THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: September 25, 2009
Biosafety Website: 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, 1st 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. 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

PRINCIPAL INVESTIGATOR: Leo Lau
SIGNATURE: 
DEPARTMENT: Surface Science Western
ADDRESS: Room G-1 Western Science Centre
PHONE NUMBER: 519-661-2173
EMERGENCY PHONE NUMBER(S):
EMAIL: llau22@uwo.ca

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 external funding from J. Craig Venter Institute to support collaboration
GRANT TITLE(S):

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 (kleung7@uwo.ca)

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

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? ✓ YES O 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? O 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:
_____
_____
_____
Please attach the CFIA permit.
Please describe any CFIA permit conditions:
_____
_____
_____

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
Shewanella oneidensis MR-1	O Yes ✓ No	O Yes ✓ No	O Yes ✓ No	1	J. Craig Venter Institute	✓ 1 O 2 O 3
Shewanella oneidensis Δ-Cg mutant	O Yes ✓ No	O Yes ✓ No	O Yes ✓ No	1	J. Craig Venter Institute	✓ 1 O 2 O 3
Shewanella oneidensis OmcA/MtrC mutant	O Yes ✓ No	O Yes ✓ No	O Yes ✓ No	1	J. Craig Venter Institute	✓ 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 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	O Yes ✓ No		Not applicable
Rodent	O Yes ✓ No		
Non-human primate	O Yes ✓ No		
Other (specify)	O Yes O No		

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	O Yes Ø No		
Rodent	O Yes Ø No		
Non-human primate	O Yes Ø No		
Other (specify)	O Yes O 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 O 1 O 2 O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES Ø 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		O Yes O No O Unknown		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No O Unknown		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No O Unknown		O 1 O 2 O 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? O YES Ø NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below O 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? O YES, complete table below O 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 O YES, please specify _____ O NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ O NO
- SV 40 Large T antigen O YES O NO
- E1A oncogene O YES O NO
- Known oncogenes O YES, please specify _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ O NO

4.5 Will virus be replication defective? O YES O NO

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

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

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O YES Ø 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? O YES O 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? O YES, number: _____ O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? O YES Ø 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 O YES, specify: _____ O NO

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

_____

_____

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7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

- Pound source dogs O YES Ø NO
- Pound source cats O YES Ø NO
- Cattle, sheep or goats O YES Ø NO
- Non-human primates O YES, please specify species _____ Ø NO
- Wild caught animals O YES, please specify species & colony # _____ Ø NO
- Birds O YES Ø NO
- Others (wild or domestic) O YES, please specify _____ Ø NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES Ø NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin_____

8.4 How much of the toxin is handled at one time*? _____

8.5 How much of the toxin is stored*? _____

8.6 Will any biological toxins be used in live animals? O YES, Please provide detais:_____ O NO

*For information on biosecurity requirements, please see:
http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES Ø NO
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-time" use, give location: _____

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

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____

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10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O 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? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O 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? O YES O NO
If NO, please forward the permit to he 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 O NO

11.2 Has an Import Permit been obtained from HC for human pathogens? O YES ☑ NO not needed

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? O YES ☑ NO not needed

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ ☑ 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 _____

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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/

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:

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Brief description of work

Freeze-dried bacterial samples, namely *Shewanella oneidensis* MR-1, *S. oneidensis* Flg mutant and *S. oneidensis* OmcA/MtrC mutant, will be provided by Dr. Yuri Gorby and Dr. Greg Wanger at the J. Craig Venter Institute in San Diego. The bacterial samples being transferred are non-pathogenic. At Western, the received samples will be cultured or stored for future use. The grown cells will be harvested and air-dried for atomic force microscopic analysis.

Shewanella oneidensis

From MicrobeWiki, the student-edited microbiology resource

A Microbial Biorealm page on the genus *Shewanella oneidensis*

Contents

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 (Fe203) 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 Nealson 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