	Δ*comCDE* mutant of CH1, Sp^R; s.g.cAM373-positive	This study
S. sanguinis SK36		4
S. sanguinis SK36-S	Sm^R	This study
S. anginosus ATCC 33397		ATCC
S. anginosus ATCC 33397-S	Sm^R	This study
S. mutans ATCC 25175		ATCC
S. mutans ATCC 25175-S	Sm^R	S. Flannagan, University of Michigan
S. suis ATCC 43765		ATCC
S. suis ATCC 43765-S	Sm^R	This study
E. faecalis OG1X/pAM373	Sm^R responder	5
E. faecalis OG1X/pAM4020	Sm^R Em^R; pAM373 with Em^R Tn*917lac* insert; responder	5
E. faecalis JH2-2/pAM378	Rf^R Fa^R; pAM373 with Tc^R Tn*918* insert; responder	6
E. faecalis JH2-2/pAM378/pAMS470	Rf^R Fa^R Tc^R Em^R donor	3
JH2-2/pAMS470	$Rf^R$$Fa^R$$Em^R$ filter mating control; not pheromone responsive	3

* Streptococcal strains designated -S were selected for spontaneous resistance to streptomycin (Sm) by passage and growth on Todd Hewitt agar with increasingly higher concentrations of

antibiotic. Restriction enzyme digestion fragment lengths and 16S rDNA sequence comparisons were done to confirm that spontaneous Sm-resistant strains were each derived from the respective parent strain.

†. Antibiotic resistance levels for maintenance and/or selection were: 25 μg rifampicin (Rf) ml-1, and 25 μg fusidic acid (Fa) ml-1 for *E. faecalis* JH2-2 chromosomal markers, 1000 μg Sm ml-1 for OG1X and streptococcal chromosomal markers and 125 μg spectinomycin (Sp) ml-1 for CH9278 chromosomal marker, 10 μg tetracycline (Tc) ml-1 for the pAM373-derivative pAM378, 5 μg erythromycin (Em) ml-1 and 10 μg Em ml-1 for the enterococcal/streptococcal replicative plasmid, pAMS470, and the pAM373-derivative, pAM4020, respectively.

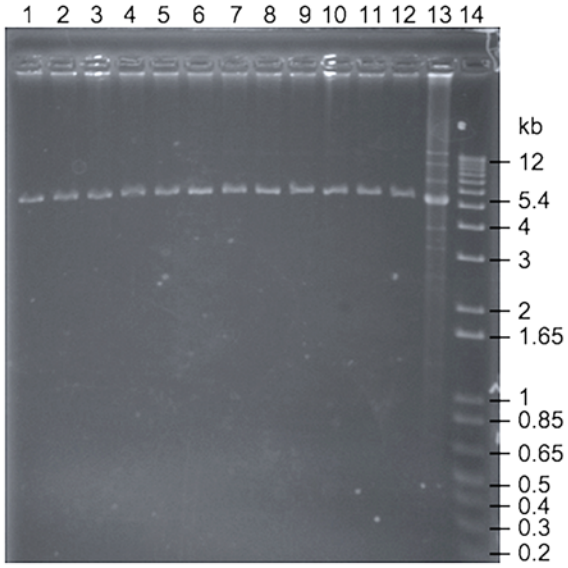
Supplemental Method

Construction of *S. gordonii* strain CH9278

To control for the role of competence in DNA uptake during filter matings, a *comCDE*-deficient mutant strain was constructed in *S. gordonii* by allelic replacement. A 458-bp region of the CH1 chromosome encoding the region upstream of the *comCDE* promoter was amplified by PCR with primers (engineered restriction digest sites underlined) Xho2149F (5'AT<u>CTCGAG</u>CTATTGAAGGTCAGCAATTTCC3') and EcoComCR (5'AT<u>GAATTC</u>GCTTCATATGCAAGTAAATATAACTG3'); a 320-bp CH1 chromosomal region encoding the 5-prime end of *comE* and the downstream chromosomal region was amplified with Bam2145F (5'AT<u>GGATCC</u>AGAGATATTCTATATATTGAGACGAC3') and Sst2145R (5'AT<u>GAGCTC</u>AGTTTATCTCGCTTTGTCAACAG3'). The resulting amplicons were purified by agarose electrophoresis, eluted (Qiagen), digested, and cloned into compatible restriction sites located upstream and downstream, respectively, of the *aad9* gene encoding spectinomycin resistance [7] in pGEM-7SpR [8] using *E. coli* DH5α (Stratagene) cloning strain grown in Luria-Bertani medium incubated aerobically at 37 ^{o}C. After confirmation of nucleotide sequence fidelity, the resulting plasmid was digested with *Xho*I and *Sst*I, and the released 1936-bp

double-stranded DNA fragment was transformed into serum-competent strain CH1 cells [2] and selected for growth on spectinomycin agar. A selected transformant, strain CH9278, had the chromosomally integrated *aad9* gene replacing the *comCDE* promoter, *comC* and *comD* structural genes and the DNA encoding the first 167 amino acids of ComE as confirmed by sequence analysis.

Figure S1. Donor and transconjugant plasmid analysis.



*Hin*dIII-digested plasmid DNA from JH2-2/pAM378/pAMS470 donors (lane 13) and two randomly-chosen putative transconjugants from an independent mating with each recipient species. Lanes: 1-2 *S. gordonii* CH1-S; 3-4 *S. gordonii* CH9278; 5-6 *S. sanguinis* SK36-S*;* 7-8 *S. anginosus* ATCC 33397-S: 9-10 *S. mutans* ATCC 25175-S; 11-12 *S. suis* ATCC 43765-S. Lane 14: DNA size ladder (Thermo Fisher Scientific Waltham, MA). Expected sizes based upon nucleotide sequence analysis and individual *Hin*dIII-digested plasmid preparations from JH2-2/pAM378 and JH2-2/pAMS470 (data not shown) are pAM378: 13, 10, 6.9, 6.8, 5.5, 3.9, 3.2, 2.5, 1.3, and 0.6 kb; pAMS470: 5.4 kb.

Figure S2.

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Accession Number        Aligned Deduced Amino Acid Sequences
WP_012000638[a] MKKIYTLALLVFSVFILAACSSQEAWLNGTWKGEKNKKTYVFEEKQGKWTILNGKEKIAE
WP_048774577[b] MKKIYTLALLVFSVFILAACSSQEAWLNGTWKGEKNKKTYVFEEKQGKWTILNGKEKIAE
WP_045503232[c] MKKIYTLALLVFSVFILAACSSQEAWLNGTWKGEKNKKTYVFEEKQGKWTILNGKEKLAE
WP_061410676[d] MKKIYTLALLVFSVFILAACSSQEAWLNGTWKSEKTKDTYVFEEKQGKWNILNGKEKIAE
WP_060970353[e] MKKIYTLALLVFSVFILAACSSQEAWLNGTWKSEKTKSTYVFEEKQGKWNILNGKEKIAE
WP_060553535[f] MKKIYTLALLVFSVFILAACSSQEAWLNGTWKSEKTKGTYVFEEKQGKWNILNGKEKIAE
                ******************************** ** * *********** ******* **

WP_012000638  KGEVKDKKGDQFSIVDANGNRHQIKKVDENTIEYQPFTSDGISATNGESFKFKKDK
WP_048774577  KGEVKDKKGDQFSIVDANGNRHQIKKVDENTIEYQPFTSDGISATNGESFKFKKNK
WP_045503232  KGEVKDKKGDQFSIVDANGNRHQIKKVDENTIEYQPFTSDGISATNGESFKFKKDK
WP_061410676  KGEVKDKKGDQFSIVDANGNRHQIKKVDENTIEYQTFTSDGISATNGESFKFKKEK
WP_060970353  KGEVKDKKGDQFTIVDANGNRHQIKKVDENTIEYQSFTSDGISATNGESFKFKKDK
WP_060553535  KGEVKDKKGDQFTIVDTNGNRHQIKKVDENTIEYQPFTSDGISATNGESFKFKKDK
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Comparison of CamG like proteins by multiple sequence alignment. CamG-like proteins were identified in multiple oral streptococcal strains by a BlastP search [9] of NCBI databases (as of June 22, 2017). Entries that encoded proteins with amino acid sequences identical to s.g.cAM373 in their signal sequences (bold font) located immediately upstream of the cysteine lipoprotein cleavage site were aligned by CLUSTAL omega [10]. Conserved amino acids are represented by an asterisk.

Strains included in each accession number are noted by a superscript letter as follows:

[a]*S. gordonii* strain CH1, FDAARGOS_257

[b]*S. cristatus* strain 771SOLI; *S. gordonii* strains FSS2, MW10, and PV40

[c]*S. gordonii* strain G9B

[d]*S. gordonii* strain DD07

[e]*S. gordonii* strains Blackburn and FSS8; *S. sp.* CCH8-G7

[f]*S. gordonii* strains ATCC10558, CCUG33482, and Channon

References for Supplemental Material.

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