Actinomyces and *Fusobacterium* Drive Co-aggregation in a Subgingival Biofilm Model

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Objective: The oral cavity is home to over 700 bacterial species, and the success of their colonization is linked to their ability to adhere to surfaces and co-exist in biofilms. Numerous studies on bacterial coaggregation have utilized bacteria isolated from different subjects. This study utilized bacteria isolated from the same periodontal pocket, to identify naturally occurring co-aggregation partnerships found under the gumline.

Methods: The bacterial strains were previously isolated from a single eight mm periodontal pocket. The six bacterial strains utilized in this study are *Porphyromonas gingivalis* (1-1), *Veillonella dispar* (1-3), *Parvimonas micra* (1-4), *Fusobacterium nucleatum polymorphum* (1-5), *Actinomyces oris* (1-8), and *Streptococcus anginosis* (1-10). Bacterial species were grown anaerobically in TSBY broth, and resuspended in co-aggregation buffer to a final OD600 of 2.0. Equal volumes of two bacteria were mixed and allowed to stand at 24°C for four hours, with OD600 readings taken every 30 minutes. Plots of optical density over time were used to calculate slope, and significant differences identified with a students T-Test. Surface adherence and co-aggregation partners were visualized with fluorescent staining and microscopy.

Results: 1-5 was present in the three strongest coaggregations, when paired with 1-4, 1-8, and 1-3. 1-8 was the next strongest driver of co-aggregation, when paired with 1-1. Contrary to published reports, 1-10 was not a strong co-aggregation partner. 1-8 binds most strongly to surfaces.

Conclusions: These results imply that 1-8 adheres to the tooth surface, followed by secondary colonization by 1-5 and 1-1. 1-5 is then able to recruit other oral bacteria such as 1-4 and 1-3. 1-10 does not appear to act as a pioneer or strongly associate with the periodontal biofilm. More research on these bacterial relationships will be key to understanding how these pathogens colonize the subgingival sulcus.

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