

**THE UNIVERSITY OF WESTERN ONTARIO  
BIOLOGICAL AGENTS REGISTRY FORM**  
**Approved Biohazards Subcommittee: July 9, 2010**  
**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 (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological 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 biological agents 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 Biohazards 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	<u>Thomas Linn</u>
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Location of experimental work to be carried out: Building(s) \_\_Dental Sci Bldg\_\_ Room(s) 3004E, 3013\_\_

\*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 15.0, Approvals).

FUNDING AGENCY/AGENCIES: \_\_NSERC\_\_  
 GRANT TITLE(S): \_\_Role Sigma Factors in Regulating B. cereus Virulence\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

List all personnel working under Principal Investigators supervision in this location:

<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
<u>Ryan Lum-Tai</u>	<u>rlumtai@uwo.ca</u>	<u>Biosafety Aug 5, 2010</u>
_____	_____	<u>Whimis Dec 9 2009</u>
_____	_____	<u>Lab &amp; Waste Aug 15, 2010</u>
_____	_____	<u>Health &amp; Safety Aug 12, 2010</u>
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_____	_____	_____

**Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.**

The biological agent is *Bacillus cereus* 14579. This is an attenuated *Bacillus cereus* strain that lacks the emetic plasmid so it cannot cause the emetic form of food poisoning. Although it is classified as Level 2 in Canada, it is classified as Level 1 in the USA.

The main stock is kept as a small (1 ml) frozen culture at -80°C. Petri plates of LB media are streaked out from the stock culture to use for daily experiments. Most experiments involve the use of small liquid cultures (usually 10 – 20 ml). Most experiments involve: 1) lysing the cells to extract RNA for RT-PCR analysis, 2) constructing specific deletion mutations of sigma factor genes, 3) comparison of the growth of the resultant mutants to the wildtype strain under a variety of growth conditions.

After an experiment is completed all material that was in contact with the bacteria is autoclaved to kill and remaining live bacteria.

In order to survive and grow bacteria have to cope with a wide range of conditions that they encounter in the natural environment or during infection of a host organism. They do so by changing their program of gene expression in response to the varied conditions. The experiments we are conducting will examine the role of RNA polymerase sigma factors in regulating gene expression in *Bacillus cereus* in response to cues signaling an altered environment. *B. cereus*, which is best known for food poisoning, is a member of the “*Bacillus cereus* group” that also includes *B. thuringiensis* and *B. anthracis*.

In the classical *E. coli* model system it has been demonstrated that alternative sigma factors are activated in response to stresses such as increased temperature or starvation for iron, carbon or nitrogen sources. These sigma factors bind to the core RNA polymerase and direct it to transcribe a set of genes (regulon) that allows the bacteria to cope with the specific stress. When a pathogenic bacterium encounters a potential host organism it is subjected to a variety of stresses such as iron limitation, oxidative burst, increased temperature and reduced pH. We began by examining whether the expression levels of alternative sigma factors of *B. cereus* are increased in response to these stresses. Genome sequencing of *B. cereus* indicates it has 20 putative alternative sigma factor genes. Primer pairs were designed for each gene and reverse transcriptase-PCR (RT-PCR) was used to monitor transcription of these genes under different growth conditions. Increased temperature and increased acidity in particular showed dramatic induction of different subsets of sigma factor genes. Part of the work will be to continue to quantify, by real time-PCR, the induction of sigma factor genes under various growth conditions.

A main approach of our work is the genetic analysis of the role of these alternative sigma factors. That involves the construction of null (deletion) mutations for the specific sigma factor genes of interest. Gene cloning and recombinant DNA tools are poorly developed in *B. cereus* as compared to *E. coli*. Therefore we have expended considerable effort in the development of *E. coli*-*B. cereus* shuttle vectors and *B. cereus* expression vectors that will be required for these experiments. Additional modification and improvement of these vectors will be ongoing. A major effort has been made to develop a protocol for *B. cereus* to generate precise in-frame deletions by allele replacement. Although that has led to a useable protocol to produce *B. cereus* strains with either single or multiple sigma factor gene deletions we are continuing to streamline and increase the efficiency of this technique. The deletion mutants are being compared to the wildtype for growth and survival in response to in vitro stresses and we plan in the future to examine their survival and virulence in a mouse infection model.

An ultimate goal of this work is to define the regulon for each sigma factor by using transcriptome sequencing to quantify the genome expression in: 1) the wild-type, 2) the sigma factor deletion mutant 3) and a strain engineered to overproduce that sigma factor. The identification of candidate genes will be confirmed by RT-PCR.

This combination of experiments should delineate what sigma factors in *B. cereus* are involved in responding to various environmental conditions and in turn lead to an identification of the set of genes and functions the bacterium uses to cope with the specific stresses. This information in general should increase our understanding of transcriptional networks in bacteria and specifically make us better prepared to deal with *B. cereus* infections.

## 1.0 Microorganisms

1.1 Does your work involve the use of biological agents?  YES  
 (non-pathogenic and pathogenic biological agents 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? No, the organism is already in my lab.  
 If YES, please give the name of the species. Bacillus cereus 14579  
 What is the origin of the microorganism(s)? American Type Culture Collection  
 Please describe the risk (if any) of escape and how this will be mitigated: Only small cultures of the organism are used with standard aseptic technique. At the end of experiments all material is autoclaved or treated with bleach.

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
E.coli DH5a	<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	0.5 l		<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
E.coli XL1-Blue	<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	0.5 l		<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
B. cereus 14579	<input checked="" type="radio"/> Yes <input type="radio"/> No	<input checked="" type="radio"/> Yes <input type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	0.4 l	ATCC	<input type="radio"/> 1 <input checked="" type="radio"/> 2 <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"/> 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 type="radio"/> No		Not applicable
Rodent	<input type="radio"/> Yes <input type="radio"/> No		
Non-human primate	<input type="radio"/> Yes <input type="radio"/> No		
Other (specify)	<input type="radio"/> Yes <input type="radio"/> 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	<input type="radio"/> Yes <input type="radio"/> No		
Rodent	<input type="radio"/> Yes <input type="radio"/> No		
Non-human primate	<input type="radio"/> Yes <input 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  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"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <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 from transformation or tranfection
Bacillus cereus 14579	Modified versions of pBKJ236	S. Stibitz, FDA Bethesda, MD USA	Deleted versions of sigma factor genes	.Deletion of sigma factor gene from chromosome, bacteria will be attenuated

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

\*\* Please attach a plasmid map.

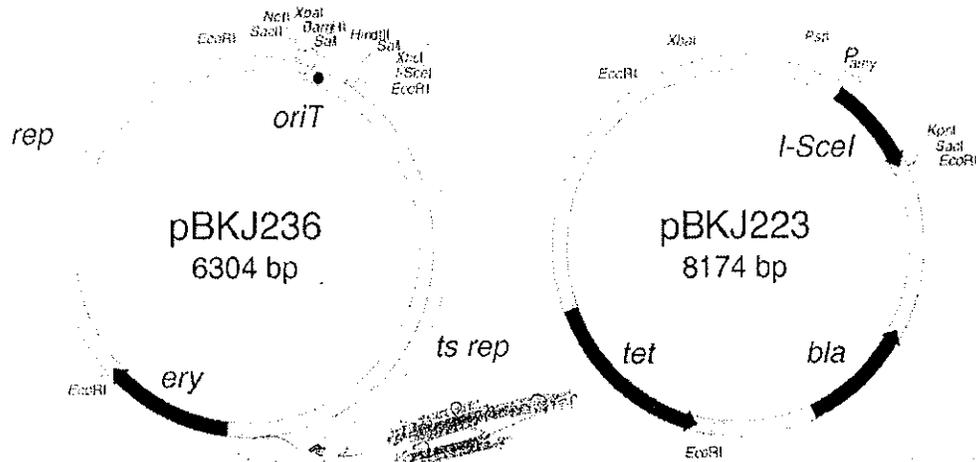
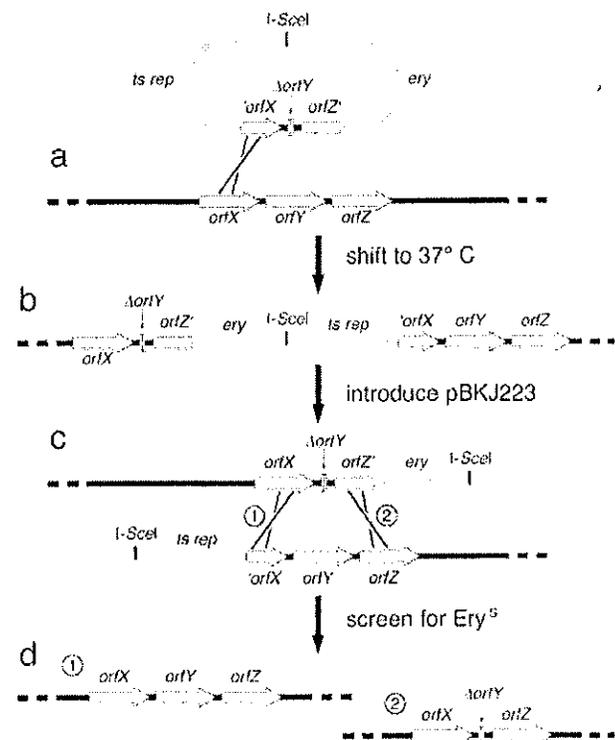


FIG. 1. Plasmids used in allelic exchange. pBKJ236 was created by the addition of an approximately 250-bp Bam-HindIII fragment from pSS1916 (25) containing the *oriT* of RE4 into the same sites of pJRS233 (16) and the subsequent addition at the KpnI site of the complementary oligonucleotides CATAGGGATAACAGGGTAATTAATTCCGGTAC and CGAATTC AATTACCCTGTTATCCCTATGGTAC containing an I-SceI site. To create pBKJ223, two PCR-generated fragments were added between the Sall and KpnI sites of pUTE29 (8). One fragment, from XhoI to NdeI, was created using the primers CGCGAATTCCTCGAGAAGCITGAAGAAGACCATAAAAAATACCTTGTC and CGCTCTAGACATATGCGTTCCTTCATTTTCITATACAAAATATATTTT with *Bacillus amyloliquefaciens* chromosomal DNA as a template and was based on that described by Cohen et al. (5). This fragment contains the promoter for amylase and the ribosome-binding site of *B. anthracis pagA* (incorporated into one primer). The promoter sequence differs from the published sequence (5, 15) at several positions: an AT-to-TA transversion at positions -80 and -79 (with respect to the initiating codon) and a T-to-G change at position -63, presumably due to PCR errors. The other fragment, from NdeI to KpnI, was created using the primers CGCTCTAGACATATGCATCAAAAAACCAGGTAATGAAC and CGCGGTACCTTATTTTCAGGAAAGTTTCGGAGGAGAT with pUCRP12 (17) as a template and comprised the ORF for the I-SceI enzyme.

resistance. A derivative of pUTE29 (8), this plasmid contains the gene for the I-SceI enzyme under the control of a hybrid amylase promoter and gram-positive ribosome-binding site. Transformants are streaked twice on solid medium containing tetracycline, and then single colonies are scored for loss of erythromycin resistance. Following screening by PCR for the incorporation of the desired mutation, the pBKJ233 plasmid is lost spontaneously by streaking the cells twice on medium lacking tetracycline and scoring a small number of colonies for tetracycline sensitivity. The replicational instability of the pUTE29 vector has been previously described (21).

In order to demonstrate the utility of this approach, a number of mutations were introduced into *B. anthracis* 7702 (Sterne). We have also used this method with success with a similar strain, 34F2 (data not shown). The structures of the lesions are presented in Table 1, and the efficiency of the pBKJ233-encoded I-SceI nuclease in stimulating the second crossover event and allelic exchange is presented in Table 2. In separate control experiments with the strain harboring the pBKJ240 plasmid integrant, the pUTE29 vector showed no such stimulation (data not shown). Figure 3 shows the results of the PCR analysis demonstrating the incorporation of the altered allele in the resulting mutant strains. The *plcR* locus was used as an initial test of this method because it has previously been documented that the *plcR* gene is nonfunctional in *B. anthracis* due to a frameshift mutation (1). Both a clean, in-frame deletion of the *plcR* gene ( $\Delta plcR240$ ) and a deletion marked with a spectinomycin resistance cassette (*plcR241::spc*) were successfully introduced. The *scrB* gene was chosen since it presented a target with a potentially scorable colonial phenotype. This gene is predicted to encode sucrose-6-phosphate hydrolase, which is essential for the metabolism of sucrose by the sucrose phosphoenolpyruvate-dependent phosphotransfer-

ase system (7, 9). Analysis of the *B. anthracis* (Ames) genome suggested that no other pathway for catabolizing sucrose existed. Indeed, both the insertion mutation (*scrB239::spc*) and a missense mutation (*scrB237*) (G214Q) conferred a sucrose uti-



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 from transduction

\* 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 Possibly in the future. If no, please proceed to section 7.0

6.2 Name of animal species to be used Mouse

6.3 AUS protocol # Not yet determined

6.4 Will any of the agents listed in section 4.0 be used in live animals  YES, specify: B. cereus14579

6.5 Will the agent(s) be shed by the animal:  YES  NO, please justify:  
The bacteria will be ingested and pass through the digestive tract.

## 7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)?  YES  No If no, please proceed to section 8.0

7.2 Please specify the animal(s) used:

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

## 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(s) \_\_\_\_\_  
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD<sub>50</sub> (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?  YES, Please provide details: \_\_\_\_\_  NO

\*For information on biosecurity requirements, please see:

[http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity\\_Requirements.pdf](http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf)

## 9.0 Insects

9.1 Do you use insects?  YES  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?  Initiate and maintain colony, give location: \_\_\_\_\_  
 "One-time" use, give location: \_\_\_\_\_

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

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

9.7 Do you use insects that require a permit from the CFIA permit?  YES  NO  
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

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

**10.0 Plants**

10.1 Do you use plants?     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 YES, Please attach the CFIA permit & 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 \_\_\_\_\_     NO  
If no, please proceed to Section 12.0

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

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens?     YES     NO

11.4 Has the import permit been sent to OHS?     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 biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE \_\_\_\_\_ 

**13.0 Containment Levels**

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

13.2 Has the facility been certified by OHS for this level of containment?  
 YES, permit # if on-campus \_\_Room 3004E DSB\_\_\_\_  
 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 Thomas Lin Date: SEPT 3, 2010

14.2 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 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 biological agents listed, such as a needlestick injury:

We are not injecting the organism. This strain is attenuated and cannot cause an emetic reaction. It is still capable of causing a diarrheal reaction, but a substantial amount has to be ingested and even then it is self limiting and over in 24 – 48 hours.

**15.0 Approvals**

1) UWO Biohazards Subcommittee: SIGNATURE: \_\_\_\_\_  
Date: \_\_\_\_\_

2) Safety Officer for the University of Western Ontario  
SIGNATURE: \_\_\_\_\_  
Date: \_\_\_\_\_

3) Safety Officer for Institution where experiments will take place (if not UWO):  
SIGNATURE: \_\_\_\_\_  
Date: \_\_\_\_\_

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

Special Conditions of Approval:

## Bacteria

ATCC® Number: **14579™** Order this Item Price: **\$40.00**

[Preceptrol® Culture](#)

Organism: *Bacillus cereus* Frankland and Frankland

Designations: [BCRC 10603, CCM 2010, CCUG 7414, CIP 66.24, DSM 31, HAMBI 1887, HAMBI 1905, IAM 12605, JCM 2152, LMG 6923, NBRC 15305, NCCB 75008, NCIMB 9373, NCTC 2599, NRRL B-3711, VKM B-504]

Depositor: RE Gordon

[Biosafety Level:](#) 1

Shipped: freeze-dried

Growth Conditions: [ATCC medium3](#): Nutrient agar or nutrient broth

**Temperature:** 30.0°C

Permits/Forms: In addition to the [MTA](#) mentioned above, other [ATCC and/or regulatory permits](#) may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please [click here](#) for information regarding the specific requirements for shipment to your location.

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Home > Emergency Preparedness > Laboratory Security > Material Safety Data Sheets (MSDS) - Infectious Substances > Bacillus cereus - Material Safety Data Sheets (MSDS)

## Bacillus cereus - Material Safety Data Sheets (MSDS)

### MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

#### SECTION I - INFECTIOUS AGENT

**NAME:** *Bacillus cereus*

**SYNONYM OR CROSS REFERENCE:** *Bacillus cereus* food poisoning

**CHARACTERISTICS:** Large (1 x 3-4 µm), aerobic, gram-positive rod; spore forming; motile; produces heat stable and heat labile toxins

#### SECTION II - HEALTH HAZARD

**PATHOGENICITY:** Opportunistic pathogen; intoxication characterized by two forms: an emetic form with severe nausea and vomiting and a diarrheal form with abdominal cramps and diarrhea; both forms are usually mild and self-limiting (24 hrs); immunocompromised individuals are susceptible to bacteremia, endocarditis, meningitis, pneumonia; also associated with posttraumatic endophthalmitis (ocular infection - rare)

**EPIDEMIOLOGY:** Worldwide; common cause of foodborne disease, especially in Europe

**HOST RANGE:** Humans

**INFECTIOUS DOSE:** Greater than  $10^6$  organisms by ingestion ( $>10^5$  organisms/g of food)

**MODE OF TRANSMISSION:** Ingestion of foods kept at ambient conditions after cooking; emetic form frequently associated with cooked rice

**INCUBATION PERIOD:** Emetic form 1-6 hours, average 4 hours; diarrheal form 6-24 hours, average 17 hours

**COMMUNICABILITY:** Not communicable from person to person

#### SECTION III - DISSEMINATION

**RESERVOIR:** Ubiquitous organism of the soil; commonly found in low levels in raw, dried and processed foods

**ZOONOSIS:** None

**VECTORS:** None

#### SECTION IV - VIABILITY

**DRUG SUSCEPTIBILITY:** Sensitive to chloramphenicol, aminoglycosides, vancomycin, clindamycin, erythromycin

**DRUG RESISTANCE:** Resistant to penicillin, ampicillin, cephalosporins, trimethoprim

**SUSCEPTIBILITY TO DISINFECTANTS:** Spores are relatively resistant; inactivated by 2% glutaraldehyde, 5% sodium hypochlorite; prolonged contact times required

**PHYSICAL INACTIVATION:** Spores destroyed by heating at 100°C for 10 min; ionizing radiation destroys spores with 540 krad

**SURVIVAL OUTSIDE HOST:** Spores are relatively resistant to heat and desiccation; survive cooking

## SECTION V - MEDICAL

**SURVEILLANCE:** Monitor for symptoms and confirm by identification of organism in suspected food and faeces of patients

**FIRST AID/TREATMENT:** Supportive therapy

**IMMUNIZATION:** None available

**PROPHYLAXIS:** None available

## SECTION VI - LABORATORY HAZARDS

**LABORATORY-ACQUIRED INFECTIONS:** None reported to date

**SOURCES/SPECIMENS:** Contaminated food sources, stool

**PRIMARY HAZARDS:** Ingestion of contaminated material

**SPECIAL HAZARDS:** None

## SECTION VII - RECOMMENDED PRECAUTIONS

**CONTAINMENT REQUIREMENTS:** Biosafety level 2 practices, containment equipment and facilities for activities involving clinical specimens and cultures

**PROTECTIVE CLOTHING:** Laboratory coat; gloves when skin contact with infectious materials is unavoidable

**OTHER PRECAUTIONS:** Good personal hygiene and frequent handwashing

## SECTION VIII - HANDLING INFORMATION

**SPILLS:** Allow aerosols to settle; wearing protective clothing, gently cover spill with absorbent paper towel and apply 5% sodium hypochlorite starting at the perimeter and working towards the centre; allow sufficient contact time before clean up

**DISPOSAL:** Decontaminate all wastes before disposal; steam sterilization, chemical disinfection, incineration

**STORAGE:** In sealed containers that are appropriately labelled

## SECTION IX - MISCELLANEOUS INFORMATION

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**Date prepared:** November 1999

**Prepared by:** Office of Laboratory Security, PHAC

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