METABOLOMICS AS A NOVEL APPROACH TO STUDY MIXED SPECIES BIOFILMS OF STREAM BACTERIA EXHIBITING MUTUALISTIC AND ANTAGONISTIC RESPONSES

V. J. Washington, S. G. Villas-Bôas and G. D. Lewis

School of Biological Sciences, The University of Auckland, New Zealand. Email - vwas002@ec.auckland.ac.nz

Experimental objective / Purpose

1. To investigate the metabolic interactions of bacterial species using metabolic footprint profiling.
2. As proof of concept, microbes exhibiting mutualistic and antagonistic associations were chosen for this study.

Experimental design

Screening - mutualistic and antagonistic bacterial pairs

↓

Metabolic footprinting - microtitre plates

↓

GCMS - MCF | GCMS - TMS | Direct Infusion Mass Spectrometry (DIMS)

GCMS - Gas Chromatographic Mass Spectrometry
MCF– Methylchloroformate derivatization - specific for amino and nonamino acids
TMS - Trimethylsilyl derivatization - for general non-volatile metabolites

Background

Metabolic footprint profiling is a new approach in metabolomics designed to assess the growth of microbes in their environment based on the profile of their extracellular metabolites. This study focussed on the mutualistic pair of *Serratia plymuthica* strain *DSM 4540* and *Janthinobacterium lividum*, and the antagonistic pair of *Serratia plymuthica* strain *DSM 4540* and *Pseudomonas syringae*. Initial data showed that paired mixes presented distinct profiles of metabolites compared to pure cultures. This clearly demonstrated that metabolic interaction between organisms in co-culture occurred and we showed that metabolomics is the most appropriate approach to characterize the physiological relationships in microbial communities.

Conclusions

- Serratia physically responds to co-culture by forming a cell based structure not present in pure culture.
- Metabolic footprint profile of *Janthinobacterium* culture presents a distinctly different primary metabolism pattern as compared to *Serratia* and *Pseudomonas* under tested conditions.
- Metabolic footprint profile of the mixed cultures is not the sum of the profiles of individual components of the mix suggesting direct metabolic interactions.
- Metabolic footprint profile of the mutualist mix reveals a unique pattern to the antagonist mix indicating a more intensive exchange of metabolic products in the mutualist pair.

Assessment of bacterial interactions on nutrient media

Mutualistic Responses

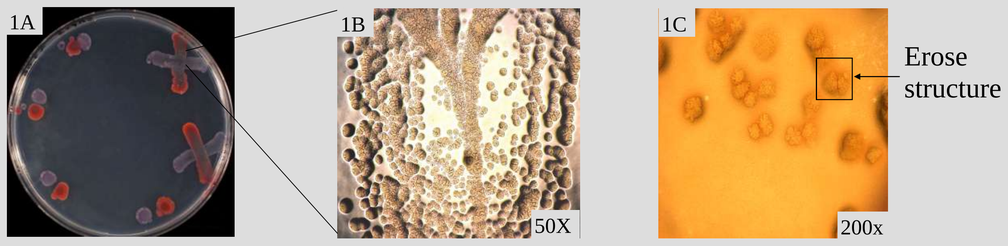

Figure 1 A) Shows colony mixing - *Serratia plymuthica* colony (red) and *Janthinobacterium lividum* colony (purple) on agar. B) Mixing zones from cross streaked cultures (Fig1A) showing unusual granular characteristics not seen in either culture. C) Shows an enlarged view of the granular structures.

Antagonistic Responses

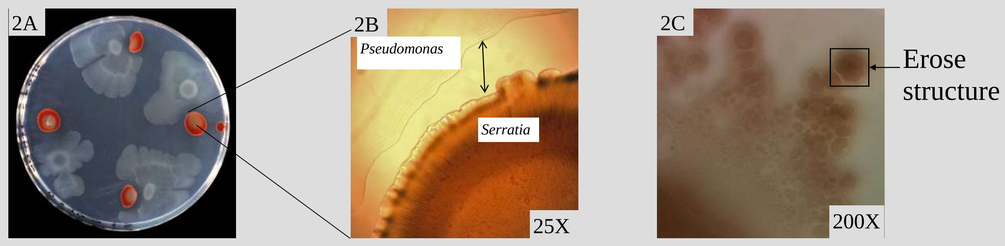

Figure 2 A) *Serratia plymuthica* colony (red) and *Pseudomonas syringae* colony (white) on agar. B) Colony boundaries of both cultures spotted close together showing zone of separation between the colonies. C) Unusual 'erose' structures formed by *Serratia plymuthica* when cultures are forcibly mixed.

Mass trees of metabolites - Central carbon metabolism

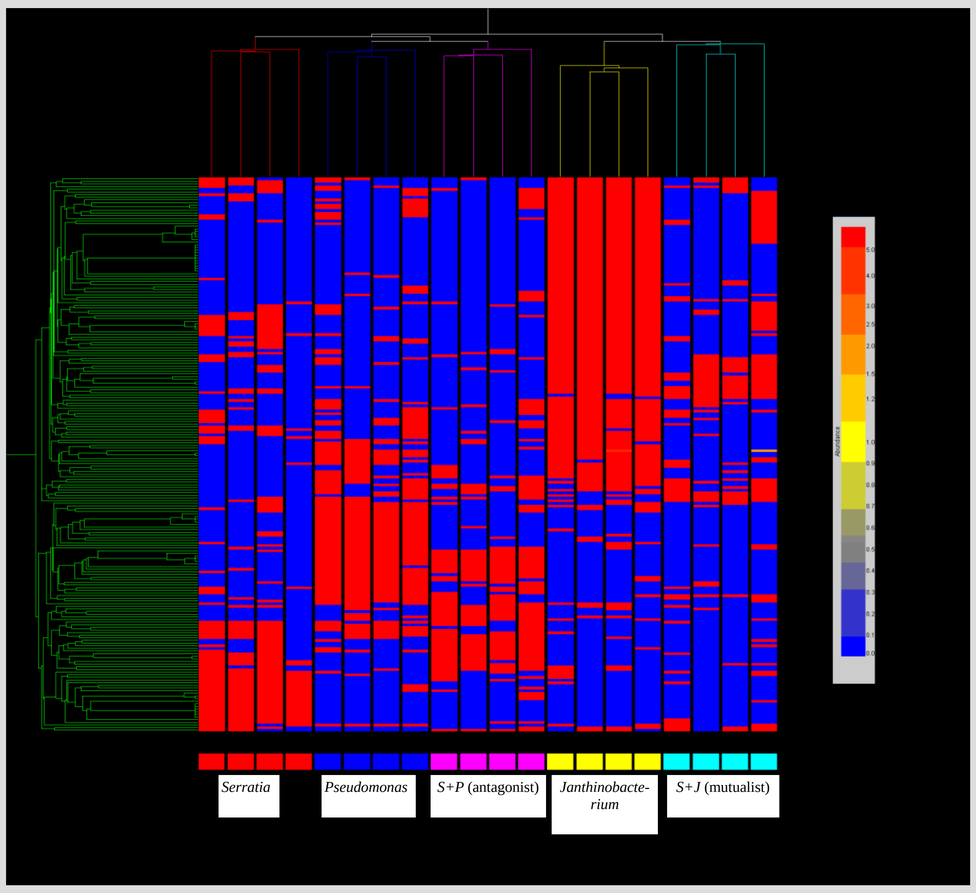

Figure 3 Mass trees of raw GCMS data (identified and unidentified metabolites) for pure and mixed cultures. Figure shows clustering of data classes (on the top) based on the similarity of their metabolite profiles.

PCA-MCF and TMS data

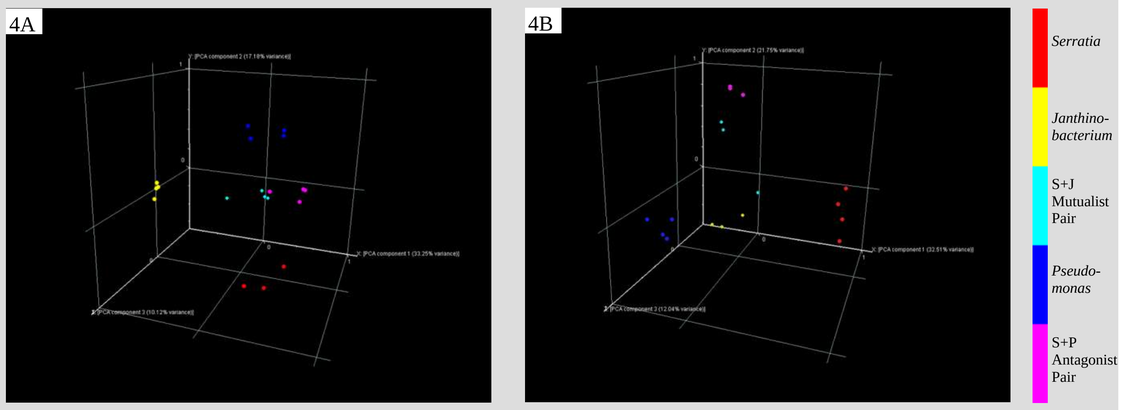

Figure 4 Principal Component Analysis (PCA) of GCMS raw data based on statistically significant MS fragments ions (4156 fragments for MCF and 747 for TMS). Samples from same data classes clustered together and clearly separated from each other. MCF results show that samples from mixed-species clustered between their respective pure cultures.

ANOVA - Heat map of significant identified components

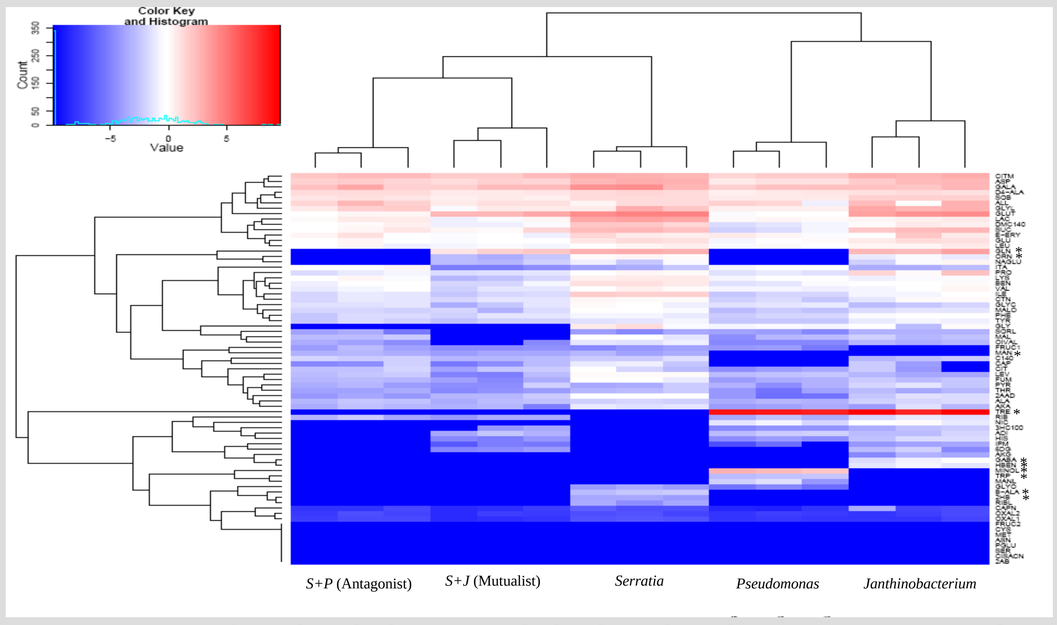

Figure 5 Heat map of 73 identified compounds for the 5 data classes (n=3). Top 10 of 59 compounds with 95% significance ($p<0.05$) are shown with an asterisk *

Ongoing work

1. Analyses of DIMS data and possible biodiscovery of novel metabolites produced by stream biofilm bacteria.
2. Flow cytometry studies to estimate relative numbers of bacteria in mixed-species cultures.
3. Generation of hypotheses to explain the observed changes in metabolite profiles.

www.streambiofilm.org.nz