

# Bacterially mediated manganese deposition in novel 'anelli' within the biofilms of an impacted urban stream

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## Introduction

The purpose of this work is to identify bacteria responsible for the formation of manganese containing anelli within stream biofilms, and study their distribution. Manganese oxidising bacteria are part of a diverse group of organisms found commonly within many disparate environments, which deposit manganese and iron biominerals within biofilms and flocs [1]. The purpose of microbial manganese oxidation is poorly understood, but may be associated with energy production, mobilisation of nutrients, protection and/or detoxification [1, 2]. Manganese has a high sorptive capacity for heavy metals, metalloids, and other ions, as well as a strong oxidising potential, and therefore frequently induces co-precipitation of cations present within the surrounding environment [1]. Within urban streams contaminants such as heavy metals may therefore potentially be concentrated within stream biofilms in the presence of manganese oxidising bacteria.

## Results

Biofilms grown for 8 – 124 days at the Henderson site were dominated by brown, manganese-containing, doughnut-shaped structures (anelli; 10 – 20 µm diameter; Figure 1) in which bacteria-like objects were sometimes present, surrounded by a ring enriched in manganese and iron (see Figure 1).

A bacterium, putatively designated JOSHI\_001 was isolated from Henderson biofilm (see Figure 2). This bacterium was found to grow as anelli when grown as biofilms in liquid culture.

Colonies were observed to grow up to 500 µm in diameter, with a brown ring of precipitated manganese giving an approximately circular form and irregular undulate margins (see Figure 2D). The bacterial component of these microcolonies was present as a globule within the central pore, which was confirmed by staining with DAPI and safranin (see Figure 2B-C).

The isolate, putatively designated JOSHI\_001 was found to be a non-motile Gram negative rod, measuring approximately 1.5 µm in length and 0.6 µm in width (see Figure 2A).

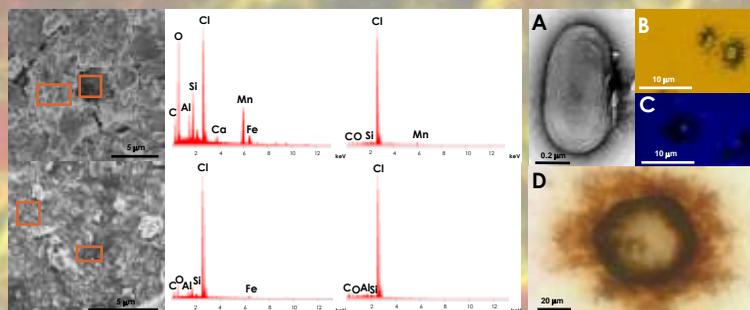


Figure 1. Anelli structure and composition. A) An anelli with bacteria-like objects within pore surrounded by a ring enriched in manganese. B) Non-anelli biofilm. 14-day Henderson biofilms viewed with SEM and analysed with EDX to show the elemental composition of 1) anelli ring, 2) anelli pore, 3) non-anelli biofilm and 4) slide background.

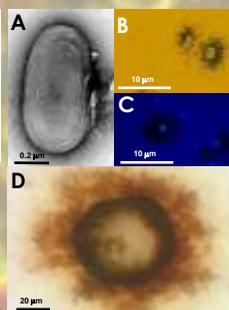


Figure 2. JOSHI\_001 colonies. A) 4-day uranyl acetate stained culture viewed under TEM. B) 1-day colonies stained with safranin. C) 2-day colonies, DAPI-stained. D) mature colony.

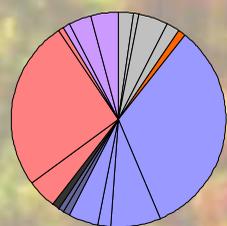


Figure 4. Whole community molecular analysis of Henderson biofilm community based on 16S rRNA gene sequences.

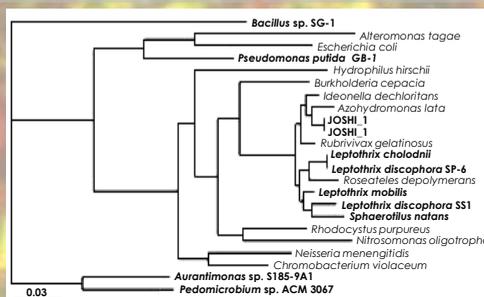


Figure 3. Maximum Likelihood phylogenetic tree showing the identity of JOSHI\_001, within the order Burkholderiales and of the class beta-proteobacteria, based on 16S rRNA gene sequences. Outgroup – Bacillus sp. SG-1. Mn-depositing bacteria in bold font.

Phylogenetic analysis of the 16S rRNA gene of this bacterium indicated that JOSHI\_001 is a beta-proteobacterium of the class Burkholderiales (see Figure 3), within the Rubrivax group. Identity of 95 – 97 % was seen with 16S rRNA gene sequences from various strains of the genera *Ideonella*, *Rubrivax*, *Roseateles*, *Azohydromonas*, and *Aquabacterium*, as well as members of the manganese depositing genus *Leptothrix*.

Whole community molecular analysis of the bacterial component of biofilms grown on glass slides within Henderson Creek for 21 – 49 days showed members of the beta-proteobacteria to be only a small proportion (< 2 %) of the community (see Figure 4).

Within biofilms grown at the four sites along the Oratia Stream reach, the presence of anelli was seen to increase with increasing levels of urban impact moving down the catchment area (see Figure 5). This correlated to increasing concentrations of dissolved iron and manganese (see Figure 6). No correlation was seen between the levels of dissolved organic carbon (DOC) and number of anelli (see Figure 6).

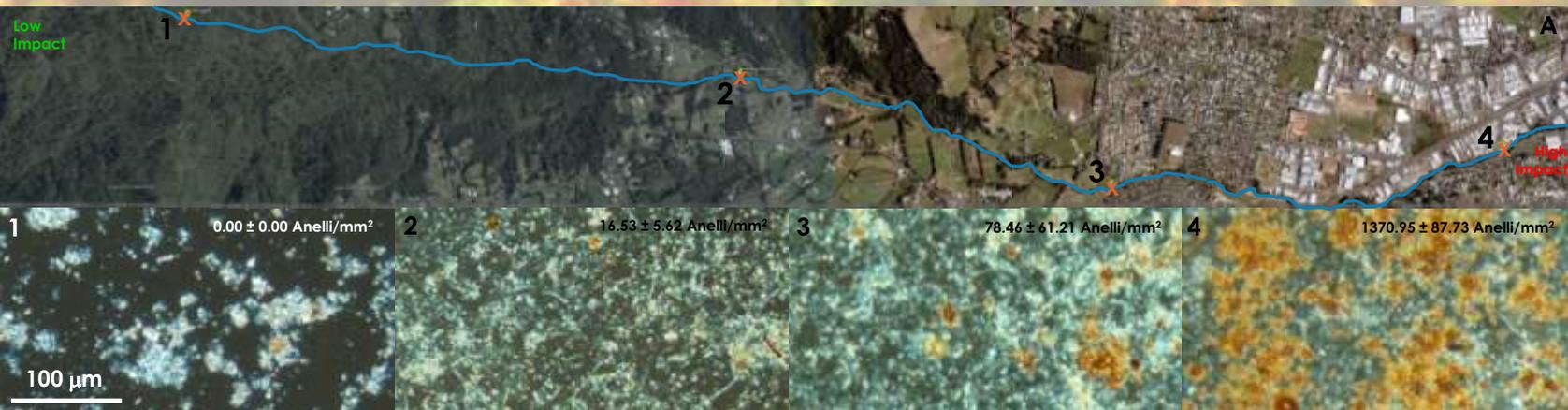


Figure 5. Anelli growth at four sites along Oratia Stream reach. A) Image of Oratia Stream and catchment, sourced from Google Earth, and biofilm from 1) Rangemore (upstream forested), 2) Kellys Road (rural), 3) Pars Cross (urban border) and 4) Henderson (commercial).

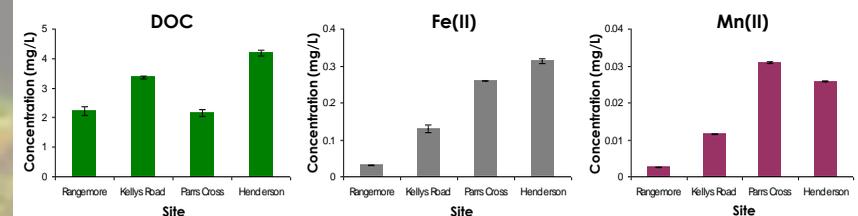


Figure 6. Analysis of water from Oratia Stream sites for levels of dissolved organic carbon (DOC), dissolved iron (Fe(II)) and dissolved manganese (Mn(II)).

## Conclusions

- A novel bacterium, designated JOSHI\_001, has been isolated, which is responsible for the active deposition of iron and manganese within doughnut shaped structures (anelli) in stream biofilms.
- This bacterium appears to be a minor component of the biofilm bacterial population, although anelli may dominate the physical structure of the biofilms.
- Anelli growth is enhanced within the highly impacted Henderson site.

## Methods

**Field Sites:** Biofilms were grown on glass or acetate slides suspended vertically within the water column at four sites (upstream bush, rural, urban border and commercial) along Oratia Stream, Waitakere, Auckland. The Henderson (commercial) site, characterized by a high level of anthropogenic impact, was the principal study site. **Biofilm analysis:** Air dried slides were examined *in situ* on the growth substrate under dark-field illumination (unstained) or with scanning electron microscopy (SEM), and electron dispersive X-ray analysis (EDX) was used for determination of elemental composition. **Molecular analysis of biofilm community:** DNA was extracted from biofilms with a bead beating method as per Miller et al. [3]. A nested PCR of bacterial 16S rRNA genes using primers P836 (forward) and P838 (reverse) [4] for PCR I and 530F (forward) and 243R (reverse) [5] for PCR II. This was followed by restriction analysis (enzymes HaeIII and MspI), cloning and sequencing (Macrogen Inc., Seoul, Korea) as per Heyndrickx et al. [6]. Resultant sequences were subjected to a BLASTn search (<http://www.ncbi.nlm.nih.gov/>) using default settings. **Enumeration of anelli:** Slides were grown in triplicate at each site along Oratia Stream. The number of anelli observed at 28 sample points on each slide was counted, and the stream average calculated as the average of these values. **Isolation of anelli-forming bacterium:** Culture was undertaken from slurries of Henderson Creek biofilm on 1/10 R2A agar supplemented with 1 mg/L MnSO<sub>4</sub>. Growth was also achieved in a liquid medium composed of 0.07 g/L Yeast Extract and 1 mg/L MnSO<sub>4</sub> in tap water. Cultures were incubated at 20 °C in dark, with liquid cultures shaken on an orbital shaker at 120 rpm. Bacterial colonies were viewed in place on agar in their native state, or stained with Safranin for 1 minute or 4,6-diamidino-2-phenylindole (DAPI; 10 mg/mL incubated in dark for 45 minutes). Bacterial suspensions were stained with uranyl acetate for 30 seconds and viewed under TEM. **Molecular identification of anelli-forming isolate:** 16S rRNA gene fragments amplified by PCR with primers P836 and P838 [4] were sequenced. The results of a BLASTn search on these sequences, plus 16S rRNA gene sequences from other manganese depositing bacteria and various members of the alpha- and beta-proteobacteria were used to generate a phylogenetic tree estimated with Maximum Likelihood, in Geneious (v. 3.7.0) using the GTR model of sequence evolution. Bootstrap values were obtained with 1000 replicates. Alignments for all trees were performed in Geneious using default settings. **Chemical analyses:** Water samples were analysed by Hill Laboratories, Hamilton, New Zealand, and included determination of dissolved organic carbon (W.DOC), dissolved basic mdatelut settings suite (W.MWBd), dissolved manganese (W.MnSi) and dissolved iron (W.FeSi) to trace level.

## References

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