Arginine Catabolism by Bacteria in the Oral Microbiome and the Prevention of Tooth Decay

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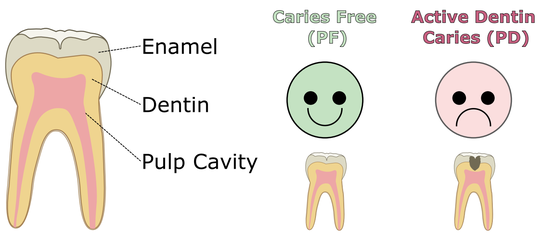
*arc*A *arc*B *arc*C

Background

Tooth decay is the most common preventable chronic disease, globally affecting more than two billion people, most of whom are children. The development of caries on teeth is primarily a consequence of acid production by cariogenic bacteria that inhabit the plaque microbiome. Arginine catabolism through the bacterial arginine deiminase metabolic pathway (ADS) has anticariogenic properties through the production of ammonia, which modulates the pH of the oral environment. Given the potential preventative capacity of the ADS pathway, the exploitation of ADS competent oral bacteria through pre- or probiotic applications is a promising therapeutic target to prevent tooth decay, yet the pervasiveness and rate of expression of the ADS pathway in diverse mixed microbial communities in oral health and disease remains an open question. Here we use a multi-omics approach to characterize the microbial community and ADS pathway expression in healthy and late-stage cavitated teeth.

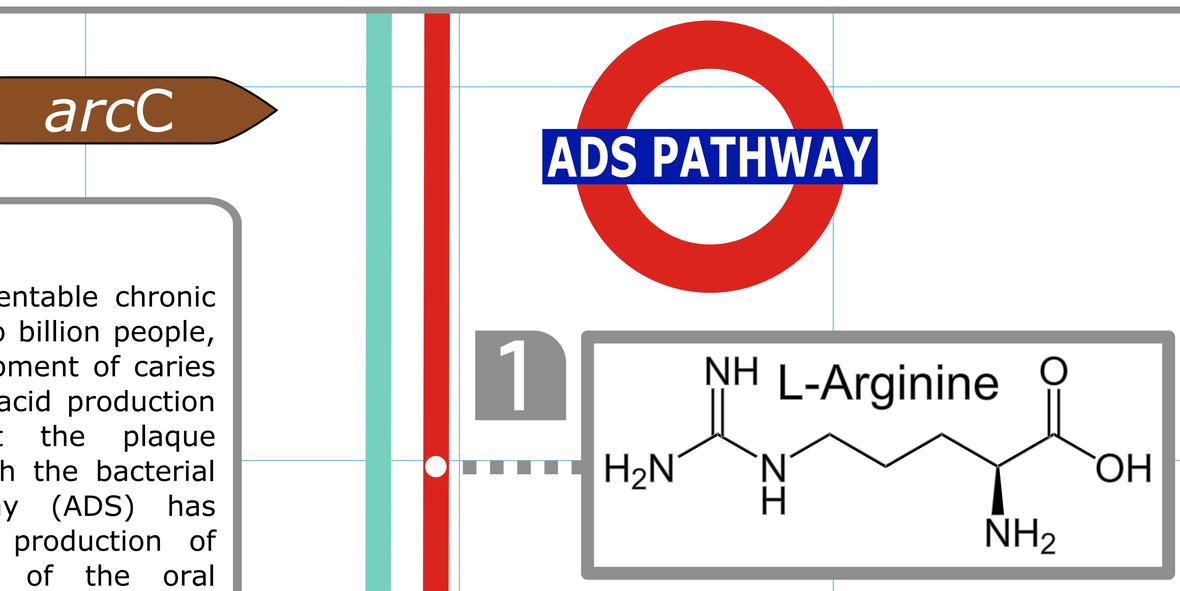
Methods

- 60 supragingival plaque samples (PD = 27, PF = 33



Three biological datasets generated for each sample

- Gene expression profiles characterized by metatranscriptomic sequencing
- Microbial community composition characterized by *rpo*C amplicon sequencing
- *In vitro* ADS expression characterized by citrulline quantification assay



Different bacteria express ADS in health or disease

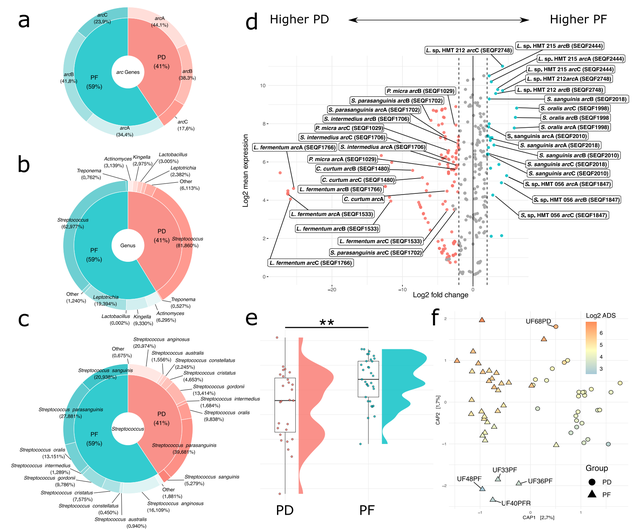


Fig 1. Significant difference in ADS activity comparing PD and PF. (a) Proportion of transcripts mapping to each of the three *arc* genes. (b) Proportion of transcripts mapping to different *Streptococcus* species. (d) Differential expression of *arc* genes. Colored points are statistically significant. (e) Shannon diversity for PD and PF samples. (f) Beta diversity colored by citrulline expression.

- ADS expression higher in PF (primarily *Streptococcus* and *Leptotrichia* sp.) but also expressed in PD by distinct bacterial groups.
- Expression is **lineage or strain specific**, not all members of a species have high expression

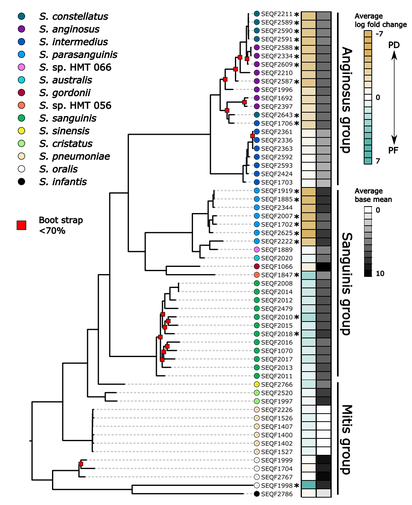
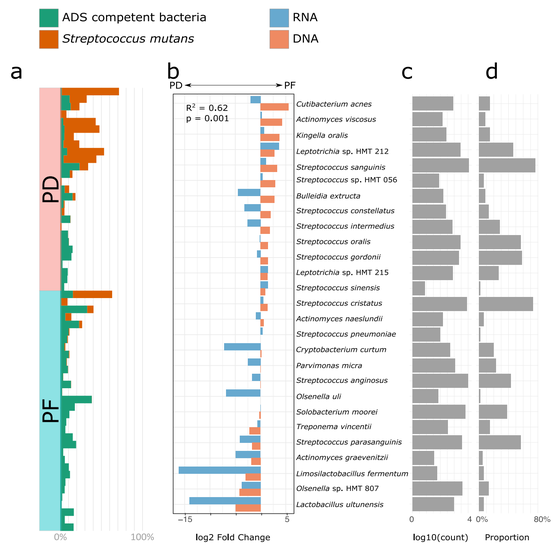


Fig 2. Differential expression and abundance of ADS operon varies by *Streptococcus* lineages. Maximum likelihood phylogeny of concatenated ADS operon. Asterix (*) denotes lineage where all three *arc* genes were up or down regulated. Heatmap indicates log fold change and base mean for corresponding lineages.

Gene expression profiles reflect community composition



- Differential expression driven primarily by distinct communities in PF and PD
- Potential probiotic targets have high ADS expression independent of community abundance (e.g., *Leptotrichia* HMT 212, 215 and *S. oralis* SEQF1998)

Fig 3. Species level ADS operon expression significantly correlated to community composition. (a) Relative proportion of amplicon reads for ADS competent bacteria as compared to *Streptococcus mutans* across all samples. (b) Comparison of log fold changes for ADS competent bacteria in the amplicon and metatranscriptomic datasets. (c) Log 10 normalized read count of amplicon sequences for each species corresponding to Figure c. (d) Proportion of samples in amplicon dataset where species listed in Figure c were detected.

Conclusions

Results of this study highlight potential candidates for probiotic panels including oral bacteria where pH modulation through the ADS pathway have previously been described (e.g., *Streptococcus* sp.) as well as those that are less well characterized (e.g., *Leptotrichia* sp.). While our multivariate approach substantiates the role of the ADS pathway in health and disease, it highlights the importance of accounting for taxonomic shifts in the interpretation of functional differences in mixed microbial ecosystems and suggests that probiotic development may benefit from further strain-level investigations for ADS activity and pH modulation potential in mixed microbial communities.

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