

# CHE-0133237 - Fundamental Studies of In Situ Biosurfactant Production and the Attendant Impact on Metal Interactions with Soil Surfaces

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## Project Description

This novel collaboration combines the expertise of an environmental microbiologist with that of two chemists to better understand the role of biosurfactants produced by bacteria in toxic heavy metal fate and transport in the environment. Surfactants produced by microorganisms are one mechanism by which the bioavailability of organic and inorganic compounds is enhanced. In particular, *Pseudomonas aeruginosa* produces two types of rhamnolipid biosurfactants (monorhamnolipid and dirhamnolipid) that have been shown to bind toxic heavy metals and alter mineral surface properties. These properties can influence solubilization and sorption of metals from insoluble mineral oxides or can potentially be exploited for bioremediation purposes. The efficiency of these processes will profoundly influence the fate and transport of metals in surface and subsurface soils as well as groundwater environments.

## Broader Impacts

This interdisciplinary research has implications beyond metal fate and transport in soil. Biosurfactants are one class of secondary metabolites produced by soil microorganisms. Our results make clear that secondary metabolite conditioning of soil surfaces has the potential to influence processes in soil that ultimately mediate soil formation and function. Soil function, the ability of soil to respond to environmental and anthropogenic perturbation and to support agriculture, will become increasingly important as world population and stress on natural resources increases. Thus, this project serves as a model for cross-cutting interdisciplinary efforts needed to advance science at the interface between disciplines.

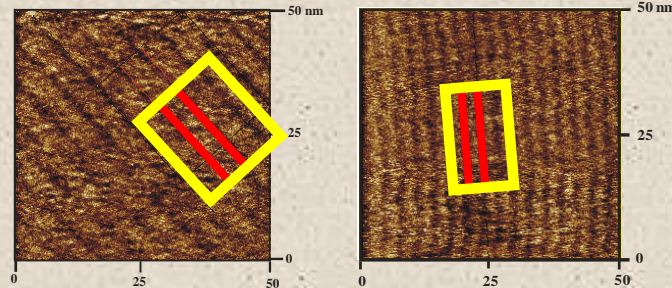
This project also has multidisciplinary educational activities that reflect the creative fusion of these two disciplines. Chemistry graduate students working on this project pursue formal minors in environmental chemistry and the environmental microbiology graduate students pursue formal minors in chemistry. A new undergraduate laboratory experience on chromatographic purification (SPE) and analysis (RPLC) of rhamnolipids from bacterial cultures has been completed and will be introduced this fall into an analytical chemistry course for nonmajors (CHEM 323), largely from microbiology and environmental sciences.



Chemistry and Microbiology personnel supported by this project  
From left to right: Valeria Ochoa Herrera, Julie Neilson, Ariel Lebron-Paler, Sadie Iverson, and Vanessa Stedronsky

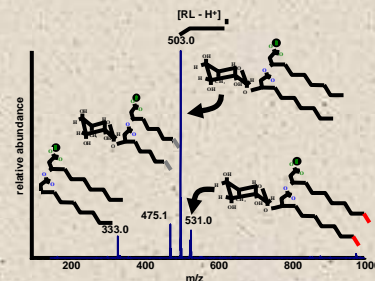
## Examples of Advances and Discoveries

### First AFM images of biosurfactant on a surface



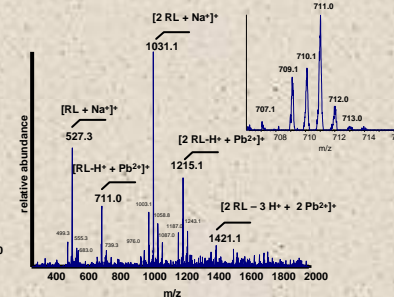
AFM images of rhamnolipid assemblies on highly ordered pyrolytic graphite (HOPG). Image A shows organized assemblies of rhamnolipid alone with spacing of 5.50 nm (see red lines). Image B shows organized assemblies of rhamnolipid-Cu<sup>2+</sup> complexes with spacing of 3.12 nm.

### Development of a purification protocol for monorhamnolipid



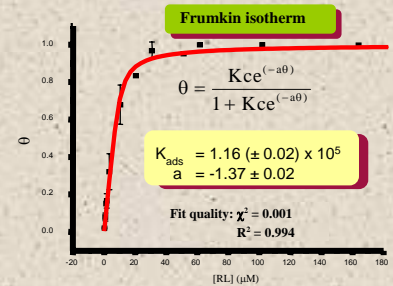
ESI-MS negative ion mode spectrum of monorhamnolipid (RL) produced by *P. aeruginosa* ATCC 9027 after extraction and purification. This spectrum clearly shows the grade of purity obtained using the purification protocol developed in our lab. The m/z at 475, 503 and 531 are monorhamnolipids with different lipid alkyl chain lengths. The peak at m/z 333 corresponds to the lipid chains without the rhamnose sugar moiety.

### Identification of rhamnolipid-Pb<sup>2+</sup> complexes



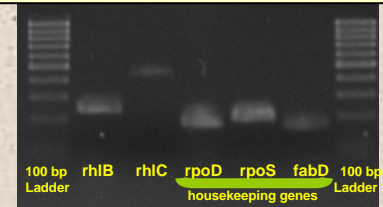
ESI-MS positive ion mode spectrum of solution of [RL<sub>2</sub>Pb(II)] complex (pH 9.3). *Inset*: Isotopic distribution of the peak at 711.0 for [RL-H + Pb<sup>2+</sup>]<sup>+</sup>. Peaks at m/z 707, 709, 710, 711 correspond to the lead isotopes <sup>204</sup>Pb, <sup>206</sup>Pb, <sup>207</sup>Pb and <sup>208</sup>Pb, respectively.

## Exploring rhamnolipid sorption by surfaces



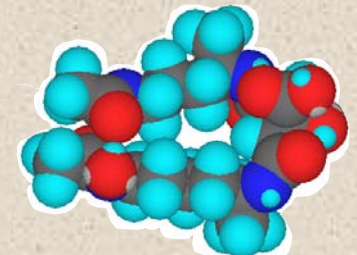
ATR-FTIR adsorption isotherm of monorhamnolipid (RL) adsorbed to  $\gamma$ -alumina at pH = 6.3. Experimental data were fit with a Frumkin model that includes attractive lateral interactions between RL units.

## Gene regulation of rhamnolipid production



We are developing a real-time reverse transcriptase PCR (RT-PCR) assay to evaluate relative gene expression for mono (rhlB) and dirhamnolipid (rhlC) production during exposure of *P. aeruginosa* to metals. Housekeeping genes (expressed all of the time) are used as internal standards.

## Discovery of a new biosurfactant



Flavolipid from *Flavobacterium* sp. MTN11 - a new class of biosurfactants