

## 2.6.14

### Lipopolysaccharide and surface proton binding characterization of *Shewanella* sp.

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#### Introduction and Methods

Cell surface charge and the nature of cell surface polymers may influence the initial adhesion of cells to surfaces; an important precursor to biofilm formation. To further understand the relationship between surface polymers and surface charge, the lipopolysaccharide (LPS) of eight planktonically grown *Shewanella* strains was characterized using silver-stained SDS-PAGE gels. In conjunction with this, cell surface proton binding affinity was determined using an acid-base titration approach with the linear programming method.

#### Results and Discussion

Titration showed that total surface ligand concentrations ( $L_T$ ) ranged from  $0.903 \pm 0.007$   $\mu\text{moles/mg}$  (*S. baltica* 63) to  $1.387 \pm 0.007$   $\mu\text{moles/mg}$  (*S. amazonensis* SB2B). A Tukey's HSD test revealed smooth strains (possessing O-side chain) exhibited significantly higher  $L_T$  values than rough strains (no O-side chain) in 69% of comparisons, suggesting the presence of O-side chains commonly increases the concentration of ionizable groups on the cell surface. Additionally, comparison of individual  $pK_a$  concentrations revealed smooth LPS strains of *Shewanella* contained relatively higher concentrations of reactive groups at  $pK_a$  5 compared to rough strains, suggesting the O-side chains contained detectable carboxyl groups. Evidently, the nature of LPS has potential to influence cell surface charge.

Titration showed that the average ZPC for the eight *Shewanella* strains was  $7.18 \pm 0.3$ . Electron microscopy revealed the distribution of cell surface polymers was very heterogeneous in many strains of *Shewanella*. It follows that under ideal growth conditions (pH ~7), close to the cell's ZPC, there could be both positive and negatively charged cells. This may be advantageous in the natural environment, where the ZPC of iron oxides varies tremendously due to the adsorption of counter ions. Thus, a phase of variably charged cells would ensure that at least some cells may always adhere to an iron oxide surface, despite the mineral's surface charge.

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### Metal and metalloid immobilization by sulfate-reducing bacterial biofilms

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Sulfate-reducing bacteria (SRB) are key participants in the biological cycling of many metals and metalloids. While much interest has focussed on precipitation of insoluble metal sulfides, SRB can also reduce metal solubility by altering pH and redox conditions and also possess enzymes capable of directly reducing metalloid oxyanions. In nature, SRB commonly occur in biofilms and the biofilm growth-mode offers potential advantages for use in bioreactors for bioremediation. Attached cells display altered phenotype and metabolism, while the presence of an extensive extracellular matrix influences metal immobilization through ligand binding, entrapment, and establishment of diffusion gradients.

Here we report on two different mechanisms for Se and Cd entrapment by SRB biofilms. Firstly, we have found that SRB can mediate formation of elemental sulfur in the presence of selenite [1]. This coprecipitation of S and Se appears to be a generalised ability of SRB, arising from sulfide biogenesis, and can take place under a range of redox conditions and in the dark. When SRB grow as an attached biofilm, the resulting sulfur-selenium deposit forms nanometer-scale aggregates that are effectively sequestered within the biofilm. The second study describes the quantitative determination of SRB biofilm interactions between metabolisable substrates and Cd. A mathematical model of bioprecipitation was developed in which CdS formation rate was determined by two steps: sulfide production and colloidal CdS flocculation [2]. This model also indicated that the rates of sulfate reduction and of flocculation were the key variables in optimising the biofilm system for Cd removal.

#### References

- [1] Hockin, S. and Gadd, G.M. (2004) *Appl Env Microbiol* **69**, 7063-7072.
- [2] White, C., Dennis, J.S. and Gadd, G.M. (2003) *Biodegradation* **14**, 139-151.