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*Interactions between bacteria obtained
from stream biofilms*

Vidya Joshua Washington

*A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy,
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Abstract

Pioneer biofilm forming bacteria are those with enhanced surface attachment properties which dominate initial attachment processes when surfaces in streams become available for colonization. These organisms multiply rapidly, promote assemblage development and are present in the mature biofilm community. The purpose of this research was to identify and describe such fresh water biofilm bacteria, and to investigate cell and metabolite-level interactions between these organisms which contribute to mixed community formation.

A subset of bacteria which displayed enhanced colonisation properties was selected from isolates which were well represented in culturable and molecular 16s rRNA gene-based databases of the stream biofilm culture collection. This included two *Janthinobacterium lividum* isolates (JL1 and JL2), *Pseudomonas costantinii* (PC), *Pseudomonas syringae* (PS), and *Serratia plymuthica* (SP) species. Each isolate displayed characteristic properties in terms of mono species biofilm structure in low nutrient media, JL1 (fluffy ball-shaped microcolonies and thin undifferentiated biofilm component), JL2 (thick undifferentiated sheet-like biofilm), PC (tower-shaped microcolonies), PS (sheet-like biofilm with well-organised voids and spaces) and SP (flat undifferentiated biofilm with good surface coverage).

Population-level responses of pioneer biofilm bacteria to co-culture with a second strain were studied in detail using two defined pairs of bacteria. The first pair, SP and JL1 demonstrated that component colonies grew together and allowed ready mixing of individual populations. The pair of SP and PS consistently maintained a clear separation of colony growth zones. Microscopic analysis of cell-level arrangements of SP and JL1 (organisms detected by Gamma and Beta proteobacteria probe specific fluorescent *in situ* hybridization respectively) showed that both bacteria maintained their mono culture structures in co-culture, and suggests that the presence of a partner did not change the overall macroscopic biofilm appearance of either strain. The co-culture biofilm of SP and PS bacteria (differentially red and green fluorescent protein labelled) showed SP cells restricted to zones within the PS-dominated biofilm, suggesting PS maintained its overall matrix structure at the expense of SP.

Extracellular metabolite profiling of SP and JL1 showed unique profiles in each mono culture. The co-culture included most of the components of the metabolite profiles of each isolate but at reduced levels. Metabolic profiles and reconstructed metabolic pathways based on metabolite output suggest possible cross-feeding in co-culture and a co-operative interaction between isolates. Extracellular metabolites of SP and PS showed differences in mono culture. The co-

culture metabolite profile was more similar to that of PS than SP, and, some molecules were present at levels higher than the PS mono culture. Nature of secreted metabolites and reconstructed metabolic pathways suggest that the interaction between bacteria of this pair was highly competitive and involved the production of inhibitory molecules.

Interspecies interactions amongst bacteria are known to be important in structuring biofilms and this research provides further knowledge into the complex web of inter bacterial interactions that influence the development of multi-species biofilm communities.

Dedication

This thesis is dedicated to my grandmother Smt. Lakshmi Ammal and parents, the late Shri G. V. Ramachandran and Smt. Saraswathy Ramachandran who always encouraged and inspired me to scale greater heights for the pursuit of knowledge. To my in-laws (my 'in-grace' set of parents), the late Shri S. G. Washington and Smt. N. S. Washington for their enormous amount of faith in me and for their generous love and support.

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