

**THE UNIVERSITY OF WESTERN ONTARIO  
 BIOHAZARDOUS AGENTS REGISTRY FORM**  
 Approved Biohazards Subcommittee: September 25, 2009  
 Biosafety Website: [www.uwo.ca/humanresources/biosafety/](http://www.uwo.ca/humanresources/biosafety/)

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biohazards being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1<sup>st</sup> edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or [biosafety@uwo.ca](mailto:biosafety@uwo.ca). If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: [www.uwo.ca/humanresources/biosafety/](http://www.uwo.ca/humanresources/biosafety/)

PRINCIPAL INVESTIGATOR

Leo Lau

SIGNATURE



DEPARTMENT

Surface Science Western

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PHONE NUMBER

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Location of experimental work to be carried out: Building(s) MSA Room(s) M00230

\*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 12.0, Approvals).

FUNDING AGENCY/AGENCIES:

NSERC Discovery Grant with

GRANT TITLE(S):

external funding from J. Craig Venter Institute to support collaboration

**PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED. A GRANT SUMMARY PAGE MAYBE ADEQUATE IF IT PROVIDES SUFFICIENT DETAIL ABOUT EACH BIOHAZARD USED.**

Names of all personnel working under Principal Investigators supervision in this location:

Kar Man Leung (kleung@uwo.ca)

## 1.0 Microorganisms

1.1 Does your work involve the use of biological agents?  YES  NO  
 (including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA?  YES  NO

If YES, please give the name of the species. \_\_\_\_\_

What is the origin of the microorganism(s)? \_\_\_\_\_

Please describe the risk (if any) of escape and how this will be mitigated:

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Please attach the CFIA permit.

Please describe any CFIA permit conditions:

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1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/Supplier	PHAC or CFIA Containment Level
<i>Shewanella oneidensis</i> MR-1	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	1	J. Craig Venter Institute	<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
<i>Shewanella oneidensis</i> Eg mutant	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	1	J. Craig Venter Institute	<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
<i>Shewanella oneidensis</i> OmpA/MtrC mutant	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	1	J. Craig Venter Institute	<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input type="radio"/> No			<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3

\*Please attach a Material Safety Data Sheet or equivalent from the supplier.

## 2.0 Cell Culture

2.1 Does your work involve the use of cell cultures?  YES  NO  
 If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture:

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	<input type="radio"/> Yes <input checked="" type="radio"/> No		Not applicable
Rodent	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input type="radio"/> No		

\* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED\*

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Rodent	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Non-human primate	<input type="radio"/> Yes <input checked="" type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input type="radio"/> No		

\*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required  1  2  3

### 3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials?  YES  NO

If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Infected With An Infectious Agent? YES/NO	Name of Infectious Agent (If applicable)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> No <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

### 4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0?  YES  NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done?  YES, complete table below  NO

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results

\* Please attach a Material Data Sheet or equivalent if available.

4.3 Will genetic modification(s) involving viral vectors be made?  YES, complete table below  NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results

\* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- ◆ HIV  YES, please specify \_\_\_\_\_  NO
- ◆ HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens  YES, specify \_\_\_\_\_  NO
- ◆ SV 40 Large T antigen  YES  NO
- ◆ E1A oncogene  YES  NO
- ◆ Known oncogenes  YES, please specify \_\_\_\_\_  NO
- ◆ Other human or animal pathogen and or their toxins  YES, please specify \_\_\_\_\_  NO

4.5 Will virus be replication defective?  YES  NO

4.6 Will virus be infectious to humans or animals?  YES  NO

4.7 Will this be expected to increase the containment level required?  YES  NO

### 5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent?  YES  NO  
 (including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)  
 If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: \_\_\_\_\_  
 Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host?  YES  NO

5.3 How will the biological agent be administered? \_\_\_\_\_

5.4 Please give the Health Care Facility where the clinical trial will be conducted: \_\_\_\_\_

5.5 Has human ethics approval been obtained?  YES, number: \_\_\_\_\_  NO  PENDING

### 6.0 Animal Experiments

6.1 Will live animals be used?  YES  NO If no, please proceed to section 7.0

6.2 Name of animal species to be used \_\_\_\_\_

6.3 AUS protocol # \_\_\_\_\_

6.4 Will any of the agents listed in section 4.0 be used in live animals  YES, specify: \_\_\_\_\_  NO

6.5 Will the agent(s) be shed by the animal:  YES  NO, please justify:  
 \_\_\_\_\_  
 \_\_\_\_\_



**10.0 Plants Requiring CFIA Permits**

10.1 Do you use plants that require a permit from the CFIA?  YES  NO  
If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. \_\_\_\_\_

10.3 What is the origin of the plant? \_\_\_\_\_

10.4 What is the form of the plant (seed, seedling, plant, tree...)? \_\_\_\_\_

10.5 What is your intention?  Grow and maintain a crop  "One-time" use

10.6 Do you do any modifications to the plant?  YES  NO  
If yes, please describe: \_\_\_\_\_  
\_\_\_\_\_

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

10.8 Is the CFIA permit attached?  YES  NO  
If NO, please forward the permit to the Biosafety Officer when available.

10.9 Please describe any CFIA permit conditions:  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

**11.0 Import Requirements**

11.1 Will any of the above agents be imported?  YES, please give country of origin USA  
If no, please proceed to Section 12.0  NO

11.2 Has an Import Permit been obtained from HC for human pathogens?  YES

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens?  YES

11.4 Has the import permit been sent to OHS?  YES, please provide permit # \_\_\_\_\_  NO

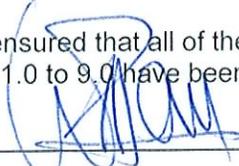
NO } Not needed  
 NO

**12.0 Training Requirements for Personnel Named on Form**

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- ◆ Biosafety
- ◆ Laboratory and Environmental/Waste Management Safety
- ◆ WHMIS (Western or equivalent)
- ◆ Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE \_\_\_\_\_ 



\* DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED\*

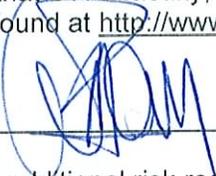
**13.0 Containment Levels**

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required.  1  2  3

13.2 Has the facility been certified by OHS for this level of containment?  
 YES, permit # if on-campus \_\_\_\_\_  
 NO, please certify  
 NOT REQUIRED for Level 1 containment

**14.0 Procedures to be Followed**

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at <http://www.wph.uwo.ca/>

X SIGNATURE  Date: Dec. 14/2009

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, or 3 measures, that are unique to this agent.

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

14.3 Please outline what will be done if there is an exposure to the biohazards listed, such as a needlestick injury:

\_\_\_\_\_  
\_\_\_\_\_

**15.0 Approvals**

UWO Biohazard Subcommittee: SIGNATURE: \_\_\_\_\_  
Date: \_\_\_\_\_

Safety Officer for Institution where experiments will take place: SIGNATURE: \_\_\_\_\_  
Date: \_\_\_\_\_

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: \_\_\_\_\_  
Date: \_\_\_\_\_

Approval Number: \_\_\_\_\_ Expiry Date (3 years from Approval): \_\_\_\_\_

Special Conditions of Approval:

• contact Anne Marie McCusker regarding the nanotechnology safety program.

### **Cultivation details of *Shewanella oneidensis* MR-1**

Freeze-dried *Shewanella oneidensis* MR-1 samples are provided by J. Craig Venter Institute. The freeze-dried pellet is rehydrated in 1 ml of Tryptic Soy Broth (TSB), which contains 15 g of tryptone, 15 g of soytone, and 5 g of sodium chloride per liter. TSB is autoclaved at 121 °C for 15 min and cooled to room temperature before use. Rehydrated *S. oneidensis* MR-1 cells are transferred to 500 ml of TSB in a 1-liter conical flask and inoculated aerobically at 30 °C for 24 hours. The grown cells are harvested, applied to our pre-fabricated silicon chips, rinsed with deionized water, and air-dried for microscopic characterizations such as optical microscopy and atomic force microscopy.

# Shewanella oneidensis

From MicrobeWiki, the student-edited microbiology resource

A Microbial Biorealm page on the genus *Shewanella oneidensis*

## Contents

- 1 Classification
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- 2 Description and significance
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## Classification

### Higher order taxa

Bacteria; Proteobacteria; Gammaproteobacteria; Alteromonadales; Shewanellaceae; Shewanella; Shewanella oneidensis MR-1

### Species

*Shewanella oneidensis*

## Description and significance

*Shewanella oneidensis* is a Gram-negative anaerobic bacteria which is predominantly found in deep sea anaerobic habitats; but can also reside in soil and sedentary habitats. There is a presence of lipoproteins/cytochromes (MtrC and OmcA) on the outer membrane of *Shewanella oneidensis*. These cytochromes have been of particular interest in the field of research due to their potential of bioremediation of heavy metals. The entire genome of *Shewanella oneidensis* was sequenced by The Institute for Genomic Research (TIGR) in Rockville, Maryland and La Jolla, California.

## Genome structure

*Shewanella oneidensis* MR-1 has 4,566 genes in its entire genome which was completed in September, 2002. Of the total 4,566 genes: 4,324 are protein encoding genes. There are a total of 4,969,803 nucleotides present in the genome of *Shewanella oneidensis* MR-1, and the GC base concentration is 45 percent. All the genes are compacted into a single circular chromosome. *Shewanella oneidensis* MR-1 also has a circular plasmid present in its cytoplasm known as pMR-1. This plasmid has 184 genes of which 149 are protein encoding genes.

## Cell structure and metabolism

*Shewanella oneidensis* has outer membrane cytochromes (MtrC and OmcA) which reduce Fe(III) during anaerobic respiration; it does this by coupling oxidation of organic carbon to electron acceptors such as Fe(III), oxygen, nitrate, and other metals. *Shewanella oneidensis* has the ability to produce pilus like structures when its immediate environment is low in electron acceptor concentration (metals). These pili help the organism to locate and reduce metals. The main central metabolic pathway for *Shewanella oneidensis* MR-1 is the serine isocitrate lyase pathway in which formaldehyde made from pyruvate is reacted with glycine to produce serine.

## Ecology

*Shewanella oneidensis* forms biofilms in soil and sediment environments when there is an abundance of electron acceptors.

## Application to Biotechnology

Due to its ability to reduce heavy metals in the environment via cytochromes present on the outer membrane *Shewanella oneidensis* has been the target of bioremediation research.

## Current Research

- 1) Atomic Force Microscopy was used to observe if a bond forms between a hematite (Fe<sub>2</sub>O<sub>3</sub>) thin film. This technique revealed that there was a specific interaction between the cytochrome of *Shewanella oneidensis* and the hematite film.
- 2) Cytochromes of *Shewanella oneidensis* were radiolabelled in order to determine if they were lipoproteins in the outer membrane. Upon comparison of this wild-type with the mutant which didn't have outer membrane cytochromes it was determined in the radiolabelled wild-type that lipoproteins were present [4].
- 3) The knocking out of the gene SO1377 was compared to a wild-type strain and it was observed that the mutant had a depleted growth rate in aerobic conditions but not in anaerobic conditions.

## References

- 1) "Microbe." Chapter 18, pg. 370. Moselio Schaechter, John L. Ingraham, Frederick C. Neidhardt. ASM Press, American Society for Microbiology, Washington, DC 20036.
- 2) "Polyphasic taxonomy of the genus *Shewanella* and description of *Shewanella oneidensis* sp. Nov." K Venkateswaran, DP Moser, ME Dollhopf, DP Lies, DA Saffarini, BJ MacGregor, DB Ringelberg, DC White, M Nishijima, H Sano, J Burghardt, E Stackebrandt and KH Neilson Center for Great Lakes Studies, University of Wisconsin-Milwaukee, Milwaukee, WI 53204, USA
- 3) "Specific bonds between an iron oxide surface and outer membrane cytochromes MtrC and OmcA from *Shewanella oneidensis* MR-1." Lower BH, Shi L, Droubay TC, Lower SK. Pacific Northwest National Laboratory. 275 Mendenhall Laboratory, Columbus, Ohio
- 4) "The outer membrane of cytochromes of *Shewanella oneidensis* MR-1 are lipoproteins." Lower BH, Kennedy DW, Mottaz HM, Marshall MJ, Hill EA, Zachara JM. Microbiology Group, Pacific Northwest National Laboratory, Richland, WA. NCBI database [info@ncbi.nlm.nih.gov]

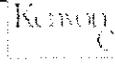
5) "c-Type cytochrome-dependent formation of U(IV) nanoparticles by *Shewanella oneidensis*." Marshall MJ, Shi L, Kennedy DW, Lai B, Wang Z. Biological sciences division, Pacific Northwest National Laboratory, Richland, WA.

Edited by student Gurpreet Dhillon; Professors: Rachel Larsen (mailto:ralarsen@ucsd.edu) and Kit Pogliano

KMG

Retrieved from "[http://microbewiki.kenyon.edu/index.php/Shewanella\\_oneidensis](http://microbewiki.kenyon.edu/index.php/Shewanella_oneidensis)"

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