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

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We discovered **ComRS quorum sensing pathways** in members of the caries-related "mutans streptococci" group, whose only genetically tractable member before this study was *Streptococcus mutans*. This discovery allowed us to induce the natural competence pathways for **genetic manipulation** in previously genetically intractable species, especially the oral pathogen ***S. sobrinus***, and led us to find **bacteriocin gene clusters** with potentially important clinical relevance.

Introduction

• **Streptococci** is by far the most dominant genus in the oral microbiome

• ***S. mutans* and *S. sobrinus*** belong to the traditional "mutans streptococci" group, and are etiological causes of **dental caries** (tooth decay)

• *S. mutans* is transformable by exploiting its **natural competence pathway**, controlled by the **ComRS quorum sensing system** using peptide XIP as the signaling molecule

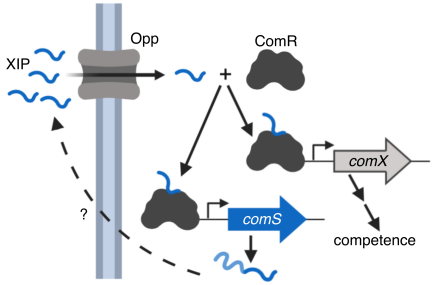

Figure 1. The ComRS quorum sensing system regulates natural competence pathway

• Besides natural competence, the ComRS pathway is known to also **regulate bacteriocin biosynthesis,** with diverse regulatory network topologies

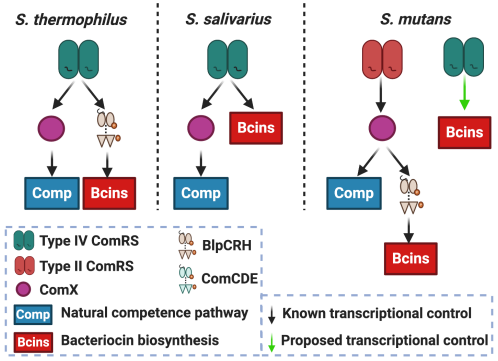

Figure 2. Diverse ComRS regulatory network topologies

Natural competence in *S. sobrinus*

• The important oral pathogen *S. sobrinus* was genetically intractable. Previous unsuccessful searches for ComRS homologs in *S. sobrinus* used *S. mutans* sequences for homolog searches in *S. sobrinus*, due to their perceived closeness.

• We serendipitously realized that *S. sobrinus* may actually be phylogenetically closer to *S. thermophilus*, and indeed we found **ComR homologs** in *S. sobrinus* based on *S. thermophilus* sequences.

• ***S. sobrinus* is now transformable.** This provides the most essential genetic manipulation capacity for modern researchers to study its pathogenesis.

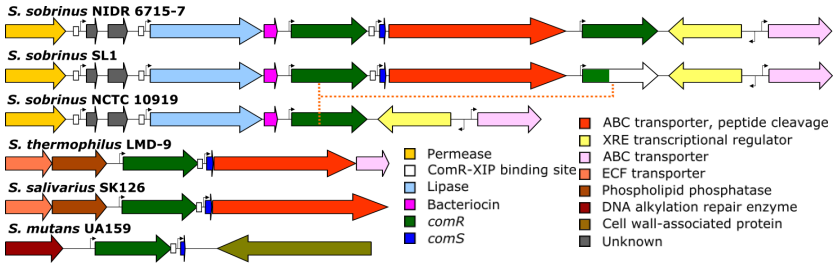

Figure 3. Genomic structures surrounding *comR* genes. The three examined strains of *S. sobrinus* have either two or one *comR* homologs (green).

S. sobrinus SL1	MNLKKIIELAITLVALMCTIVR
S. sobrinus NIDR 6715-7	MNLKKIIELAITLVALMCTIAR
S. thermophilus LMD-9	LKTLKIFVLFSLLIAILPYFAGCL
S. salivarius SK126	LKKLKLFTLFSLLITILPYFTGCL
S. mutans UA159	MFSILTSILMGLDWWSL

Figure 4. ComS sequences encoded by the ORF following comR. Predicted XIP sequences are underlined.

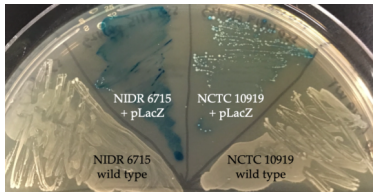

Figure 5. *S. sobrinus* NIDR 6715-7 and NCTC 10919 are transformed using the predicted XIP. SL1 is not transformable.

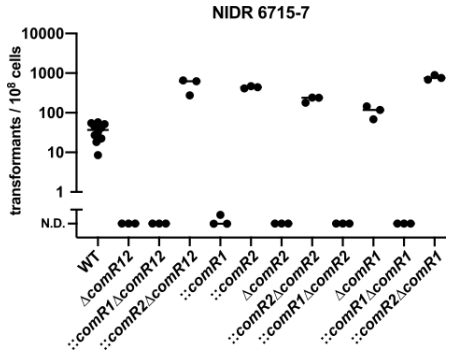

Figure 6. In *S. sobrinus* NIDR 6715-7,

• *comR2* is responsible for competence;

• *comR1* has inhibitory effects.

ComRS quorum sensing regulatory networks

• Further comparative genomics analysis led to the finding of ComRS pathways in the rest of the traditional "mutans streptococci" group members.

• **Four additional species are now transformable** using the predicted XIPs (*S. macacae* remains untransformable).

• Genomic analysis using the ComR recognition motifs revealed **bacteriocin gene clusters** under direct and indirect ComRS control.

Table 1. Genomic analysis of potential targets under direct ComRS regulation

	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	+	+	+	+	+	-	-

+: led to transformation. +: bacteriocin gene cluster.

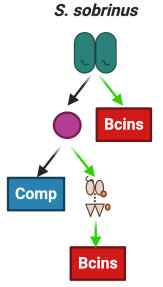

Figure 7. The predicted ComRS regulatory network in *S. sobrinus*.

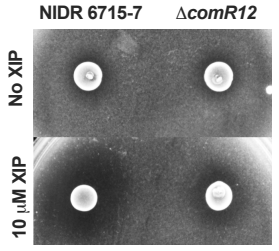

Figure 8. The ComRS system in *S. sobrinus* controls bacteriocin production targeting *S. mutans*.

Implications

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

• The possibility to study the **molecular mechanisms** of *S. sobrinus* pathogenesis in depth promises better understanding of d**ental 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 be identified along the way.