Modification Form for Permit BIO-UWO-0062

Permit Holder: Carole Creuzenet

Approved Personnel
(Please stroke out any personnel to be removed)

~~Dinath Ratnayake~~
~~Jaspreet Chanal~~
~~Dirk Lange~~
~~Melinda Demendi~~
~~Nathan Ho~~
~~Vijayakumar Somalinga~~
~~Alexandra Merkx-Jacques~~

Additional Personnel
(Please list additional personnel here)

Patrick Hopf
Sari Kichler
Jacqueline Hayworth
Rachel Ford
Anthony Wong

	Please stroke out any approved Biohazards to be removed below	Write additional Biohazards for approval below. *
Approved Microorganisms	~~Yersinia pseudotuberculosis~~	Helicobacter pylori Campylobacter jejuni
Approved Cells	Human THP1, Human CaCo2, Rodent raw	
Approved Use of Human Source Material		
Approved GMO		

actually Not new – We have been approved for this since I opened the lab in 2001!

* *PLEASE ATTACH A MATERIAL SAFETY DATA SHEET OR EQUIVALENT FOR NEW BIOHAZARDS.*
** *PLEASE ATTACH A BRIEF DESCRIPTION OF THE WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED.*

Classification: 2

Date of last Biohazardous Agents Registry Form: Aug 24, 2007

Signature of Permit Holder:

BioSafety Officer(s):

Chair,Biohazards Subcommittee:

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Approved use of Animals	mice	gerbils (I have an approved protocole).
Approved Toxin(s)		

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BioSafety Officer(s):

Chair,Biohazards Subcommittee:

In our search for genes determining virulence properties of Helicobacter pylori, we disrupt candidate genes and analyse phenotypic differences between the resulting mutants and the original wild-type strain in vitro. We also intend to perform animal assays using gerbils. The animal protocol is under preparation for full renewal. Candidate genes affecting pathogenesis in vivo will be studied further as potential therapeutic target.

In our search of genes responsible for deoxyheptose biosynthesis in Campylobacter jejuni and Yersinia pseudotuberculosis, we have disrupted several candidate genes and assessed the phenotypic differences in vitro. The information gathered helps us reconstruct a biosynthesis pathway for these unusual sugars. Not present in mammals, these sugars and the enzymes responsible for their synthesis represent novel antibiotic targets.

We have also identified several Lactobacilli strains that can either kill or inhibit the production of virulence factors by H. pylori, in a so-called probiotic effect. All assays were done in vitro so far using culture supernatants (free of any live lactobacilli). We intend to perform assays in the gerbil model to assess the efficacy of lactobacilli supernatants as prophylactic or therapeutic agents.

To investigate the mechanism involved in the probiotic effects of lactobacilli on H. pylori, we measured the production of auto-inducers, specialized molecules that allow inter-bacterial communication. This requires the use of a Vibrio harveyi reporter strain (that produces bioluminescence) and an E. coli mutant strain unable to produce the autoinducer as a negative control.

We also produce large amount of recombinant proteins and enzymes. For this, we use a variety of E. coli non-pathogenic lab strains to over-express the protein.

Finally, we have been investigating the mechanism of protein synthesis initiation in Pseudomonas aeruginosa.

BIO-UWO-0062

THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Revised Biohazards Subcommittee: January, 2007

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario where the use of biohazardous infectious agents are described in the experimental work proposed. The form must also be completed if animal work is proposed involving the use of biohazardous agents or animal carrying zoonotic agents infectious to humans. Containment Levels will be required in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) 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 (Stevenson-Lawson Building, Room 60) for forward to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Coordinator at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies) modifications must be completed and sent to Occupational Health and Safety. See website: www.uwo.ca/humanresources

PRINCIPAL INVESTIGATOR Dr Carole CREUZENET
SIGNATURE [signature]
DEPARTMENT Microbiology & Immunology
ADDRESS DSB 3031 Main Campus
PHONE NUMBER 8 3204
EMAIL ccreuzen@uwo.ca

Location of experimental work to be carried out: Building(s) DSB Room(s) 3031
*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 it being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Centre, Child and Parent Research Institute or Robarts Research Institute, University Biosafety Committee members can also sign as the Safety Officer.

TITLE OF GRANT(S):
- Virulence Factors of Helicobacter pylori (CIHR)
- Deoxyheptose synthesis in Campylobacter jejuni (NSERC)

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK, SUCH A THE RESEARCH GRANT SUMMARY(S) THAT EXPLAINS THE BIOHAZARDS USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

FUNDING AGENCY/AGENCIES CIHR & NSERC

Names of all personnel working under Principal Investigators supervision in this location:
i) Alexandra Merky-Jacques
ii) Vijay Somalinga
iii) Nathan Ho
iv) Melinda Demendi
v) Dirk Lange
vi) Jaspreet Chahal
vii) Dina Ratnayake.

* 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 microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? ☐ YES ☐ NO
If no, please proceed to Section 2.0

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?
Yersinia Pseudotuberculosis	☑ Yes ☐ No	☑ Yes ☐ No	☑ Yes ☐ No	1 L
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	
	☐ Yes ☐ No	☐ Yes ☐ No	☐ Yes ☐ No	

1.3 For above named organism(s) or biological agent(s) circle HC or CFIA Containment Level required. 1 (2) 3

1.4 Source of microrganism(s) or biological agent(s)? Dr Skurnik (FINLAND).

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 (ie. derived from fresh tissue) that will be grown in culture in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue
Human	☐ Yes ☐ No	
Rodent	☐ Yes ☐ No	
Non-human primate	☐ Yes ☐ No	
Other (specify)		

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)	Supplier / Source
Human	☑ Yes ☐ No	THP1, Caco2	VALVANO, KIN UWO, MSI
Rodent	☑ Yes ☐ No	RAW	VALVANO UWO, MSI
Non-human primate	☐ Yes ☐ No		
Other (specify)	☐ Yes ☐ No		

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

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

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 if the following will be used in the laboratory

- Human blood (whole) or other bodily fluids ☐ YES ☐ NO If YES, Specify _____
- Human blood (fraction) or other bodily fluids ☐ YES ☐ NO If YES, Specify _____
- Human organs (unpreserved) ☐ YES ☐ NO If YES, Specify_____
- Human tissues (unpreserved) ☐ YES ☐ NO If YES, Specify_____

3.3 Is human source known to be infected with and infectious agent ☐ YES ☐ NO
If YES , please name infectious agent _____

3.4 For above named materials circle HC or CFIA containment level required. 1 2 3

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
We disrupt virulence genes and donot introduce toxin genes)

4.2 Will genetic sequences from the following be involved:

- HIV ☐ YES ☑ NO
if YES specify_____
- HTLV 1 or 2 or genes from any CDC class 1 pathogens ☐ YES ☑ NO
if YES specify_____
- Other human or animal pathogen and or their toxins ☐ YES ☑ NO
if YES specify_____

4.3 Will intact genetic sequences be used from

- SV 40 Large T antigen ☐ YES ☑ NO If YES specify_____
- Known oncogenes ☐ YES ☑ NO If YES specify_____

4.4 Will a live vector(s) (viral or bacterial) be used for gene transduction ☐ YES ☑ NO
If YES name virus_____.

4.5 List specific vector(s) to be used: N/a

4.6 Will virus be replication defective ☐ YES ☐ NO

4.7 Will virus be infectious to humans or animals ☐ YES ☐ NO

4.8 Will this be expected to increase the Containment Level required ☐ YES ☐ NO

5.0 Human Gene Therapy Trials NONE

5.1 Will human clinical trials using the viral vector in 4.0 be conducted? ☐ YES ☑ NO
If no, please proceed to Section 6.0
If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? ☐ YES ☐ NO

5.3 How will the virus 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 ☐ NO

6.0 Animal Experiments

6.1 Will any of the agents listed be used in live animals? ☑ YES ☐ NO
If no, please proceed to section 7.0

6.2 Name of animal species to be used Mice

6.3 AUS protocol # Pending (OHS approval needed)

6.4 If using murine cell lines, have they been tested for murine pathogens? ☐ YES ☐ NO

7.0 Use of Animal species with Zoonotic Hazards

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

- Pound source dogs ☐ YES ☑ NO
- Pound source cats ☐ YES ☑ NO
- Sheep or goats ☐ YES ☑ NO
- Non- Human Primates ☐ YES ☑ NO If YES specify species _____
- Wild caught animals ☐ YES ☑ NO If YES specify species _____
  colony # _____

8.0 Biological Toxins

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

8.2 If YES, please name the toxin_____

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

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

9.0 Import Requirements

9.1 Will the agent be imported? ☐ YES ☑ NO
If no, please proceed to Section 10.0
If yes, country of origin_____

9.2 Has an Import Permit been obtained from HC for human pathogens? ☐ YES ☐ NO

9.3 Has an import permit been obtained from CFIA for animal pathogens? ☐ YES ☐ NO

9.4 Has the import permit been sent to OHS? ☐ YES ☐ NO
If yes, Permit # _____

10.0 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

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 _____

11.0 Containment Levels

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

11.2 Has the facility been certified by OHS for this level of containment? ☑ YES ☐ NO

11.3 If yes, please give the date and permit number: BIO UWO 0062 June 11 2006

12.0 Approvals

UWO Biohazard Subcommittee

Signature GM Kidder Date 24 Aug '07

Safety Officer for Institution where experiments will take place

Signature J Stanley Date Aug 24/07

Safety Officer for University of Western Ontario (if different than above)

Signature_____ Date_____

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

Yersinia pseudotuberculosis is an important food-borne bacterial pathogen that causes enteritis in humans. Its surface is coated by long chains of sugars called lipopolysaccharide or LPS. This LPS is known to contribute significantly to the ability of *Y. pseudotuberculosis* to cause disease, but the exact sugar components of the LPS responsible for this effect are not known.

The LPS of *Y. pseudotuberculosis* comprises an unusual sugar called a 6-deoxyheptose (DHPT). This DHPT is not found in mammals but is found also on the surface of other bacterial pathogens such as *Campylobacter jejuni*, a major cause of enteritis worldwide, and *Burkholderia mallei*, the causative agent of glanders. Hence, inhibitors of its biosynthesis could be used as novel antibacterial agents active against *Y. pseudotuberculosis*, *C. jejuni* and *B. mallei* and they would be expected to be non toxic for the host as mammals do not produce this DHPT. Demonstrating the role of the DHPT in the ability of these pathogens to cause disease is a requisite to validate the enzymes responsible for the synthesis of the DHPT as novel antimicrobial targets.

We have inactivated two genes responsible for DHPT biosynthesis in *Y. pseudotuberculosis* and demonstrated in the test tube that these genes are important for resistance of the bacterium to host factors that it is exposed to when it establishes itself in the intestinal tract. This suggests that, most likely, the mutant bacteria that harbour these inactivated genes would be less likely to cause disease. However, this needs to be demonstrated in an animal model, where host factors act upon the bacteria in a concerted manner that can not be mimicked in a test tube. Hence, we wish to use mice to demonstrate that our mutants of *Y. pseudotuberculosis* have decreased ability to cause disease.

We chose the mice as mice are a well accepted animal model in the *Y. pseudotuberculosis* field. The doses of bacteria necessary to cause disease are known, the methodology to inoculate the mice has been worked out, and the development, extent and characteristics of the disease developed are well known, including which organs the bacteria settles into.