

**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	<u>Lars Rehmman</u>
SIGNATURE	<u></u>
DEPARTMENT	<u>Chemical and Biochemical Engineering</u>
ADDRESS	<u>Thompson Engineering Building 459</u>
PHONE NUMBER	<u>ext. 89008</u>
EMERGENCY PHONE NUMBER(S)	<u>519 434 7514</u>
EMAIL	<u>rehmann@eng.uwo.ca</u>

Location of experimental work to be carried out: Building(s) TEB Room(s) 313

*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
GRANT TITLE(S): Advanced materials for the biochemical production of second generation biofuels and chemicals

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:

<u>Vivek Nagendra</u>	<u></u>
<u>Karen Schwab</u>	<u></u>
<u>Kerstin Schwanitz</u>	<u></u>
<u>Jun Zheng</u>	<u></u>

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:

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
Clostridium acetobutylicum	<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	3	DSMZ	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Clostridium beijerinckii 6422	<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	3	DSMZ	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Clostridium beijerinckii 6423	<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	3	DSMZ	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3
Clostridium saccharobutylicum	<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	3	DSMZ	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier. Not available for these strains.

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 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:

* 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/>

SIGNATURE  _____ Date: 13 April 2010 _____

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.
The microorganisms currently in use are all anaerobe (sensitive to oxygen) and are incubated air-tight bottles and transferred in an enclosed anaerobic chamber which is sterilized via UV-light. Bioreactors are not aerated but kept under positive pressure with Nitrogen, no risks of aerosol formation.

14.3 Please outline what will be done if there is an exposure to the biohazards listed, such as a needlestick injury:
Exposure to skill will be removed under running water followed by disinfection. Needle-stick injuries will be treated with standard first aid measure followed by hospital visit with information on the microbial strain that was used.

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:

Advanced materials for the biochemical production of second generation biofuels and chemicals

NSERC – Discovery grant 2010-2015. PI: Lars Rehmann

The proposed research will utilize advanced functional materials to overcome current bottlenecks in the conversion of biomass into fuels and chemicals. The targeted bottlenecks are the conversion of raw materials into fermentable sugars and the removal of toxic products from the fermenter. The initial process of interest will be the production of biobutanol, a second-generation biofuel, via *Clostridia* fermentation. The feedstock will be waste streams from industrial processes and lignocellulosic biomass. This stands in contrast to food crops such as corn currently used in bioethanol production, eliminating some of the ethical dilemmas of biofuel production and also reducing the environmental footprint. Lignocellulosic biomass (plant biomass such as lumber infected with mountain pine beetle) will initially be enzymatically converted into fermentable sugars followed by microbial fermentation into biobutanol or other products. The enzymatic step will be carried out in the presence of ionic liquids, potentially eliminating the need for additional pre-treatment. Ionic liquids (salts with melting points below 100 °C) have been shown to partly dissolve cellulose and lignin. Task-specific ionic liquids will be designed to dissolve the biomass while allowing for enzymatic activity, thereby providing a new route to produce fuels and chemicals from a variety of renewable resources.

The effect of ionic liquid mediated biomass pre-treatment processes on the fermentability via *Clostridia* will be investigated in high-throughput micro-scale experiments using a multi-mode plate-reader.

Additional so-called 'smart' polymers will be used to recover the fermentation product (initially biobutanol) from the fermenter (3L vessel). These polymers will be analogous to polymers used in targeted drug delivery. The polymers will be designed to have high affinity for the fermentation product. This affinity however can be 'switched-off' when exposing the polymers to different conditions (e.g. change in pH, temperature, medium conditions, etc.). This effect will be used to effectively harvest the reaction product from the fermenter, thereby increasing the productivity of the microorganisms due to the removal of the toxic product, followed by simple and cost effective recovery of the product from the polymer in a recovery vessel with conditions triggering the product release.


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DSM 13864 - *Clostridium saccharobutylicum* Keis et al. 2001

-see also: [Bacterial Nomenclature Up-to-Date](#)

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Name: ***Clostridium saccharobutylicum*** Keis et al. 2001

DSM No.: 13864

Other collection no. ATCC BAA-117

Information: < - D. T. Jones, New Zealand < - D. T. Jones and D. R. Woods, South Africa < - Natl. Chem. Products Ltd., South Africa; NCP 262 (*Clostridium acetobutylicum*) < - Commercial Solvent Corp., USA; BAS/B3/CSC < - Commercial Solvent Corp., USA (*Clostridium saccharo-butyl-acetonicum-liquefaciens*). Soy beans; USA. **Type strain**. Taxonomy/description (8292). Commercial production of acetone and butanol (by biotransformation of fermentable carbohydrates). (Medium 104b, 35°C, anaerobic)

Isolated from: soy beans

Medium: [104b](#), 35°C, anaerobic

Literature: [8292](#)

Supplied as: (vacuum) dried culture (actively growing cultures available on request at an extra charge)

Risk group: 1 (classification according to German [TRBA](#))

Price: EURO 50 (non-profit making institutions),
EURO 65 (other institutions): [Normal price](#).

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DSMZ 1731 - *Clostridium acetobutylicum* McCoy et al. 1926 emend. Keis et al. 2001

-see also: [Bacterial Nomenclature Up-to-Date](#)

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Name: ***Clostridium acetobutylicum*** McCoy et al. 1926 emend. Keis et al. 2001

DSM No.: 1731

Other collection no. ATCC 4259, NCIB 619

Information: <- NCIB <- NCTC (*C. acetonigenum*) <- A.C. Thaysen (*Bacillus butyricus*). DNA homology (6850). Produces acetone and n-butanol (Brit. Pat. 4845; U.S. Pat. 1,315,585) (1092). (McCoy and McClung, strain T; L.S. McClung, 2289). (Medium 411, 37°C, anaerobic)

Medium: [411](#), 37°C, anaerobic

Literature: [1092](#), [3352](#), [3353](#), [6850](#)

Supplied as: (vacuum) dried culture (actively growing cultures available on request at an extra charge)

Risk group: 1 (classification according to German TRBA)

Price: EURO 50 (non-profit making institutions), EURO 65 (other institutions): [Normal price](#).

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DSMZ 6422 - *Clostridium beijerinckii* Donker 1926 emend. Keis et al. 2001

-see also: [Bacterial Nomenclature Up-to-Date](#)

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Name: ***Clostridium beijerinckii*** Donker 1926 emend. Keis et al. 2001

DSM No.: 6422

Other collection no. NRRL B-592

Information: < - NRRL < - E. McCoy, A-39 (*Clostridium butylicum*). DNA homology (6850). Produces acetone and n-butanol (4186). (Medium 54 or 104b or 411, 35°C, anaerobic)

Medium: [54](#), 35°C, anaerobic or medium [104b](#), 35°C, anaerobic or medium [411](#), 35°C, anaerobic

Literature: [4186](#), [6850](#)

Supplied as: (vacuum) dried culture (actively growing cultures available on request at an extra charge)

Risk group: 1 (classification according to German [TRBA](#))

Price: EURO 50 (non-profit making institutions), EURO 65 (other institutions): [Normal price](#).

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DSM 6423 - *Clostridium beijerinckii* Donker 1926 emend. Keis et al. 2001

-see also: [Bacterial Nomenclature Up-to-Date](#)

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Name: ***Clostridium beijerinckii*** Donker 1926 emend. Keis et al. 2001

DSM No.: 6423

Other collection no. NRRL B-593

Information: < - NRRL < - E. McCoy, A-21 ("*Clostridium butylicum*"). DNA homology (6850). Produces 1,3-propanediol from glycerol (4188), butanol and isopropanol (4186). (Medium 54 or 104b or 411, 35°C, anaerobic)

Medium: [54](#), 35°C, anaerobic or medium [104b](#), 35°C, anaerobic or medium [411](#), 35°C, anaerobic

Literature: [4186](#), [4188](#), [6850](#)

Supplied as: (vacuum) dried culture (actively growing cultures available on request at an extra charge)

Risk group: 1 (classification according to German [TRBA](#))

Price: EURO 50 (non-profit making institutions), EURO 65 (other institutions): [Normal price](#).

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Each microorganism of the DSMZ collection is classified according to German legislation ([Biostoff-Verordnung](#)). If a strain is allocated to **Risk Group 2** (equivalent terms are Hazard Group or Biological Safety Level), this information is given in the DSMZ catalogue of strains/homepage under the respective species information, on the delivery note and on the culture vessel.

The distribution of cultures allocated to Risk Group 2* are subject to restrictions according to "Infektionsschutzgesetz" (Act dealing with the prevention and control of infectious diseases in man, IfSG from 20th July 2000, BGBl. I, p. 1045). These restrictions apply only to orders placed from within Germany. A copy of the "Erlaubnis zum Arbeiten mit Krankheitserregern" according to §44 [IfSG](#) resp. §19 "Bundesseuchengesetz" (BGBl. I 1979, p. 2262 and BGBl. I 1990, p. 2002) must be provided. Outside Germany the respective national regulations have to be observed. Risk group 2 organisms are shipped to OECD und EU member countries, to EEA - EFTA countries, and to the sovereign European microstates.

*see [TRBA](#) (Technische Regeln für Biologische Arbeitsstoffe): 'Einstufung von Bakterien in Risikogruppen' ([TRBA 466](#)) and 'Einstufung von Pilzen in Risikogruppen' ([TRBA 460](#)); Council Directive 2000/54/EEC on the protection of workers from risks related to exposure to biological agents at work (OJ L262/21 of 17.10.2000).



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Clostridium spp. - Material Safety Data Sheets (MSDS)

MATERIAL SAFETY DATA SHEET - INFECTIOUS SUBSTANCES

SECTION I - INFECTIOUS AGENT

NAME: *Clostridium* spp. (*C. histolyticum*, *C. butyricum*, *C. septicum*, *C. sordellii*, *C. novyi*, *C. ramosum*, *C. bifermentans*, *C. parapaperfringes*, *C. cadaveris*, *C. clostridiiforme*, *C. innocuum*, *C. limosum*, *C. paraputrificum*, *C. sporogenes*, *C. subterminale*, *C. tertium*)

SYNONYM OR CROSS REFERENCE: N/A

CHARACTERISTICS: Gram positive rod usually with subterminal spores; anaerobic

SECTION II - HEALTH HAZARD

PATHOGENICITY: Involved with gas gangrene of wounds; *C. septicum* - bacteremia and infections associated with malignancy; *C. butyricum* - necrotizing enterocolitis in infants; opportunistic pathogen

EPIDEMIOLOGY: Worldwide, especially in areas where contact with contaminated soil is likely

HOST RANGE: Humans, animals

INFECTIOUS DOSE: Not known

MODE OF TRANSMISSION: Introduced through a wound contaminated with soil

INCUBATION PERIOD: Usually 1-3 days

COMMUNICABILITY: Not directly transmitted from person to person

SECTION III - DISSEMINATION

RESERVOIR: Intestine of animals including humans; soil; animal feces

ZOONOSIS: None

VECTORS: None

SECTION IV - VIABILITY

DRUG SUSCEPTIBILITY: Susceptible to clindamycin, chloramphenicol, erythromycin, tetracycline

SUSCEPTIBILITY TO DISINFECTANTS: Sporeformers are fairly resistant; moderate susceptibility to 1% sodium hypochlorite; susceptible to glutaraldehyde (prolonged contact time)

PHYSICAL INACTIVATION: Sporeformers are fairly resistant to heat (spores destroyed by moist heat 121°C for at least 15 min)

SURVIVAL OUTSIDE HOST: Spores survive for long periods outside host

SECTION V - MEDICAL

TOXICITY: ...
SYMPTOMS: ...
FIRST AID: ...
MEDICAL TREATMENT: ...

SECTION VI - LABORATORY HAZARDS

HAZARD IDENTIFICATION: ...
CLASSIFICATION: ...
PRIMARY HAZARDS: ...
SPECIAL HAZARDS: ...

SECTION VII - RECOMMENDED PRECAUTIONS

PERSONAL PROTECTION: ...
ENVIRONMENTAL PRECAUTIONS: ...
HYGIENE: ...
OTHER PRECAUTIONS: ...

SECTION VIII - HANDLING INFORMATION

STABILITY: ...
REACTIVITY: ...
DISPOSAL: ...
TRANSPORT: ...

SECTION IX - MISCELLANEOUS INFORMATION

Date prepared: ...
Date Approved by: ...
Prepared by: ...
Approved by: ...

Date Modified: 1997-10-11

----- Original Message -----

Subject:Re: Fwd: Clostridium spp. - Containment Level Request

Date:Thu, 15 Apr 2010 10:43:12 -0400

From:Permit-Permis <permitpermis@phac-aspc.gc.ca>

To:Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

The four species of Clostridium listed are all Risk Group 1 in Canada.

The DSMZ classification does not always match the Canadian Risk Group classification, so you can not rely of the DSMZ classification for Canadian importation requirements.

Regards,

Denis Laframboise

Regulatory Technologist/ technologiste en réglementation

Pathogen Regulation Directorate (formerly Office of Laboratory Security) /

Direction de la réglementation des agents pathogènes (anciennement le Bureau de la sécurité des laboratoires)

Public Health Agency of Canada/ Agence de santé publique du Canada

100 ch. Colonnade Rd. AL: 6201A Ottawa, Ontario, Canada K1A 0K9

Tel: (613) 957-1779

Fax: (613)941-0596

Subject: Clostridium spp. - Containment Level Request
From: Jennifer Stanley <jstanle2@uwo.ca>
Date: Wed, 14 Apr 2010 11:44:49 -0400
To: Geneviève Lacroix <genevieve.lacroix@phac-aspc.gc.ca>

Hello there

There is a researcher who is proposing work with *Clostridium spp.*

The stains have been classified by the German "TRBA" (this appears to be their biosafety guidelines) as Risk Group 1 - see the four websites below.

<http://www.dsmz.de/microorganisms/html/strains/strain.dsm006422.html>

<http://www.dsmz.de/microorganisms/html/strains/strain.dsm006423.html>

<http://www.dsmz.de/microorganisms/html/strains/strain.dsm013864.html>

<http://www.dsmz.de/microorganisms/html/strains/strain.dsm001731.html>

I know that Health Canada normally classifies *Clostridium spp.* as Level 2. **Is there any equivalency with the German guidelines here or do we need to use Level 2 for these strains of bacteria?**

Regards,
Jennifer