Quorum sensing systems couple regulations of natural competence and bacteriocin biosynthesis with diverse network topologies in cariogenic streptococci

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Background

The human oral microbiome is dominated by members of the genus *Streptococcus* (1). The species *Streptococcus mutans* and *Streptococcus sobrinus*, traditionally grouped into "mutans streptococci", are etiological causes of dental caries (tooth decay) (2), the most prevalent human disease (3). The mechanisms behind *S. sobrinus'* pathogenic properties are unknown due to a lack of genetic tools (4), including any means to introduce DNA into the cells. *S. mutans* and many other streptococci are transformable by exploiting their natural competence pathways, including the ComCDE and ComRS systems (5). Both systems involve peptide pheromones (CSP and XIP) and function as quorum sensing systems to regulate the expression of ComX, the alternative sigma factor controlling the expression of natural competence genes (Fig. 1A) (6). In many streptococci, the ComRS systems are also coupled to bacteriocin production, linking antimicrobial defenses to virulence and competence (Fig. 2A) (5). Despite the importance of ComRS systems, no functional competence pathway has ever been discovered in *S. sobrinus* (7).

Results

***S. sobrinus* natural competence pathway.** Previous unsuccessful searches for ComRS homologs in *S. sobrinus* used *S. mutans* sequences for homolog searches in *S. sobrinus*, due to their perceived phylogenetic closeness (7). We serendipitously realized that *S. sobrinus* may actually be closer to *Streptococcus thermophilus*, and indeed we found *comR* homologs in *S. sobrinus* based on *S. thermophilus* sequences (Fig. 1B). In two of the three examined strains (SL1 and NIDR 6715-7), an unannotated short ORF following a ComR recognition motif in the intergenic region after the first *comR* homolog (*comR1*) is predicted to encode the signaling peptide precursor ComS, and we predicted the corresponding XIP to be its last seven amino acids. Two *S. sobrinus* strains (NIDR 6715-7 and NCTC 10919) are now transformable using the XIP in the newly discovered ComRS system (Fig. 1C). Gene knockout analysis confirmed that in NCTC 10919, the single *comR* is responsible for competence pathway activation (Fig. 1D), but in NIDR 6715-7, the second *comR* homolog (*comR2*) is the gene responsible for competence pathway activation, while *comR1*, the gene directly associated with *comS*, has inhibitory effects (Fig. 1E). This also explained why strain SL1, with a premature stop codon in its *comR2* (Fig. 1F), is not transformable. Sequence alignment revealed that the single *comR* in strain NCTC 10919 was likely a recombination product between the two *comR* genes in its common ancestor with SL1, eliminating the pre-existing premature stop codon to restore a functional *comR* (Fig. 1F).

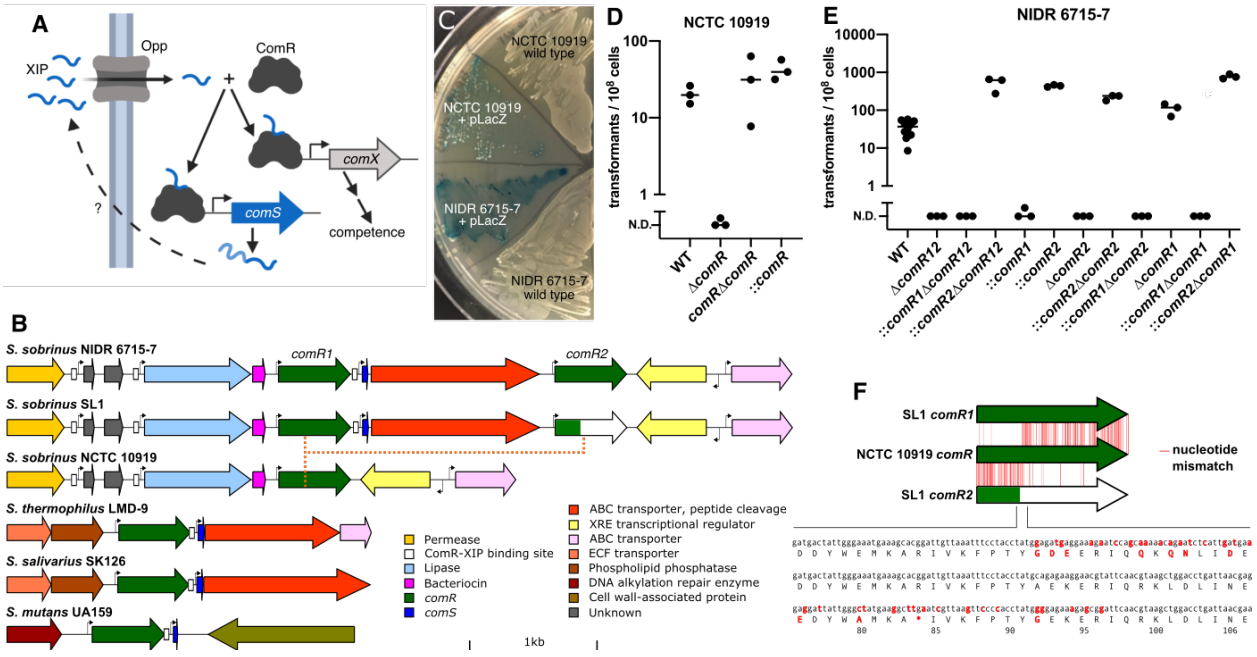

Figure 1. A. The ComRS competence pathway in *S. mutans* forms an autocrine signaling loop. An unknown protein cleaves the leader peptide from ComS and exports activated XIP. XIP is imported where it facilitates dimerization of the transcriptional regulator ComR. The ComR/XIP complex binds to a DNA motif to activate transcription of *comS* and *comX*. ComX is an alternative sigma factor for genes including the late competence genes. **B.** The ComRS gene cluster in strains NIDR 6715-7 and SL1 contain two homologs of *comR* that we call *comR1* and *comR2*. The type strain SL1 contains a truncated *comR2* gene (green/white) and cannot be transformed. Strain NCTC 10919 has a single homolog of *comR* and no *comS* gene or a peptide export/cleavage gene. **C.** *S. sobrinus* strains NIDR 6715-7 and NCTC 10919 can be transformed using exogenous XIP. Both strains were transformed with a plasmid expressing LacZ. When plated with X-gal, the plasmid-carrying strains produce a blue color, but the wild-type strains do not. **D.** The single *comR* homolog in strain NCTC 10919 is required for transformation. **E.** Strain NIDR 6715-7 cannot be transformed if the region from *comR1* to *comR2* is deleted. Additional copies of *comR1* on a plasmid does not rescue transformation, but strains complemented with extra *comR2* can be transformed. The horizontal bars represent the mean of the biological replicates (black dots). **F.** The *comR* gene in strain NCTC 10919 appears to be a fusion of parts of *comR1* and *comR2*. A recombination event that produced *comR* would have removed the premature stop codon found in the *comR2* gene of strain SL1.

Natural competence pathways in other "mutans streptococci" species. Further comparative genomics analysis led to the finding of ComRS pathways in the rest of the traditional "mutans streptococci" group members. Following this, four additional species are now transformable using their predicted XIPs, leaving only one out of the seven "mutans streptococci" members untransformable, a major leap from the starting situation where six out of seven were genetically intractable.

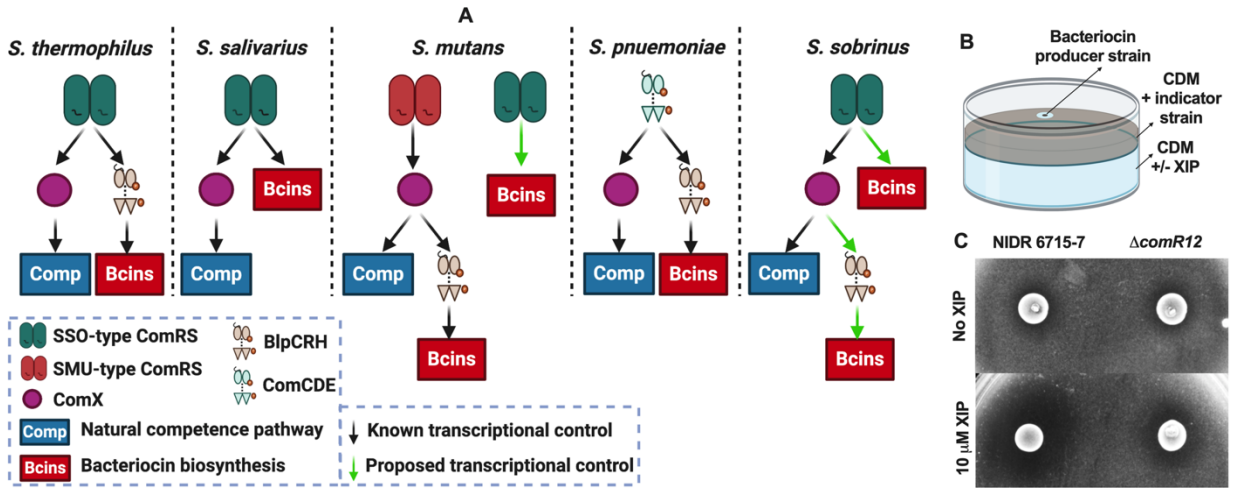

Figure 2. A. Streptococci use the ComRS or ComCDE/BlpCRH pheromone systems to couple competence and bacteriocin biosynthesis with different network topologies. **B.** Schematic for inhibition assay developed for this project. **C.** *S. sobrinus* NIDR 6715-7 inhibits *S. mutans* growth in the presence of XIP via the ComRS pathway.

Coupling of bacteriocin biosynthesis to natural competence in the ComRS networks. Additional genomic analysis using the ComR recognition motifs revealed a series of bacteriocin gene clusters under direct ComRS control in "mutans streptococci", with an additional cluster found in *S. sobrinus* under the control of BlpCRH two-component system, in turn controlled by ComX (Fig. 2A). Inhibition assay (Fig. 2B) revealed that the *S. sobrinus* bacteriocin machinery can target *S. mutans* when its ComRS system is activated (Fig. 2C), a relationship with potentially important clinical relevance toward our understanding of the oral microbiome dynamics.

Table 1. Summary of genes in the ComRS regulatory networks in mutans streptococci

	comX	Type II *comS*	Type IV *comS*	Cluster 1	Cluster 2	Cluster 3	Cluster 4
S. sobrinus	+	-	+	+	-	-	-
S. downei	+	-	++	-	-	+	+
S. criceti	+	-	+	-	-	-	-
S. mutans	+	+	+	+	+	-	-
S. ratti	+	+	+	-	+	-	-
S. ferus	++	+	+	-	+	-	-
S. macacae	+	+	+	+	+	-	-

Each column represents genes/gene clusters that are related in homology or genomic location. +: ComS with associated XIP that led to transformation. +: bacteriocin gene clusters.

Significance

Before this study, *S. mutans* was the only genetically tractable species in the cariogenic "mutans streptococci" group. Now all but one are transformable. The possibility to study the molecular

mechanisms of *S. sobrinus* pathogenesis at the genetic level promises better understanding of dental caries development and new prevention/treatment methods. The study of quorum sensing regulation of natural competence and bacteriocin biosynthesis provides new angles to our understanding of the oral microbiome dynamics. Bacteriocins specific to oral pathogens may also be identified along the way to replace the current more broad-spectrum antimicrobials, the former being important for preserving the healthy oral microbiome (8–10).

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