LAKE SUPERIOR
STATE UNIVERSITY

December 1, 2015

Britton Ranson Olson, PhD
School of Biological Sciences
Lake Superior State University

Dear Dr. Ranson Olsen,

I am pleased to inform you that the Sabbatical Committee will be recommending to the Provost that you be granted two semesters of sabbatical release for the academic 2016-17 year. The committee was impressed with the content of the proposed research as well as the potential positive impact of your studies for expanded learning opportunities for Lake State students.

Sincerely,

Ron Hutchins, PhDc

Academic Dean/Co-Chair of 2015 Sabbatical Committee

cc. Maurice Walworth, Provost and VP for Academic Affairs

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LAKE SUPERIOR STATE UNIVERSITY

OFFICE OF THE PROVOST

APPLICATION FOR SABBATICAL LEAVE

(Refer to Section 15.4 of the Faculty Association Agreement)

I. Name Britton Ranson Olson Date Nov 13, 2015

Department Biology Ext. No. 2157

Home Address [redacted] Home Phone [redacted]

II. Application for leave during the following (*indicate semester and/or year*):

☐ Fall ☐ Spring ☒ Full Year

III. Number of years of faculty service (*minimum of 5 years required*) 7

IV. Year your tenure at LSSU was earned (*tenure required*) 2012

V. Semester or year of last sabbatical (*if applicable*)
(*minimum of 5 years since last sabbatical required*) —

VI. I agree to return to the University and to provide a complete written report (electronic) to the Provost upon the completion of my sabbatical semester(s) as denoted in section 15.4 of the Faculty Associate Contract.

Britton Ranson Olson 11/13/15

Signature of Faculty Applicant Date

Provost Office

NOV 13

VII. Signature of your Dean indicating his/her awareness of the application:

[signature] 11/15/15

Signature of Dean Date

Lake Superior State University

VIII. Attachments:

a. Title and Description of Sabbatical Project (Required and described on the next page)
b. Support Documents (Optional but strongly suggested)
c. Curriculum Vitae (Required)

Title and Description of Sabbatical Project.

Provide a document that describes your proposed sabbatical activities. The document should include at a minimum the following components:

- *Project Abstract/Executive Summary:* A summary of the sabbatical project and outcome (150 word maximum).
- *Project Description: A detailed description of the sabbatical project with the following sections:*
  - *Introduction:* Provide an introduction to the topic/field of study.
  - *Background:* Provide information regarding previous work/activities related to the project.
  - *Outcome:* Describe the work to be completed and state the specific outcome(s) of the project. This section must address at least one of the following.
    i. The strength of the relationship between the sabbatical leave proposal involving applied or theoretical research related to professional activities and the advancement of knowledge within disciplinary areas.
    ii. The strength of the relationship between the sabbatical leave proposal involving an external, professionally-related experience/study in a business, industrial, health care, scientific or educational setting and the improvement of instructional/professional activities at the University.
    iii. The strength of the relationship between the sabbatical leave proposal involving travel or advanced study and its yield in improving the quality of instruction at the University.
  - *Timeline:* Provide a timeline for the proposed project activities.

LAKE SUPERIOR STATE UNIVERSITY

Application for Sabbatical Leave **November 13, 2015**

Britton Ranson Olson, Biology

Project Abstract

My research interests are directed towards understanding the toxicity mechanisms of perfluorinated chemicals (PFCs). As part of this research, bacterial strains exhibiting varying sensitivities to PFCs have been identified and characterized, thereby allowing for genetic and phenotypic comparisons to better understand how exposure to PFCs affects cellular processes and what imparts tolerance. The first part of this project would be directed towards the development and refinement of a PFC transport assay for the goal of understanding how these chemicals interact with the cells and whether they can be moved across the cell wall. The second part of the project would consist of creating a proposal to submit to the National Science Foundation (NSF) Research in Undergraduate Institutions (RUI) program. This proposal would consist of the development of a cutting edge gene-editing technology that our students would have the very unique experience to work with as undergrads.

Project Description

Introduction

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) are manmade, biologically recalcitrant chemicals, which are considered ubiquitous contaminants of the environment. Documented effects of PFOA and PFOS include growth inhibition and induction of oxidative stress, and so also cell membrane and DNA damage in a variety of eukaryotic and prokaryotic organisms that carry out different energy metabolisms (1, 2, 3, 4, 5, 6, 8). Although data have linked PFCs to these adverse effects, the molecular mechanisms of these or any other toxic consequences of exposure are not fully resolved. We have identified bacterial models useful for investigating PFOA and PFOS effects at a cellular and molecular level. *Escherichia coli* is insensitive to both PFCs, as apparently either the chemicals cannot gain access to cellular components and/or metabolic processes affected by the chemicals, or *E. coli* lacks the targets altogether (7). The PFC-sensitive *Rhodobacter sphaeroides* 2.4.1. strain happens to grow by aerobic and anaerobic energy metabolisms, including photosynthesis, making it possible to compare toxicity in both the presence and absence of oxygen and light. Further, and what

promises to be particularly useful towards understanding how PFOS and PFOA toxicity mechanisms might differ, spontaneously arising *R. sphaeroides* mutants have been isolated that display increased tolerance to both PFCs (7). That one such mutant has an increased capacity to process or potentially limit cellular uptake of the chemical and avoid toxicity is another advantageous tool for us to investigate.

Background

May, 2013. Aerobic and anaerobic respiration growth profiles presented at the General Meeting of the American Society for Microbiology in Denver, Colorado.[a]

> Data presented showed *Escherichia coli* K-12 was insensitive, but *R. sphaeroides* 2.4.1 was sensitive to both PFOA and PFOS. Inhibition of *R. sphaeroides* growth by PFOA was limited to aerobically metabolizing bacteria, while both aerobic and anaerobic growth were inhibited by PFOS, although inhibition increased when oxygen was available.

March, 2014. Metabolic profile effects presented at the Michigan branch of the American Society for Microbiology, Davenport University.[b]

> Data demonstrate a change in the enzyme activity of *R. sphaeroides* for both carbon and phosphorus cycling based on increased peroxidase, phenol oxidase, and acid and alkaline phosphatase activity. As oxidative stress is suspected to be induced by PFCs, peroxidase activity of *R. sphaeroides* was measured and compared to PFC-growth insensitive *Escherichia coli*. It was shown that the peroxidase activity in *R. sphaeroides* increased in the presence of the PFCs, while it decreased in the *E. coli* strain, and these affects were more pronounced with PFOS than they were with PFOA.

July, 2015. Hosted visiting researchers from BGSU, including my collaborator and 2012-2014 NSF program director, Dr. Jill Zeilstra-Ryalls. During this visit we completed the *R. sphaeroides* photosynthesis growth profiles.

October, 2015. Submitted proposal for sequencing spontaneous mutants displaying different sensitivities to PFCs to the Department of Energy Joint Genome Institute.[c]

> We propose to generate draft genome sequences of both wild type and mutant *R. sphaeroides* strains in order to identify gene products responsible for sensitivity of this organism to the

anthropogenic contaminants perfluorinated carbon compounds. The benefits of having this information are (1) the targets can guide us as to strategies for counteracting the harmful effects of these chemicals, and (2) it will make it feasible to undertake the development of a bacterial tool for transformation or remediation of PFCs.

[a, b, c] Supporting documents 'Identification and Application of a Bacterial Model for Toxicity Studies of PFOA and PFOS', 'Effects of PFOA and PFOS on *Rhodobacter sphaeroides* Enzyme Activity ', and 'Developing *Rhodobacter sphaeroides* as a model for investigating toxicity of perfluorinated chemicals', respectively.

Outcomes

Development of a Transport Assay

The development of a PFC transport assay is critical for us to better understand the implication of cell uptake of PFOA and PFOS. Having this test will allow us to determine whether the cells are able to transport the chemicals intracellularly, whether they integrate them into their cell membrane, or perhaps if they can lessen their toxicity via biotransformation. At this point, this data is the 'missing link' in our research and would be extremely useful in describing the various strains we have isolated and profiled. I will present the results of these studies at the 2017 General Meeting for the American Society of Microbiology.

The NSF RUI Proposal

The goal of the RUI submission is to provide funding to predominantly undergraduate institutions for the support and encouragement of research-based learning environments. This proposal would be a collaborative effort between Lake Superior State University and Bowling Green State University (collaborative efforts are strongly supported by the program, and much of the preliminary data described here resulted from my collaboration with BGSU) in which LSSU, and thus my efforts as a co-PI, would be directed towards the development of a clustered regularly interspaced short palindromic repeats (CRISPR)/Cas system, applicable to prokaryotic studies. The CRISPR/Cas system is a new and very powerful approach to carry out targeted gene editing (9). As part of the RUI proposal, LSSU would propose to implement the development of this, a prokaryotic system, in the cell model we have characterized for our PFC studies (!!!!). The development and utilization of this technology would be

very beneficial to our science majors and could be integrated into courses in the area of genetics, toxicology, biochemistry, cell and molecular biology, as well as senior project. It would bring funding to the classroom and labs, as well as a very unique opportunity for our undergrads to work with such cutting-edge applications.

Timeline

Fall 2016 semester

1. Develop and optimize the PFC transport assay.

2. Test the wildtype *E. coli* and *R. sphaeroides* strains along with the *Rhodobacter* mutants that show less sensitivity to growth with the PFCs under aerobic, anaerobic, and photosynthetic growth conditions.

Spring 2017 semester

1. Identify genetic targets, PCR primers, and appropriate vectors for delivering the CRISPR/cas system into our cell model.

2. Prepare the NSF RUI proposal, including a 1-2 week visit to the BGSU campus where myself and my collaborator, Dr. Jill Zeilstra Ryalls, a 2012-2014 NSF program director, will finalize our submission.

3. Present the results of the PFC transport data at the 2017 General Meeting for the American Society for Microbiology.

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Paper # 1041

Identification and Application of a Bacterial Model for Toxicity Studies of Perflourooctanoic Acid and Perflourooctane Sulfonic Acid

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ABSTRACT

BACKGROUND: Perflourinated compounds (PFCs) are anthropogenic contaminants found throughout the environment. Although not fully resolved, PFC exposure is reported to cause oxidative stress and, consequently, DNA and cell membrane damage. The goal of this work was to develop and use a bacterial model to examine mechanisms of toxicity of two of the most documented PFCs, perflouroctanoic acid (PFOA) and perflourooctane sulfonic acid (PFOS), at the cellular and molecular level.

MATERIALS AND METHODS: Bacteria were screened for sensitivity to PFOA and PFOS using a zone of inhibition assay. Growth rates of sensitive bacteria were compared in the presence and absence of the PFCs, under aerobic and anaerobic conditions. Mutants having altered sensitivities were either engineered or selected for, and evaluated for their ability to generate the reactive oxygen species (ROS), hydrogen peroxide.

RESULTS: *Escherichia coli* K-12 was insensitive, but *Rhodobacter sphaeroides* 2.4.1 was sensitive to both PFOA and PFOS. Inhibition of *R. sphaeroides* growth by PFOA was limited to aerobically metabolizing bacteria, while both aerobic and anaerobic growth were inhibited by PFOS, although inhibition increased when oxygen was available. We found that mutants with electron transport chain (ETC) defects were less sensitive to PFOS, while growth inhibition by PFOA was unaltered, suggesting the existence of at least one additional toxicity target for the chemicals. We also isolated spontaneous mutants having reduced sensitivities to both PFOA and PFOS, supporting the idea that there are additional cellular targets for PFC toxicity. This was further supported by the fact that, relative to wild type, while hydrogen peroxide production was diminished in the ETC mutants, it was augmented in the spontaneous mutant.

DISCUSSION: We have identified a bacterial model for examining PFC sensitivity. Using this model, it has already been possible to determine that (1) mutants having reduced capacities to generate ROS have improved tolerance towards PFOS but not PFOA, and (2) PFCs appear to have multiple mechanisms of toxicity. Equally useful for studies to understand cellular and molecular targets of these chemicals, we have identified a bacterial model that is reduced in sensitivity to both PFOS and PFOA, which will allow us to explore tolerance mechanisms.

INTRODUCTION

Perflouroctanoic acid (PFOA) and perflouroctane sulfonic acid (PFOS) are manmade chemicals that are used in surfactant and fireproofing technologies, and as non-stick surface coatings in stain resistant, grease-proofing, and cosmetic applications. Their usefulness stems in part from their inherent resistance to degradation, which means that they are also bioaccumulative by nature. Once they became recognized as ubiquitous environmental contaminants, efforts were made to reduce their use. However, several industrial applications are exempt from disuse ordinances and their production continues. This means that their environmental burden persists and the effects of PFOA and PFOS exposure remain a relevant issue.

Little is known regarding the cellular effects of PFCs, but both PFOA and PFOS have been implicated as inducers of oxidative stress (1-7). Here, we report the identification of a bacterial model, *Rhodobacter sphaeroides*, for investigating those and other potentially harmful consequences. Using this model, we were able to demonstrate that there are both oxygen-dependent and also oxygen-independent adverse effects of exposure to PFCs. In addition, *E. coli* was identified as a model in which to explore mechanisms of PFC tolerance, since the bacterium appears to be completely insensitive to both PFOA and PFOS.

MATERIALS AND METHODS

Strains and growth studies. Bacterial strains and media used were as indicated. Conditions with respect to oxygen availability were manipulated by shaking liquid cultures slowly (50 rpm) for low oxygen or vigorously (150 rpm) for high oxygen growth. Anaerobic conditions were achieved by growing the cells in completely filled screw-capped tubes, and supplementing them with yeast extract (0.1%, w/v) and dimethyl sulfoxide (DMSO; 0.06M) as an alternate electron acceptor. Optical densities were measured at 660 nm.

Mutant strain construction. A DNA fragment of the *ctaC* gene encoding a subunit of the aa_3-type cytochrome *c* oxidase of *R. sphaeroides* (8), was inserted into the pKNOCK-Kn suicide vector. After mobilizing the plasmid into *R. sphaeroides*, recombinants were selected for on medium with Kn. The integrity of mutant candidates was confirmed by PCR and sequencing of the products.

Hydrogen peroxide assay. Net amounts of hydrogen peroxide produced by *R. sphaeroides* were measured for cells cultured under low oxygen conditions and harvested at an OD (660 nm) of 0.17-0.19; each reaction was performed in triplicate. Resting cells were provided with 1 mM malate and 2.5 mM pyruvate as substrates for 20 minutes and the net amount of hydrogen peroxide present was determined by measuring the fluorescence emitted from the hydrogen peroxide-dependent dimerization of homovanillic acid catalyzed by horseradish peroxidase, as described previously (9).

RESULTS

PART 1: *R. sphaeroides* is sensitive to PFOS and PFOA but *E. coli* is not.

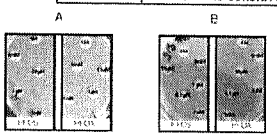

FIGURE 1. Zone of inhibition tests with PFOS or PFOA (1M colloidal slurry PFOS). Shown are: panel A, *E. coli* K12 on minimal M63 medium and panel B, *R. sphaeroides* on Sistrom's succinate minimal medium (10).

PART 2: Oxygen is a factor with respect to PFC toxicity effects, but PFOS is toxic even in the absence of oxygen.

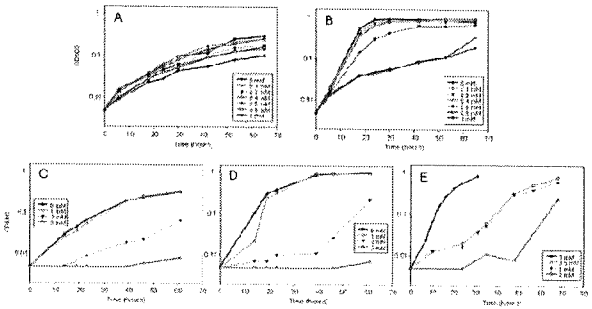

FIGURE 2. Growth of *R. sphaeroides* in the presence and absence of varying amounts of PFCs. Panels are: A, anaerobic dark growth with PFOA; B, low oxygen growth with PFOA; C, anaerobic dark growth with PFOS; D, low oxygen growth with PFOS; and E, high oxygen growth with PFOS.

PART 3: Electron transport chain mutants have higher tolerances towards PFOS but not PFOA.

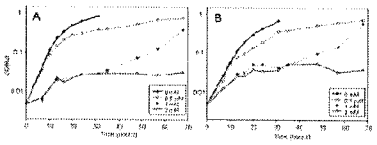

FIGURE 3. Growth of *R. sphaeroides* electron transport chain mutants (compare to the wild type, Fig. 2E) . Bacteria were grown under high oxygen conditions in varying amounts of PFOS. Panels are: (A) growth of an aa_3-type cytochrome *c* oxidase null mutant, (B) growth of an cbb_3-type cytochrome *c* oxidase null mutant.

PART 4: Unlike electron transport chain mutants, spontaneous mutants isolated in the presence of PFOA are less sensitive than the wild type to both PFCs

Table 1. Sensitivities to PFOS and PFOA of *R. sphaeroides* mutants relative to the wild type.

Bacterial strain	Diameter of the zone of inhibition (in mm)*	
	PFOS	PFOA
Wild type 2.4.1	16	50
Mutant isolate I	10	36
Mutant isolate II	11	36
Mutant isolate III	11	36
Mutant isolate IV	10	34

*Disk diameter is 8 mm.

PART 5: Varying levels of net H_2O_2 production among different strains of *R. sphaeroides* suggests there are multiple mechanisms of PFOS toxicity.

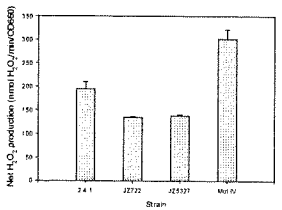

FIGURE 4. Net H_2O_2 production detected when semi-aerobically grown *R. sphaeroides* are provided with malate and pyruvate (see also Table 1.)

DISCUSSION

R. sphaeroides is sensitive to PFOA and PFOS, while *E. coli* K12 is not. Therefore, these bacteria are useful prokaryotic models for studying mechanisms of PFC toxicity on the one hand and PFC tolerance on the other. We have already exploited the sensitive model to examine the role of oxygen in PFC toxicity. We found that, while oxygen is required for PFOA-associated growth inhibition, growth inhibition by PFOS persists even in the absence of oxygen. Thus, while oxidative stress may be one component of PFC toxicity, there are also oxygen-independent mechanisms as well.

Though electron transport chain mutants have increased tolerance to PFOS, they remain sensitive to PFOA, despite their reduced hydrogen peroxide production capacities. This, along with differences between PFC sensitivities of these mutants *versus* those of spontaneous mutants isolated in the presence of PFOA, again suggests that more than one toxicity pathway exists, and that PFOA and PFOS affect the cells differently. Having established a PFC sensitive model in *R. sphaeroides* and an insensitive model in *E. coli* K12, we are in the position to further identify the cellular and molecular targets of PFOA and PFOS toxicity and resistance.

REFERENCES

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Effects of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonic Acid (PFOS) on *Rhodobacter sphaeroides* Enzyme Activity

Erin Mulroney (UG) and
Dr. Britton Ranson-Olson
LAKE SUPERIOR STATE UNIVERSITY
Sault Ste. Marie, MI

Abstract

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) are man-made environmental contaminants that have been identified in numerous biological sampling projects, and that these perfluorinated compounds (PFCs) are still produced overseas, there is a need to investigate the potential effects that these chemicals have on a cellular level. *Rhodobacter sphaeroides* is a good model to demonstrate such effects because it has been shown to be sensitive to both PFOA and PFOS and it possesses similar metabolic processes to those found in eukaryotic mitochondria. The results of this study demonstrate a change in the enzyme activity of *R. sphaeroides* for both the carbon and phosphorus cycling based on increased peroxidase, phenol oxidase, and acid and alkaline phosphatase activity. As oxidative stress is suspected to be induced by PFCs, peroxidase activity of *R. sphaeroides* was measured and compared to PFC-growth insensitive *Escherichia coli*. It was shown that the peroxidase activity in *R. sphaeroides* increased in the presence of the PFOS, while it did not change significantly with PFOA. It is unknown why *E. coli* is insensitive to PFC, and so to investigate whether biotransformation might be a possible mechanism, the peroxidase activity was measured in a *R. sphaeroides* strain grown in the supernatant of *E. coli* cultures pre-incubated in PFC. It was shown that activity was lowered in cultures grown with the supernatant containing PFOA, suggesting a possible method for reducing the harmful effects of this PFC. Overall, this study supports oxidative stress as a potential cellular effect of PFOS, while PFOA mechanisms remain unknown.

Introduction

Perfluorooctanoic acid (PFOA) and perfluorooctane sulfonic acid (PFOS) do not occur naturally in the environment, but have been manufactured for use in consumer products, including water- and stain-repellent surfaces, adhesives, nonstick coatings and lubricants [1]. As a result of their multiple uses and extreme persistence [2], these man-made perfluorinated compounds (PFCs) are ubiquitous contaminants of humans and wildlife [1]. Previous experiments performed have generally found adverse effects to the liver and kidney [3], and to the reproductive [4], nervous [5] and immune systems [6] with exposure to PFOA or PFOS, yet the their effects at the cellular level remain poorly understood.

One potential mechanism, oxidative stress, is suspected to be induced by PFCs, resulting in the production of reactive oxygen species (ROS) and cell membrane and DNA damage [1,7]. This can be induced through impairment of mitochondrial functions, such as the electron transport chain. Eukaryotic mitochondria share an evolutionary association with the α-proteobacteria *Rhodobacter sphaeroides*, and effects measured in this bacterium may emulate those occurring in the eukaryotic cell [8]. *R. sphaeroides* 2.4.1 has been shown to be growth sensitive to both PFOA and PFOS, whereas *Escherichia coli* does not demonstrate sensitivity to either PFC [7]. Due to these sensitivities and similarities, *R. sphaeroides* has been identified as a useful model for representing PFOA and PFOS effects on a eukaryotic cell [7].

The potential for PFCs to induce changes in cellular metabolism was assessed by measuring the enzymatic activity of phenol oxidase, peroxidase and acid and alkaline phosphatase for *R. sphaeroides* treated with PFOS and PFOA. Because previous studies observed an increase in ROS formation when cells are exposed to PFCs [7], it is hypothesized that changes in peroxidase activity will be demonstrated by this study's assays.

Objectives

1) To measure the enzyme activity of carbon (phenol oxidase and peroxidase) and phosphorus cycling (phosphatase) in order to detect metabolic changes when *R. sphaeroides* 2.4.1 is treated with PFOA or PFOS. 2) To compare the peroxidase activity of *R. sphaeroides* with PFCs and the insensitive bacterial model *E. coli*. 3) To explore the mechanism which allows *E. coli* to be insensitive to PFOA and PFOS.

Methods

Strains and Growth Studies

R. sphaeroides strains were grown in liquid Sistrom's succinate minimal medium (SIS) at 28°C and the *E. coli* strain was grown in liquid Luria-Bertani (LB) at 37°C. All strains were aerated in a shaking water bath. JZ1968 was selected for the addition of 50 g/ml of spectinomycin and 50 g/ml of streptomycin.

Treatment with Supernatant and PFCs

E. coli and JZ1968 cultures were treated with PFOA or PFOS for 24 hours in the same growth conditions followed by centrifugation for 20 minutes at 7000 rpm. 5 ml of each supernatant was transferred to a separate test tube, to which 0.5 ml of previously untreated JZ1968 was added. These were shaken at 28°C for 24 hours followed by an A_{660} measurement.

Enzyme Activity Testing

Four enzymatic assays were performed based on previously described methods with some modifications [9, 10]. All tests were performed in triplicate. A negative control was included that did not receive culture, nor treatment with PFCs. All tests were performed on cultures with an optical density range of 0.05-1.245.

Phenol Oxidase Activity Assessment:
-250 µl of culture
-975 µl of a 50 mM acetate buffer, pH 5
-25 µl of L-3,4-dihydroxyphenylalanine (L-DOPA) substrate
-Incubated at 28°C for one hour
-Centrifuged at 10,000 rpm for 3 minutes
-Absorbance of supernatant measured at 460 nm (measure of dopachrome product)

Peroxidase Activity Assessment:
-Same as phenol oxidase procedure, with the exception of adding 50 µl of hydrogen peroxide

Acid Phosphatase Activity Assessment:
-250 µl of culture
-750 µl of phosphatase substrate in 50 mM acetate buffer, pH 5
-Shaken at 28°C for 5 minutes
-Centrifuged at 10,000 rpm for 3 minutes
-Supernatant received 100 µl of 1 M NaOH treatment to stop the reaction
-Absorbance of supernatant measured at 405 nm

Alkaline Phosphatase Activity Assessment:
-Same as acid phosphatase, with the exception of substituting acetate buffer for 1 M TRIS buffer, pH 8

Results

Table 1. Enzyme activity (µM/million bacteria/hour) of *R. sphaeroides* 2.4.1 with PFOA treatment.

Concentration of PFOA	No PFOA	1 nM	1 µM	1 mM
Phenol Oxidase Activity	2.926921	3.609220	3.480376	8.956073
Peroxidase Activity	0.6399011	0.8159745	0.8016821	0.527977
Acid Phosphatase Activity	0.667402	0.620164	0.556233	0.3609
Alkaline Phosphatase Activity	0.128108	0.048017	0.056914	0.172023

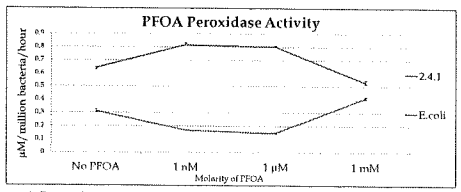


Figure 1. Peroxidase activity of *R. sphaeroides* 2.4.1 and *E. coli* with PFOA treatment.

Table 2. Enzyme activity (µM/million bacteria/hour) of *Rhodobacter sphaeroides* 2.4.1 with PFOS treatment.

Concentration of PFOS	No PFOS	1 nM	1 µM	1 mM
Phenol Oxidase Activity	0.135575	0.205177	0.223018	0.538544
Peroxidase Activity	0.545036	0.661223	0.639183	1.221733
Acid Phosphatase Activity	2.663642	0.638154	3.611544	12.83509
Alkaline Phosphatase Activity	0.0899	0.254934	0.117772	0.29559

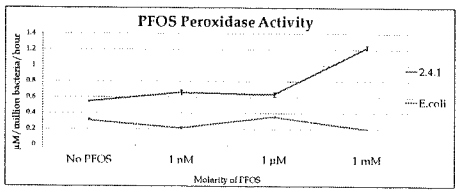


Figure 2. Peroxidase activity of *R. sphaeroides* 2.4.1 and *E. coli* with PFOS treatment.

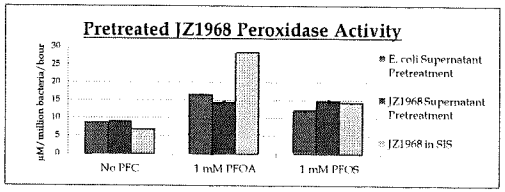
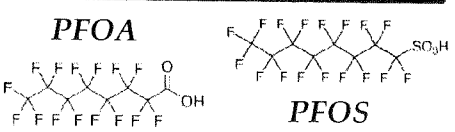


Figure 3. Peroxidase activity of *R. sphaeroides* JZ1968 treated with PFC and *R. sphaeroides* JZ1968 treated with supernatant previously incubated with PFC and *R. sphaeroides* JZ1968 or *E. coli*.



Conclusions

The results of this study demonstrate a change in the enzyme activity of *R. sphaeroides* for both the carbon and phosphorus cycling. When treated with PFOA or PFOS, the enzyme activity of the bacterial model demonstrates increased phenol oxidase, peroxidase, and acid and alkaline phosphatase activity. This establishes that not only growth of *R. sphaeroides* is affected by PFC exposure, but also functionality of the cell. This relationship shows greater effects with PFOS treatment than PFOA, suggesting different mechanisms for toxicity.

Peroxidase activity is linked to hydrogen peroxide production and usage, and the formation of ROS [11]; therefore, PFCs could pose a health risk via changes to redox regulation. As PFOS treatment seemingly induced enzyme activity while PFOA did not, these data support a potential role for oxidative stress for one of the PFCs, but again suggest that PFOS and PFOA impact the cells in different ways.

Finally, this study also demonstrated a potential method of reducing the toxicity effects of PFOA, since pretreatment with both the *R. sphaeroides* and *E. coli* strains produced peroxidase activity significantly less in JZ1968 than without pretreatment. Perhaps the cells can modify the PFOA chemically, or integrate it into their cell wall. What ever the case, more exploration into this subject is needed but there remains the potential for bioremediation applications.

Acknowledgements

Thank you to The LSSU Undergraduate Research Committee for funding. The approval of the Dr. Barb Keller and Dr. Nancy Kirkpatrick made this independent study possible. The help of Ben Southwell, for the use of the LSSU Environmental Research Lab facilities, and Mary St. Antoine is also appreciated.

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Developing *Rhodobacter sphaeroides* as a model for investigating toxicity of perfluorinated chemicals.

DOE – Joint Genome Institute Small Scale Microbial Proposal

DESCRIPTION

We propose to generate draft genome sequences of both wild type and mutant *Rhodobacter sphaeroides* strains in order to identify gene products responsible for sensitivity of this organism to the anthropogenic contaminants perfluorinated carbon compounds. Based on the median total length of previously sequenced strains of *R. sphaeroides*, the genome sizes will be approximately 4.6 Mb (median GC% is 68.8%).

JUSTIFICATION

Perfluorinated carbon compounds (PFCs) are very resistant to degradation, and so bioaccumulative. The surface tension-reducing properties of PFCs have made them useful in a wide variety of commercial applications. Their use has been such that they are now detectable throughout our industrialized and also natural environments. Though some nations have committed to reducing PFC production, many applications, including photography and imaging, electronics, metal plating and finishing, remain exempt. The properties of PFCs are also very well suited for use in proton exchange membrane fuel cells. These current and developing applications justifies studies to understand their impact and to develop ways to reduce that impact.

While harmful organismal effects (especially animals) resulting from PFC exposure are documented in the literature, the toxicity mechanisms have not been fully resolved. Descriptions of the adverse impact of PFCs on microorganisms of any sort are limited. It is our goal to address how these chemicals affect organisms on the cellular and molecular level. We sought to identify a bacterial model that would provide all the advantages of such microorganisms (e.g. rapid genetic testing, existence as a single-celled organism which eliminates exogenous components such as sex hormones and so simplifies in vivo exploration of toxicity mechanisms). Some of the literature suggested that an important intracellular target of PFCs is the mitochondrion, which predicts that an alphaproteobacterium would be a good choice. Therefore, we investigated the effect of two of the most prevalent and highly monitored PFCs, PFOA and PFOS on growth of *R. sphaeroides*, which is regarded as a close free-living relative of mitochondria. Since PFOA and PFOS have been found in both animals and plants, *R. sphaeroides* is a particularly useful bacterial model as its ability to grow both chemotrophically in the presence and absence of oxygen, and phototrophically (anaerobically) make it possible to explore the role of oxygen and light with respect to PFC toxicity. We found that *R. sphaeroides* wild type strain 2.4.1 is sensitive to both chemicals but to different degrees, and their impact on growth varies according to the growth conditions (Figure 1).

To begin to explore the role of oxygen with respect to the inhibition of growth, we analyzed two *R. sphaeroides* 2.4.1 cytochrome *c* oxidase mutants in which one or the other of the two terminal oxidases is absent. These are the low oxygen affinity mitochondrial-like aa_3-type cytochrome *c* oxidase and the high oxygen affinity cbb_3-type cytochrome *c* oxidase. We found that cells lacking cbb_3-type cytochrome *c* oxidase are less sensitive to PFCs, but the aa_3-type cytochrome *c* oxidase mutant does not differ from wild type. This result suggests that one mechanism of PFC toxicity involves the respiratory chain, which is consistent with reports in the literature that attribute PFOA and PFOS toxicity in part to their action as oxidative stress inducers. However, this leaves unexplained sensitivity observed under anaerobic-dark or light conditions, which predicts there are additional targets for PFCs. Toward identifying PFC targets, we have taken two approaches: (1) We evaluated sensitivities among wild type strains. (2) We isolated spontaneous mutants having diminished sensitivities that arose in populations grown under different conditions (all are derived from wild type strain 2.4.1). In all, including our cytochrome *c* oxidase mutants, we now have a total of 25 *R. sphaeroides* strains with varying sensitivities to the PFCs. While five wild type strains of *R. sphaeroides*, including strain 2.4.1, have previously been sequenced, we propose to resequence them, because it is known that there is variability in genome content even between different laboratory isolates.

UTILIZATION

A comparison of the draft sequences of the genomes for our set of *R. sphaeroides* strains with varying sensitivities will enable us to develop a list of gene product targets of PFC action. The benefits of having this information are (1) the targets can guide us as to strategies for counteracting the harmful effects of these chemicals, and (2) it will make it feasible to undertake the development of a bacterial tool for transformation or remediation of PFCs.

Having these *R. sphaeroides* strains in hand, together with their defined relative sensitivities, genomic sequencing is the most practical and direct means to develop a list of possible targets for PFC action. The availability of draft genome sequences of mutants having different sensitivities and also several wild type strains will reinforce each other. Thus, sensitive strains could have sequences in common, while mutant strains that are less sensitive should point to sequences that are missing or altered in

the more tolerant strains. Because we have mutants that were isolated under different growth conditions, the list of possible targets can be refined by considering sequence differences within the context of already available transcriptomic and proteomic data for *R. sphaeroides* 2.4.1 grown under different conditions (aerobically, anaerobically in the dark, phototrophically). These targets can then be confirmed using genetic tools available for this organism to achieve gene inactivation and/or complementation.

Knowing what sequence are responsible for higher tolerances will better position us to develop strategies to effectuate biotransformation and/or remediation by these or other bacteria. For example, it is important and necessary to know whether or not tolerance is solely due to an ability to exclude the PFCs from acting on (intracellular) targets, that it comes about because targets are missing or altered, or that strains are enhanced in their ability to transform the chemicals to less active species. Each of these points to a different avenue for further investigation toward developing appropriate ways to either reduce/eliminate harmful effects or to treat them.

The draft genome sequences will significantly expand our preliminary results, and so considerably strengthen a research proposal to secure funding for additional studies from appropriate agencies (e.g. the Department of Energy and the National Science Foundation). As was true of the preliminary investigations undertaken to develop a bacterial model, and to identify an appropriate set of useful strains for sequencing, these additional studies would be performed by the collaborative efforts of two investigators at two different institutions. Lake Superior State University is geographically isolated, and the smallest publically funded undergraduate university in the state of Michigan. Bowling Green State University is a research university that supports undergraduate research experiences, and also grants both Master's and Ph. D. degrees. We aim to provide research opportunities at our respective host institutions, and thereby enhance recruitment, retention, and graduation of undergraduate students in multidisciplinary studies encompassing molecular biology, bacterial genetics, biochemistry, metadata analysis, systems biology, while also supporting research and mentoring training of graduate students. Through our collaboration we can optimize the use of instruments and other resources available at each institution for present and future studies.

Draft genome sequences of more wild type strains will expand our knowledge of *R. sphaeroides* genome complexity. This was the first bacterium in which two chromosomes were identified, and the number and sequence content of the endogenous plasmids is known to vary considerably. Learning more about the genetic variability among these bacteria will inform studies of evolution, physiology, gene regulation, and applied research involving this organism.

COMMUNITY INTEREST

While the degree of risk associated with exposure remains uncertain, it is well-established that PFCs are global environmental contaminants, and the level of concern is sufficient to have prompted both state (including Minnesota, New Jersey, Ohio, and Washington) and national (Centers for Disease Control) health agencies to implement monitoring programs. Interest by the broader scientific community is demonstrated by the 230 signatures garnered for the Madrid Statement (A Blum, et al., 2015. The Madrid statement on poly- and perfluoroalkyl substances (PFASs). Environ Health Perspect 123:A107–A111), which expressed concerns about these chemicals, and urged collaboration between scientists, industry, and governments to develop a better understanding of the consequences of exposure, and to investigate ways to reduce exposure. This would not only involve curtailing production and environmental release, but also removing them from the environment.

The draft genome sequences to be generated for the strains proposed herein will address both of these issues in the following ways:

- It will provide data that can be used for identification of gene products targeted by PFCs, thereby making it possible to investigate mechanisms of toxicity. Since *R. sphaeroides* can use different energy and carbon sources, this list will be far more comprehensive than could be achieved for less metabolically versatile organisms, and also make it possible to examine the contribution of light and oxygen to PFC toxicity.
- It will provide a guide to ways and means to reduce the persistence of these chemicals in the environment. Since there is variability among strains of *R. sphaeroides*, knowing the genetic basis of that variability points the way toward developing strategies to eliminate these chemicals, or by means of bioconversion, transform them into less persistent species.
- It will generate a foundational model for testing other species of PFCs.

DOE MISSION

This project addresses the DOE JGI mission in biogeochemistry, as it will generate useable genome sequence information to learn cellular and molecular targets of ubiquitous anthropogenic environmental contaminants. On the one hand, this will enable a more complete understanding of their biological impacts and thereby make feasible studies to ameliorate adverse effects, and on the other hand it will inform us as to how to approach the development of bacterial systems that can be used to

transform or eliminate these pollutants. Certainly implicit in this mission is to improve understanding and contribute to education of students and the population in general in science, since this will help ensure both support of, and new investigators for such future studies. The Ranson-Olson and Zeilstra-Ryalls labs have decades of experience working with *R. sphaeroides*, the organism of interest here. In addition, through their collaborative efforts they have isolated and characterized the collection of strains to be sequenced, demonstrating their ability to work together to advance this project. The Zeilstra-Ryalls lab also has experience in comparative genomics of purple bacteria such as *R. sphaeroides*, with ongoing projects focused on other useful characteristics of these organisms that address their application to remediation of wastewaters and to alternative, sustainable fuel development.

SAMPLE PREPARATION

Since all of the strains to be sequenced are already available, genomic DNA will be available within 1-2 weeks after receiving notice of acceptance of this proposal. Protocols for preparing high quality DNA of sufficient quantity are well-established in the Zeilstra-Ryalls lab. Such samples are generated routinely, as they are used for DNA templates in PCR to amplify genomic sequences for cloning, and to confirm the structure of engineered mutant strains. The samples have also proven suitable for high-throughput sequencing, as they have been used successfully for other strains of *R. sphaeroides*, as well as other related (purple) bacteria.

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EDUCATION

PhD Biomedical Sciences, Health and Environmental Chemistry, Oakland University, 2007.

Dissertation: *In vitro* and *in vivo* transcription studies of the *hemA* gene of *Rhodobacter sphaeroides* 2.4.1.

MS Biological Sciences, Michigan Technological University, 2001.

Thesis: Environmentally Mediated Intellectual Manifestations.

BS Biological Sciences, Lake Superior State University, 1999.

Thesis: Which came first, the prolactin or the caregiving?

RESEARCH INTERESTS

Gene expression and environmental regulatory effects, protein and DNA interactions

RESEARCH & TEACHING POSITIONS

Associate Professor. 2012-present. Lake Superior State University.
Assistant Professor. 2007-2012. Lake Superior State University.
Graduate Research Associate. 2003-2007. Oakland University.
Graduate Research Associate. 2001-2003. Wayne State University School of Medicine.
Graduate Teaching Assistant. 1999-2001. Michigan Technological University.

TEACHING EXPERIENCE

Lake Superior State University
- Human Anatomy and Physiology I & II lecture and lab coordinator
- Freshmen Seminar
- Clinical Microbiology
- Sophomore Seminar
- Histology
- Current Topics in Molecular Biology
- Advanced Cell and Molecular Biology
- Senior Project

Oakland University
- Microbiology Labs

Michigan Technological University
- Hematology
- Clinical Lab Science
- Mycology

Sault Area Public Schools
- Substitute Teacher – K-12
- Water Safety Instructor – swimming, CPR, lifeguard certification lessons
- Summer Camp Instructor
- High School Mathematics Tutor

PUBLICATIONS & PRESENTATIONS

Mulroney E, and B Ranson-Olson. 2015. Toxicity Mechanisms of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonic Acid (PFOS) on *Rhodobacter sphaeroides*. Michigan branch of the American Society for Microbiology, Eastern Michigan University.

Mulroney E*, and B Ranson-Olson. 2014. Effects of Perfluorooctanoic Acid (PFOA) and Perfluorooctane Sulfonic Acid (PFOS) on *Rhodobacter sphaeroides* Enzyme Activity. Michigan branch of the American Society for Microbiology, Davenport University.
*winner best undergraduate poster presentation

Ranson-Olson B, and J Zeilstra-Ryalls. 2013. Identification and application of a bacterial model for toxicity studies of perflourooctanoic acid and perflourooctane sulfonic Acid. 113th General Meeting American Society for Microbiology, Denver, CO.

Ranson-Olson B, Zeilstra-Ryalls JH. 2008. Regulation of the *Rhodobacter sphaeroides* 2.4.1 *hemA* gene by PrrA and FnrL. Journal of Bacteriology. 190(20):6769-6778.

Ranson-Olson B, Jones D, Donohue T, and J Zeilstra-Ryalls. 2006. *In vitro* and *in vivo* analysis of the role of PrrA in *Rhodobacter sphaeroides* 2.4.1. *hemA* gene expression. Journal of Bacteriology. 188(9):3208-18.

Ranson-Olson B, and J Zeilstra-Ryalls. 2006. Resolving the roles of FnrL and PrrA in transcription of the *Rhodobacter sphaeroides* 2.4.1 *hemA* gene. 106th General Meeting American Society for Microbiology, Orlando, FL.

Ranson B, and J Zeilstra-Ryalls. 2005. New Insights into *Rhodobacter sphaeroides 2.4.1* Expression: Regulation by PrrA. 105th General Meeting American Society for Microbiology, Atlanta, GA.

GRANTS, MONIES, and HONORS AWARDED

Excellence in Academic Advising Award. Lake Superior State University. 2015

Distinguished Teacher Award Nominee. Lake Superior State University. 2011, 2013, 2014, 2015

Funding Innovations in Teaching Award. Lake Superior State University. $639. 2014

National Association of Advisors for the Health Professions travel grant. $1000. 2014

Golden Anchor Award for faculty making a difference in student's lives. Lake Superior State University. 2009 & 2013

Modulus Single Tube Luminometer Instrument Grant. Turner Biosystems. Principle Investigator. $9,500. 2009

Issues and Intellect: facility fees as LSSU hosts the American Society for Microbiology Michigan (ASM) branch conference entitled 'Microbiology of the Great Lakes'. Lake Superior State University. $400. 2009

Student Travel Grant. Oakland University. 2006

Corporate Activities Program Student Travel Grant. ASM. 2005

Graduate Research Associate Grant. Wayne State University School of Medicine, Department of Pathology. 2001-2003

Board of Trustees Academic Achievement Scholarship. Lake Superior State University. 1994- 1999

AFFILIATIONS

Michigan Branch American Society for Microbiology Board Member-at-large
American Society for Microbiology member
National Association of Advisors for the Health Professions member
Central Association of Advisors for the Health Professions member

PROFESSIONAL SERVICE

Advisor. LSSU Health professions co-advisor (2010-present), Mid Michigan Community College Radiology Program advisor (2011-present), allied health advisor (2008-present).
Developed 'Professional Entrance Exams & Test Taking Strategies' seminar series, offered to LSSU pre-health students (2012, 2013).

Board Member-at-large Michigan branch ASM. Coordinator/host of the MI ASM Fall 2009 Conference 'Microbiology of the Great Lakes', oral presentation judge (2008), poster presentation judge (2009, 2010, 2012).

Outreach. Implemented ASM K-12 Education and Outreach funds to the LSSU Upward Bound Program, Sault Area High School, Algoma District schools, and Brimley Community College (2009), LSSU Biomedical Camp (2008-2012).

University committees. University Policies and Procedures (2011-present), HS-IRB (2010-2013), biology, geology, and nursing faculty search (2008, 2009, 2011, 2015).

Department committees. Pre-professional advisory committee (Chair 2013-present, member 2010-2013), website (2010-present), biomedical program development (2012-2014), medical laboratory science (2011), research seminar (2008-present), alumni (2008-2012), and microscope (2009-present).