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Exploring the role of key bacteria in stream biofilms

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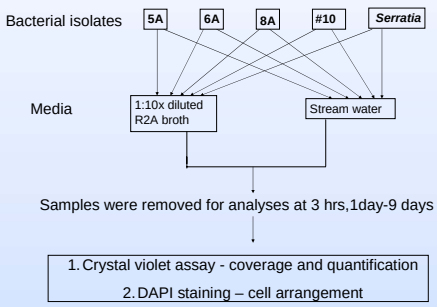
Experimental objective

To compare biofilm formation by 5 key bacterial biofilm isolates in diluted R2A broth and stream water in terms of :

- ❖Cell arrangement
- ❖Biofilm biomass
- ❖Biofilm coverage

Experimental design to investigate biofilm formation in 2 media with different nutrient levels



Methods

Isolate setup

Part1 - Crystal violet assay (Kjaergaard et al,2000)

12 well microtitre plates were inoculated (4wells/isolate) with overnight cultures of the 5 key isolates at cell concentrations of 10^6 cfu/ml. Two sets of plates were used, one with 1:10 R2A broth medium, and the other with sterilized stream water. Sufficient plates were prepared for periodic sampling.

Part2 - DAPI staining

A parallel setup was prepared which included circular 18mm dia. cover slips in each well for biofilm attachment and further examination by DAPI staining.

Culture conditions

All plates were statically incubated at 15^0 C. Plates were analyzed at intervals upto 9 days.

Preliminary screening experiments to derive the 5 key isolates

Aim: - To identify five presumptive key isolates from a set of 13 biofilm islates.

Identities of isolates: -Pure cultures of biofilm isolates were obtained from regular periodic sampling of various streams in New Zealand and stored at -80^0 C. Eleven such Auckland isolates and one Canterbury isolate were used in a preliminary screening experiment to investigate their biofilm forming capabilities and how they compared with each other.

Brief description of isolates: - All 12 isolates were Gram negative, motile rods, approx. 2.5u long x 0.5u wide, most isolates showed end to end attachment of cells whereas some showed a palisade cell arrangement. All isolates showed the presence of dense intracellular granules.

Experimental setup:-Overnight R2A broth cultures of isolates were used in a surrogate crystal violet biofilm assay (Kjaergaard et al, 2000) to assess biofilm formation over 3, 24 and 48 hrs.

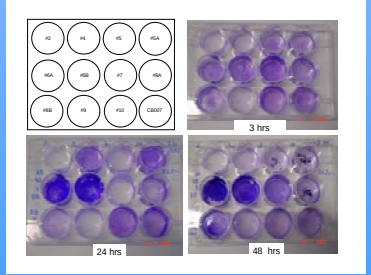


Figure1:- Crystal violet stained biofilms of 12 isolates over time periods of 3, 24 and 48 hrs. Isolates were introduced in the order specified by the key - upper left corner and evaluated over time. Uninoculated medium controls were used but are not included here.

Results

- ❖ Isolates 5A, 6A, 8A, #10 and CB007 were identified as presumptive key isolates on the basis of their early biofilm establishment and their ability to then actively grow and maintain their dominance over time.
- ❖The CB007 isolate, identified as a *Serratia* sp. was found to be dominant in the biofilm from which it was isolated by both culture and molecular techniques.

Background

Stream biofilms are microbial communities which have attached to surfaces in streams where sufficient moisture and nutrients are available**.** Research suggests the presence of key bacteria promote and contribute to the complex structural and organizational architecture of the biofilm. This research aims to select two key culturable bacteria from an existing set of biofilm isolates mainly on the basis of their ability to attach to a surface.

Conclusions

- ❖ Diluted R2A broth populations of all isolates utilized nutrients at a higher rate initially, and appeared to be unable to form dense biofilms or sustain them for longer periods of time.
- ❖ Low-nutrient stream water resulted in slow initial biomass development but ultimately more dense and structured biofilms which were sustained for longer periods than their diluted R2A broth counterparts.
- ❖ *Serratia* biofilms differentiated to leaf-like structures in stream water but not in diluted R2A broth.

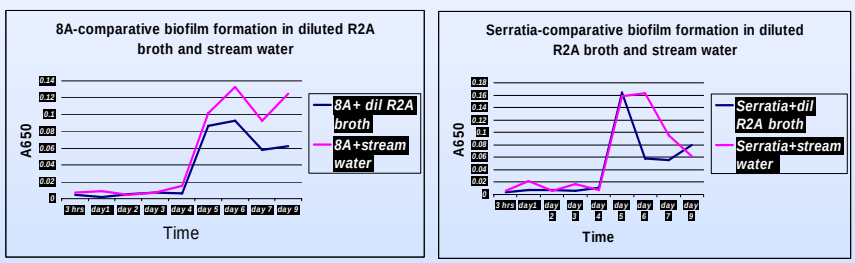


Figure2: - Quantitative estimate of biofilm biomass over time as assessed by crystal violet absorbance in 1:10 diluted R2A broth and stream water for bacterial isolates 8A and *Serratia.*

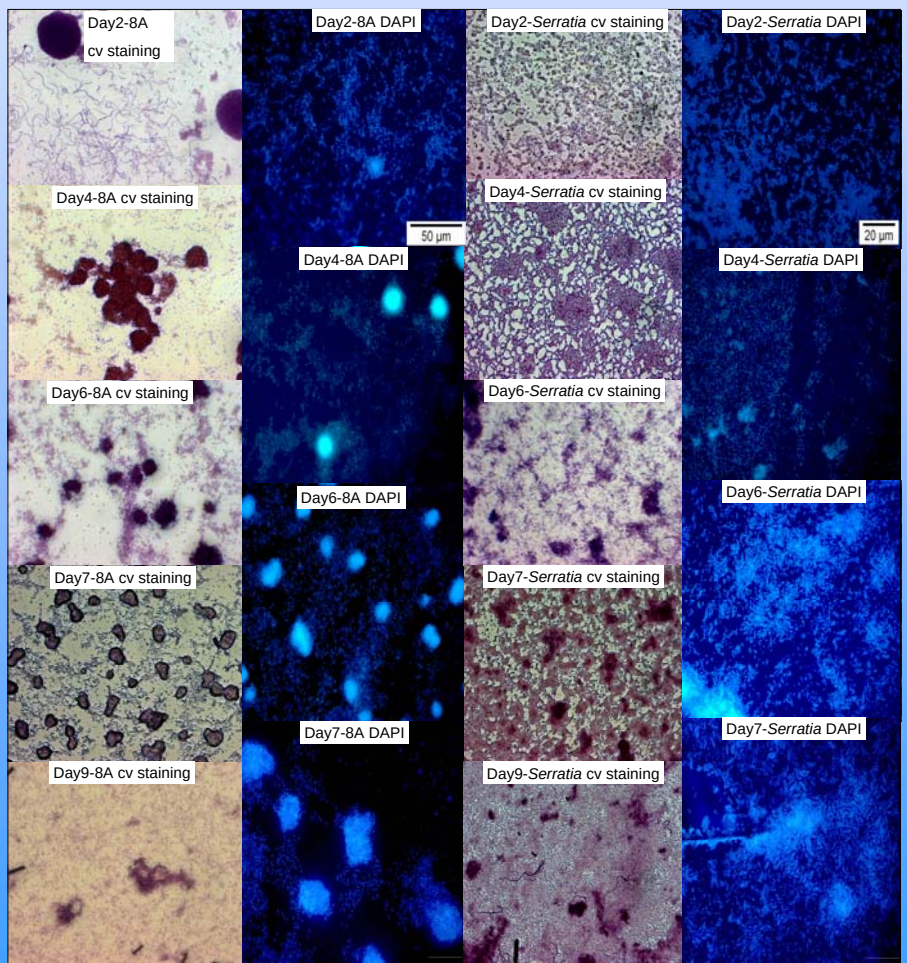


Figure3: -Biofilm formation in stream water by 8A and *Serratia* as evaluated by crystal violet (cv) staining and DAPI staining. Column 1- Cv stained biofilms of bacterial isolate 8A on day 2, day 4, day 6, day 7 and day 9 at 400x magnification. Column 2- DAPI stained pictures of 8A on day 2, day 4, day 6 and day 7 at 630x magnification. Column 3- Cv stained biofilms of bacterial isolate *Serratia* sp. on day 2, day 4, day 6, day 7 and day 9 at 400x magnification. Column 4- DAPI stained pictures of *Serratia* on day 2 (1000x magnification), day4, day 6 and day 7 at 630x magnification.

Reference: - Kjaergaard,K.,Schembri,M.A., Hasman,H. & Klemm, P. (2000). Antigen 43 from *Escherichia coli* induces inter- and intraspecies cell aggregation and changes in colony morphology of *Pseudomonas fluorescens.* Journal of Bacteriology, 182(17), 4789-4796.

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