Adhesion Prevention: A Neglected Strategy in the Control of Dental Biofilms
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Dental caries is a dieto-bacterial disease associated with the oral cavity. The disease and the associated tooth decay (demineralization) are correlated with the utilization of dietary sugars by the oral biofilm which leads to a decrease in pH and an increase in the proportion of acidogenic and aciduric species, especially Streptococcus mutans. In the oral cavity, dental biofilm or plaque formation is detectable in minutes, rapidly forming on teeth and dental implants. Micro-organisms derived from the oral mucosa and saliva adhere to the pellicle in a critical first step, necessary for the development of dental biofilm. Antiseptic mouth rinse solutions are used in many situations for controlling plaque, however adhesion prevention is often neglected in the fight against tooth decay. The effect of an enzyme containing mouthwash on bacterial adhesion was examined using the MBEC biofilm model. Mouthwashes containing enzymes prevented bacterial adhesion after a one minute treatment compared with a commercially available mouthwash. These results highlight the relevance of preventing bacterial adhesion when developing oral hygiene strategies.

Theme: Signalling and communication in biofilms
Biofilm formation and development in Rhodobacter sphaeroides involves the chemosensory system which is regulated by a two component regulatory system
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Rhodobacter sphaeroides is a purple, non-sulphur α-proteobacteria which is able to form biofilms. It has a complex chemosensory system consisting of three main operons and unlinked loci. It is known that the two major chemotaxis operons, cheOp2 and cheOp3, are essential for chemotaxis and expressed in planktonic cells, however little is known about their role and expression in biofilm bound cells. In this study, we looked at the role of chemotaxis in biofilm development and the expression of cheOp2 and cheOp3, using a plasmid based GFP reporter system, when cells are grown in biofilms. It was shown that both operons are expressed throughout the biofilm lifecycle and that they are expressed in cells which are located in different areas of the biofilm.

We then looked at control of expression and identified a two-component regulatory system, which when deleted, caused biofilms to be altered in their physical appearance and have increased production of exopolysaccharide and increased adhesion to surfaces. Furthermore deletion of the two-component regulatory system alters expression of the chemosensory genes. The data suggests that environmental sensing via this two-component regulatory system is involved in the switch from the free-living to surface-attached growth.

F Plasmid-Mediated Signaling during Escherichia coli Biofilm Formation
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Abstract: The F plasmid of E. coli allows horizontal DNA transfer between an F+ donor cell and an F- recipient. Expression of the pilus genes is tightly controlled by a number of factors, including the following plasmid-encoded regulatory proteins: TraJ, and the autoregulators TraM. However, the unusual expression of F pili between two F+ cells (F- phenocopies) has been observed during the development of E. coli biofilm. The F+ × F+ mating was resulted from the secondary characteristics of stationary phase-like sessile bacterial population during the formation of microcolonies. Here, we found that traM and traJ genes were up-regulated in microcolony biofilm, and later promoted the development of microcolony to mature biofilm. We then demonstrated that the interaction between traM and traJ involved in the F+ × F+ pilation. The localization of TraMJ expression was found on the substratum inside microcolonies indicated that F pili are the initial cell-to-cell adhesion. We showed that TraMJ signal were quorum sensing-like molecule. TraM and TraJ were secreted and assembled outside bacterial cells. In addition, the interaction between TraMJ was regulated by H-NS from the host cell, and each molecule could be produced from different cells. These indicated the role of F transfer in adaptive physiology in starved or stationary-phase cells during biofilm development.

Monoculture and mixed biofilms of Listeria monocytogenes and Pseudomonas fluorescens – effect of different culture media and temperatures
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Like most microorganisms, *Listeria monocytogenes* and *Pseudomonas fluorescens* are able to form biofilms and are rarely found as monoculture biofilms in natural environments. Previous works showed that associations between bacteria from different genus commonly found in food-processing environments may affect their growth, attachment and biofilm formation. This work studied *L. monocytogenes* and *P. fluorescens* monoculture and multispecies biofilm formation, and investigated how different culture media and temperatures may influence such bacterial interactions.

*L. monocytogenes* strains assayed were CECT 4031\(^{\dagger}\), 747 and 994 (food isolates), 1559 (environmental isolate) and 1562 (clinical isolate). *P. fluorescens* strains used were ATCC 27663 and PF7A (food isolate). Each strain was tested for monoculture and mixed culture biofilm formation with each one of the other bacterium’s strains. Assays were performed during three days in 96-well microtitre plates, at 4\(^{\circ}\)C, 22\(^{\circ}\)C and 37\(^{\circ}\)C. Brain Heart Infusion (BHI) and Skim Milk (SM) were the culture media and biofilm formation was assessed by Crystal Violet staining.

Overall results showed that both media and temperature affect biofilm formation, as monoculture and as multispecies biofilms, and confirmed that the influence of different bacterial genus on biofilm formation is dependent on strains. Although a decrease of biomass was observed on multispecies biofilms formed at 22\(^{\circ}\)C in SM and at 37\(^{\circ}\)C in BHI, significantly higher OD values were found at 4\(^{\circ}\)C in both media, and at 22\(^{\circ}\)C in SM, indicating that the combination of these two bacteria on meat and dairy food processing environments may seriously compromise food safety potentiating higher contamination levels.

**Concurrent Quorum Sensing and Quorum Quenching in a Simultaneous Nitrification, Denitrification & Phosphorus Removal Sludge Community**

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Cell-to-cell communication or quorum sensing (QS) signalling is critical for coordination of social behaviours of bacteria. Despite several reports on the isolation of acyl homoserine lactone (AHL) signal producers from activated sludge, limited studies have documented the occurrence of AHL-mediated communication within the sludge microbial community. We have investigated the potential involvement of AHL-mediated communication between bacterial populations and the turnover of AHL signalling molecules in a Simultaneous Nitrification, Denitrification & Phosphorus Removal (SNDPR) sludge community of a Sequencing Batch Reactor (SBR). Using a culture-based AHL detection assay, AHL signal production was readily detected in the SNDPR sludge community. However, AHL signals were almost undetectable in experiments where a bioassay strain that expresses the GFP reporter gene in the presence of AHLs (i.e., a bacterial strain carrying a broad range plasmid expressing *luxR* and the *luxI* promoter fused to the *gfp* gene) was added to the sludge community. One possibility for this discrepancy, is that signal-degradation or quorum quenching (QQ) activity occurs simultaneously in the mixed community which impacts on the *in situ* detection of AHLs. Indeed, *in situ* assays of SNDPR sludge samples spiked with synthetic AHL signals, demonstrated that the exogenously added signals were consistently degraded in a concentration- and time-dependent manner. Further analyses using crude protein preparations of the SNDPR sludge confirmed that the signal degradation was mediated by enzymatic activities. These findings demonstrate the simultaneous QS and QQ activities in a SNDPR sludge community, providing a rare insight into AHL-mediated communication by microbial communities in complex systems.

**Capture and retention of Cryptosporidium parvum oocysts by Pseudomonas fluorescens biofilms on PVC pipe**

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Association of *Cryptosporidium* oocysts with biofilm communities can influence the propagation of this pathogen through both environmental systems and water treatment systems. The purpose of the present work was to determine the adhesion ability of *Pseudomonas fluorescens* ATCC 13525 and evaluated the capacity of *P. fluorescens* biofilm to retain and capture *C. parvum* oocyst in poly (vinyl chloride) (PVC) pipe, commonly used in irrigation system.

The experimental system was made of one PVC pipe reactor, one peristaltic bomb, one glass bottle and, two hoses, previously sterilized. The system worked with circulation flow rates of 20 ml.min\(^{-1}\) for 12 h per day, during 5 days. Adhered cells were removed by manual scratching a stainless steel rod with a neoprene disc on the reactor wall and they were quantified through colony forming units (CFU). Oocysts were concentrated and quantified by direct immunofluorescence technique. Hydrophobicity was evaluated through contact angle measurements by the sessile drop technique. The degree of hydrophobicity was expressed as the free energy of interaction.