THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: February 18, 2011
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 (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, 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 Biohazards 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	Dr. Argyrios Margaritis
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EMERGENCY PHONE NUMBER(S)	" "
EMAIL	amarg@uwo.ca

Location of experimental work to be carried out: Building(s) SEB, TEB Room(s) 2035, 2033 & 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 15.0, Approvals).

FUNDING AGENCY/AGENCIES: NSERC
GRANT TITLE(S): LIGNOCELLULOSIC BUTANOL PRODUCTION FROM ANGRICULTURAL WASTE RESIDUES USING SOLVENTOGENIC CLOSTRIDIA

UNDERGRADUATE COURSE CODE (IF APPLICABLE): _____
List all personnel working under Principal Investigators supervision in this location:

Name	UWO E-mail Address	Date of Biosafety Training
Dr. Peter M. Kilonzo Postdoctoral Research Associate	pkilonz2@uwo.ca or pkilonzo@uwo.ca	2005-Present

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.

↙ Details?

Yeast (*S. cerevisiae, Pichia pastoris*) and bacteria (*C. acetobutylicum, C. beijerinckii*) are received from freeze-dried vials obtained from ATCC then grown in shake flasks after steam sterilization of liquid medium. All preparation work is done within the laminar sterile hood Biosafety cabinet level 1. After use, the petri-dishes that have the remaining Clostridia cells are autoclaved and then stored in special yellow bags for disposal. Prior to disposal, spent liquid media are autoclave at 121°C and 1 atm for 60 min to destroy all cells and spores present.

Please include a one page research summary or teaching protocol.

1 Abstract:

Clostridium acetobutylicum ATCC 824 was grown on a variety of different sugars [D-(+)-glucose, D-(+)-xylose, D-(+)-mannose, D-(+)-galactose, D-(-)-arabinose, and D-(+)-cellobiose] found in agricultural waste hydrolysates and assayed for acetone, butanol, and ethanol (ABE) production. The order of sugar utilization by the culture was D-(+)-glucose > D-(+)-cellobiose > D-(+)-mannose > D-(+)-xylose > D-(-)-arabinose > D-(+)-galactose. The high D-(+)-glucose utilization of 98% resulted to higher cell density of 2.49 g/L with the highest growth rate of 0.293 h^{-1}, than other sugars. In this system, ABE concentration of 18.3 g/L, was triggered by a total acid concentration of 11.5 g/L, but growth cessation took place at a total butanol and acid concentration of 24.1 g/L. Although the culture utilized 92% sugar from D-(+)-cellobiose and 86% from D-(+)-xylose, the resultant biomass concentration (1.53 and 1.82 g/L) was very low. In these system, a total acid concentration between 14 and 15 g/L triggered 19.0 and 13.9 g/L ABE g/L from D-(+)-cellbiose and D-(+)-xylose, respectively. Relatively high yield (0.33 g/g), productivity (0.47 g/L.h), and low growth rate of 0.069 h^{-1} resulted from the D-(+)-cellobiose system. In both systems, growth cessation occurred at a total butanol and acid concentration between 25 and 27 g/L. Culture grown on mixed sugars utilized 70-90% total sugars. A total acid concentration between 23 and 28 g/L triggered only 13-16 g/L ABE, with relatively high yield and productivity of 0.37 and 0.43 g/L.h, whereas growth inhibition occurred at a total butanol and acid concentration between 30 and 39 g/L.

Keywords: solvents, acetone-butanol-ethanol, butanol, acids, individual sugars, mixed sugars, *C. acetobutylicum* ATCC 824

2 Abstract

Production of glucoamylase by recombinant *Saccharomyces cerevisiae* C468/pGAC9 (ATCC 20690) in a continuous stirred tank bioreactor was studied at different dilution rates. Plasmid stability was found to be growth (dilution rate) dependent; it increased with the dilution rate. Bioreactor productivity and specific productivity also increased with the dilution rate. A kinetic equation was used to model the plasmid stability kinetics. The growth rate ratio between plasmid-carrying and plasmid-free cells decreased from 1.397 to 1.215, and segregational instability or probability of plasmid loss from each cell division decreased from 0.059 to 0.020 as the dilution rate increased from 0.10 to 0.37 1/h. The specific growth rates increased with dilution rate, while the growth rate difference between plasmid-carrying and plasmid-free cell populations was negligible. This was attributed to the low copy number of the hybrid plasmid pGAC9. Thus, the growth rate had no significant effect on plasmid instability. The proposed kinetics was consistent with experimental results, and the model simulated the experimental data well.

Keywords: *Saccharomyces cerevisiae*, Glucoamylase, Kinetics, Dilution rate, Plasmid stability

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? • YES O NO
(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? 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:
N/A
_____
_____
_____

1.2 Please complete the table below:

Name of Biological Agent(s)* (Be specific)	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
BACTERIA : *Clostridium acetobutylicum* ATCC 824 (Production of biofuel-biobutanol from sugars) *Clostridium beijerinckii*	O Yes • No	O Yes • No	O Yes • No	210mL	ATCC	• 1 O 2 O 2+ O 3

(Production of biofuel-biobutanol from starch)						
YEAST: *Saccharomyces cerevisae* (production of enzymes from sugars)	O Yes • No	O Yes • No	O Yes • No			• 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 2+ O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 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 • 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)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	O Yes • No			
Rodent	O Yes • No			
Non-human primate	O Yes • No			
Microorganism	• Yes O No	Bacteria Yeast	1 1	ATCC ATCC

*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 O 2 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 • 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/UNKNOWN**	**Name of Infectious Agent (If applicable)**	**PHAC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Blood (fraction) or other Body Fluid		O Yes O Unknown		O 1 O 2 O 2+ O 3
Human Organs or Tissues (unpreserved)		O Yes O Unknown		O 1 O 2 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? • YES NO If no, please proceed to Section 5.0

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

Yeast Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection
Saccharomyces cerevisiae 468	*pGAC9*	yeast 2 μ plasmid (2 μ micron circle),	*LEU* gene glucoamylase gene from *Aspergillus awamori*	. The *S. cerevisiae* host strain C468 (α *leu2-3 leu2-112 his311 his3-15 mal-*) (ATCC 62995) is haploid, with auxotrophic markers for leucine and histidine and carries mutation (*mal-*) blocking the utilization of maltose as carbon source. Therefore, the host cell is complementary to the leucine prototrophy by inserting the selectable marker (*LEU* 2) into the expression plasmid and the presence of the glucoamylase gene on the plasmid allows the host cell to grow on maltose

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

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?
O 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 O YES, please specify _____ • NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ • NO
- SV 40 Large T antigen O YES • NO
- E1A oncogene O YES • NO
- Known oncogenes O YES, please specify _____ • NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ • NO

4.5 Will virus be replication defective? O YES • N/A

4.6 Will virus be infectious to humans or animals? O YES • N/A

4.7 Will this be expected to increase the containment level required? O YES • N/A

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? O 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? 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 • 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:

_____

_____

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)? O YES • No If no, please proceed to section 8.0

7.2 Will live animals be used? O YES O No

7.3 If yes, please specify the animal(s) used:

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

7.4 If no live animals are used, please specify the source of the specimens:

_____

8.0 Biological Toxins and Hormones

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

8.2 If YES, please name the toxin(s) or hormones(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 or hormone_____

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

8.5 How much of the toxin or hormone is stored*? _____

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

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

9.0 Insects

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

_____

_____

10.0 Plants

10.1 Do you use plants? 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 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? O 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? O YES O NO

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

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O 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 O 2 O 2+ O 3

13.2 Has the facility been certified by OHS for this level of containment?

• YES, date of most recent biosafety inspection: MARCH 01/2011

O NO, please certify

O NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): _____

14.0 Procedures to be Followed

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

NO ADDITIONAL RISK REDUCTION REQUIRED

14.2 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash:

NO CONCERN FOR THE BIOLOGICAL AGENT LISTED

14.3 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/

Dr. A. Margaritis

E-mail: amarg@uwo.ca

SIGNATURE _____ Date: June 06 2011

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:	**824™** Order this Item	Price:	**$205.00**
Organism:	*Clostridium acetobutylicum* McCoy et al. emend. Keis et al. deposited as *Granulobacter pectinovorum* (Stormer) Beijerinck		
Designations:	[CCRC 10639, CCUG 42182, DSM 792, IAM 19013, IFO 13948, JCM 1419, KCTC 1790, L.S. McClung 2291, LMG 5710, McCoy and McClung strain W, NCCB 29024, NCCB 84048, NCIMB 8052, VKM B-1787]		
Isolation:	plant-derived foodstuff (corn meal)		
Depositor:	ER Weyer		
Biosafety Level:	1		
Shipped:	freeze-dried		
Growth Conditions:	ATCC medium2107: Reinforced clostridial broth (modified) **Temperature:** 37.0°C **Atmosphere:** Anaerobic		
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.		

Related Links

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824.pdf

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Bacteria

ATCC® Number: **858™** Order this Item Price: **$255.00**

Organism:	*Clostridium beijerinckii* Donker emend. Keis et al.
Designations:	LMD 25.10 [NCIB 11373, NCTC 2264, VPI 11896]
Isolation:	pasteurized garden soil
Depositor:	J van der Toorn
History:	ATCC <<--J van der Toorn<<--H.J.L. Donker 7 (<<--- A.J. Kluyver <<--- H.J.L. Donker)
Biosafety Level:	1
Shipped:	freeze-dried
Growth Conditions:	ATCC medium38: Beef liver medium for anaerobes **Temperature:** 37.0°C Duration: anaerobic
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.
References:	5543: Donker HJLBijdrage tot de Kennis der Botersuur-, butylacoholen acetonigistingen Ph.D. thesis, Delft Univ. Technol., 1926

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Fungi ,Yeasts and Yeast Genetic Stock

ATCC® Number: **62995™** Order this Item Price: **$275.00**

Organism:	*Saccharomyces cerevisiae* Meyen ex E.C. Hansen deposited as *Saccharomyces cerevisiae* Hansen, teleomorph
Alternate State:	*Candida robusta* Diddens et Lodder
Designations:	NRC 5140 [ATCC 66527, C468, CMCC 1398, LL20, NCYC 1445]
Depositors:	RK Latta
Biosafety Level:	1
Shipped:	frozen
Genotype/ORF/ Gene Name:	MATalpha leu2-3 leu2-112 his3-11 his3-15 [psi+] [cir+]
Growth Conditions:	ATCC medium 1245: YEPD **Temperature:** 25.0°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.
Applications:	transformation host [20995] [20124]
Mating Type:	alpha
Karyotype:	Ploidy: haploid
Comments:	Expression of Aspergillus awamori glucoamylase gene [20314] [21188] Sensitive to Kluyveromyces lactis toxin [20534]
Subcollection:	Yeasts

References:

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Clone

ATCC® Number:	**20690** Order this Item	Price:	**$275.00**

Designation:	pGAC9 [pYepGAC9]
Depositors:	Cetus Corp., A Belt, Cetus Corp.
Insert Source:	*Saccharomyces cerevisiae* Meyen ex E.C. Hansen
Insert Information:	DNA: cDNA Insert lengths(kb): 2.109999895095825 Gene product: glucoamylase
Biosafety Level:	1
Shipped:	frozen
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.
Vector:	Size (kb): 10.6700000762939500 Vector: pAC1 (plasmid) Promoters: Promoter enolase I Construction: pBR322, peno 46, LEU2, 2 micron Marker(s):LEU2,ampR Construct size (kb): 10.67000007629395 Features: marker(s): ampR, LEU2 promoter: enolase I replicon: pMB1, 2 micron terminator: enolase I
Comments:	Production of glucoamylase [12209] The insert contains the full-length cDNA and 3' poly(A), minus the four introns. [12209]
Media Description:	ATCC medium 1212: Yeast synthetic minimal medium
References:	12209: Nunberg J, et al. Glucoamylase cDNA. US Patent 4,794,175 dated Dec 27 1988

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