

CAREER: The Role of Microorganisms in Arsenic Contamination of Groundwater

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ABSTRACT: *Arsenic exposure through drinking water affects the health of millions of people world wide. The health effects of exposure to arsenic through drinking water range from skin ailments to certain cancers (Hopenhayn, 2006). A multidisciplinary approach to studying how arsenic is released into groundwater is needed to effectively answer many of the questions that exist. A study of microorganisms from groundwater wells showed that both iron and arsenate reducing bacteria may contribute to the elevation of groundwater arsenic levels. Researchers at the University of Manchester have shown that iron-reducing bacteria can be stimulated by the addition of organic carbon to release arsenic into the water phase using sediments from West Bengal (Islam et al, 2004.). Collaboration with the Williamson Research Centre at the University of Manchester, UK has allowed us to investigate the microbial populations associated with groundwater wells in Northport, ME by applying molecular techniques to characterize the microbial communities of groundwater samples of varying arsenic concentrations. The collaboration and techniques learned have allowed our research to progress in new directions and has broadened our research networks and introduced us to new potential collaborations within the University of Maine.*

INTRODUCTION

Dr MacRae's research team at the University of Maine has been studying microorganisms that have the potential to affect groundwater arsenic concentrations. The research has primarily focused on an aquifer in Northport, Maine. An arsenate reducing microorganism, *Sulfurospirillum* species NP4 was isolated from high arsenic well water. In addition to arsenic, *Sulfurospirillum* species NP4 is able to utilize a variety of additional terminal electron acceptors (TEAs) in energy-generating reactions coupled with the oxidation of a small number of carbon sources (MacRae et al., 2007). This isolate can also fix nitrogen using the *nif* operon and carbon using the reverse TCA cycle (manuscript in preparation). By employing molecular techniques, *Sulfurospirillum* species NP4 was found to correlate well with the concentration of As(III), the more toxic and mobile form of arsenic, in well water samples from the Northport aquifer. The total arsenic concentration correlated with the *Geobacter* population (Weldon and MacRae, 2006). The genus *Geobacter* was examined because it contains many iron-reducing bacteria and relatively few known arsenate-reducing bacteria.

The collaboration established under the IREE program had two primary purposes: to acquire expertise working with iron reducing bacteria by working with one of the leading

groups in metal-reducing bacteria and to establish contacts with researchers who have worked on arsenic contamination in several international settings. The geomicrobiology group at the University of Manchester, UK has experience combining the tools commonly used in geology to characterize minerals with anaerobic microbiological techniques, and it was anticipated that we could apply these techniques to arsenic-contaminated iron surfaces or substrates to learn more about how they are affected by microbial activity. We also wished to apply molecular techniques to environmental samples from Maine, as had been done by their group with materials from India and Cambodia. Jennifer Weldon's work focused on applying molecular techniques to the samples from Northport, Maine.

Professor Jonathan Lloyd's Geomicrobiology Laboratory is housed within the Williamson Research Center for Molecular Environmental Science at the University of Manchester. This large, interdisciplinary group has conducted numerous field and laboratory studies involving microbial transformation of inorganic constituents in the subsurface and their effects on water quality. They have done extensive international work on the arsenic problem in West Bengal, Southeast Asia and Australasia. Relevant expertise in the department includes the use of advanced molecular tools and microscopy for the characterization of environmental microbial populations.

The PI, Jean MacRae, worked at the University of Manchester from January 5-July 18, 2007. Jennifer Weldon joined the group from June 3-29, 2007.

RESEARCH ACTIVITIES AND ACCOMPLISHMENTS OF THE INTERNATIONAL COOPERATION

The Graduate researcher brought Northport groundwater samples to the UK in order to conduct microbial community analysis. DNA was extracted and clone libraries have been constructed for some of the water samples, and this work continues in our lab. The molecular techniques are now being used to more fully characterize the microbial communities in the aquifer that we have studied in Maine to see if there are any additional links between microorganisms and water chemistry at the site.

DNA was first extracted from the samples, and a preliminary assessment of the diversity of the microbial community was made using

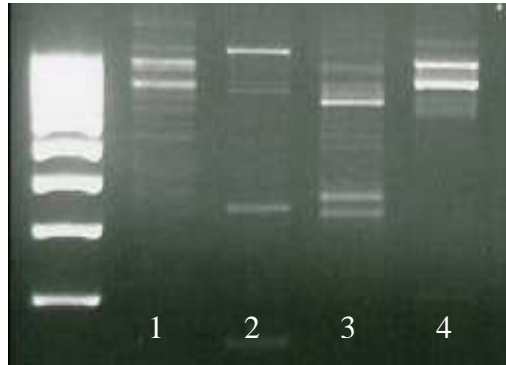


Figure 1: 1% agarose gel from RISA analysis of extracted DNA from groundwater samples

ribosomal intergenetic spacer analysis (RISA). This shows the variability in the fragment length between the 16S and 23S ribosomal DNA in the samples. In Figure 1, the lane on the left is a reference DNA size ladder. The 4 other lanes are polymerase chain reaction (PCR) products using the RISA primer set. Samples 1 and 4 are from wells with low arsenic levels and samples 2 and 3 are from wells with high arsenic levels. It can be seen that in both samples 1 and 4 the dominant (brightest) bands are of approximately the same size, although some of the minor bands are different. The pattern of bands obtained in the high arsenic

wells are dissimilar, and both are very different from the low arsenic samples. This is a preliminary indication that there is variability in the groundwater microbial consortium when comparing low and high arsenic wells. It also appears that there is variability when comparing two wells from the high arsenic region of the aquifer.

Once the RISA analysis was completed and indicated significant differences in the well populations, 16S ribosomal DNA was amplified using the 8F and 519R primer set (Holmes et al., 2002). The amplified products were purified and cloned using the Invitrogen TOPO[®] TA cloning kit with TOP10[®] chemically competent cells. Following cloning, the individual colonies that appeared to contain an insert were tested using PCR primers specific to the cloned insert. After ascertaining the presence of the 16S rRNA gene fragment, the samples were compared using restriction fragment length polymorphism (RFLP). RFLP uses 2 restriction endonucleases to cut the sample DNA into fragments of different length, which are then separated by electrophoresis, stained with ethidium bromide and photographed under short wave UV light. The different patterns of bands allow the selection of unique samples from the clones.

RFLP analysis was only completed on one of the 4 samples that were brought to Manchester, however the knowledge gained has allowed the remaining samples to be completed upon return to Maine. Now the unique samples have all been sequenced.

In addition to the cloning, microcosm experiments that consisted of groundwater incubated in the presence of sand that was uncoated, coated with ferrihydrite, or coated with ferrihydrite that was coprecipitated with arsenic were analyzed. In addition to the variety of surfaces some of the bottles were amended with acetate as a carbon source or carbon dioxide and hydrogen for carbon fixation. DNA was extracted from those samples. The extracted DNA was analyzed using RISA analysis to give a preliminary indication of how unique the microbial populations were in each of the samples. A portion of the sand from the microcosms was removed and was studied using the Environmental Scanning Electron Microscope (ESEM). In that analysis some of the samples had bacteria that looked similar in shape to *Sulfurospirillum* species NP4 and some had morphology similar to various *Geobacter* species.

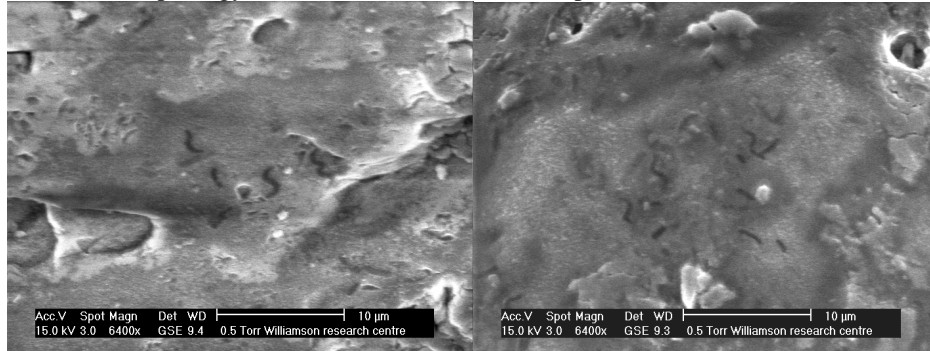


Figure 2: The ESEM image on the left shows “S” shaped *Sulfurospirillum* like organisms. The ESEM image on the right shows “S” shaped organisms and rod shaped organisms similar to *Geobacter* species. Both images were from a sample that was amended with carbon dioxide and hydrogen and had sand that was coated with iron that was coprecipitated with arsenic.

BROADER IMPACTS OF THE INTERNATIONAL TRAVEL

The international travel allowed the PI and her graduate student to broaden the scope of their work to include microscopic analysis of surfaces colonized by relevant microbial types, and to expand the analysis of microbes in the arsenic-contaminated Maine aquifer. With this experience, these lines of inquiry may be pursued at the University of Maine by developing collaborations with researchers who have the appropriate research tools on campus but have not yet applied them to the kind of samples we will generate. Prior to this trip we had only worked on solution phase chemistry of both the groundwater and culture media. Experience gained in the UK will allow us to expand our explorations to iron reduction as it is relevant to the cycling of arsenic in the subsurface.

Graduate student participant Jennifer Weldon corresponds with members of the lab when technical issues relating to molecular biology techniques arise.

The graduate participant, Jennifer Weldon, had the opportunity to interact with a large interdisciplinary research group and to experience a different academic setting. She also met students from a variety of European and Asian countries. The opportunity to work side by side other researchers that are employing the same molecular techniques that she is using has proved to be invaluable to her, as well as conversing with individuals whose expertise is in fields where she is still a novice. The training she received while in Manchester has greatly hastened the pace of her research using the molecular biology tools that she became familiar with in Manchester. The training and experience she gained in Manchester is now guiding her as she completes her dissertation research.

DISCUSSION AND SUMMARY

Understanding the microbial role in causing increased arsenic levels in groundwater is a difficult task. There are many different types of organisms that can be involved in the process such as those that alter the redox state of iron or arsenic. There has been much research devoted to understanding this problem in response to the health crisis that arsenic in drinking water caused in Bangladesh and West Bengal, India as well as other locations. Even with all of this research there are still many questions that are still remaining. More understanding is needed to understand what factors control microbial release of arsenic. Also, more information is needed to fully understand what is happening at the surface of aquifer materials where arsenic release occurs.

The work done in the context of the collaboration between the graduate student and her host at Manchester University has been a first step in assessing the microbial diversity in an aquifer in Northport, ME that has regions of elevated arsenic levels. The techniques that were learned in Manchester are being continued at the University of Maine.

ACKNOWLEDGEMENTS

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BRIEF BIOGRAPHIES OF RESEARCHERS

Jean MacRae received a B.S. degree in Life Science from Queen's University in Kingston, Ontario, Canada in 1988. She received her M.S. in Microbiology in 1991 and Ph.D. in Environmental Engineering in 1997 from the University of British Columbia. She worked for Environment Canada and then Health Canada prior to taking her position in the Department of Civil and Environmental Engineering at the University of Maine in 1999. She was promoted to Associate professor in 2006. Her research interests include anaerobic microbial processes and element cycling, biodegradation and bioavailability of organic contaminants.

Jennifer Weldon attended the State University of New York Canton College of Technology where she was the Earl W. MacArthur Honor Scholar. She graduated with an associate degree in Liberal Arts and Sciences with highest honors. She obtained her B.S. degree in Chemical Engineerin in 2002 from Clarkson University and her M.S. in Civil Engineering from the University of Maine in 2005. She is now a Ph.D. candidate at the University of Maine.