Protocol #:

UNIVERSITY OF WISCONSIN LA CROSSE UWL

IBC Biosafety Protocol Application

Section I: Principal Investigator & Project Overview

A. Principal Investigator (PI)

Name[1]:	[redacted]	Department:	[redacted]
Email:	[redacted]	Employee Classification:	Faculty

B. Project Overview

Project Title:	Display of amylases on the surface of Gluconobacter oxydans to enable starch utilization
Course Number & Name[2]:	Click or tap here to enter text.

Project Type: ☒Research ☐Teaching

Application Type: ☒New ☐Renewal ☐Revision[3]

If a renewal or revision:
Protocol number: Click or tap here to enter text.
Summarize change(s): Click or tap here to enter text.
List revised protocol section(s): Click or tap here to enter text.

Funding:

Is this project associated with external award(s)? ☐Yes ☒No If yes, complete information below.

Sponsor	Status	Award # (if current)	Start Date	End Date
Click or tap here to enter text.	☐Current ☐Pending	Click or tap here to enter text.	Click or tap to enter a date.	Click or tap to enter a date.
Click or tap here to enter text.	☐Current ☐Pending	Click or tap here to enter text.	Click or tap to enter a date.	Click or tap to enter a date.

Location(s):

List all locations where biological materials will be used, stored, or handled. Add lines if needed.

Building	Room Number	Containment and/or Storage Equipment (e.g., biosafety cabinet refrigerator, freezer, dewar)
[redacted]	[redacted]	Refrigerator, freezers, incubator Autoclaves
Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.
Click or tap here to enter text.	Click or tap here to enter text.	Click or tap here to enter text.

C. Project Summary

Provide a brief description of the research project(s) in which the materials and/or organisms addressed in Section II will be used.

Acetic acid bacteria are an industrially important group of organisms because of their unique metabolism, in which they incompletely oxidize sugars rather than completely oxidize them to water and CO2. Products produced by these organisms include acetic acid, cellulose and vitamin C among other things. Industrial products are currently produced from simple sugars (e.g. glucose). This is because acetic acid bacteria can only be grown on media that is high in sugars, such as glucose, and is incapable of growth on complex carbohydrates. The proposed project aims to engineer the model acetic acid bacteria, Gluconobacter oxydans (G. oxydans), to be able to use the complex carbohydrate starch for growth and industrial production. To this end, we will create a library of 24 unique plasmids that contain genes to optimize the growth of G. oxydans on a medium containing starch. The DNA source material used for cloning will be synthetically constructed, eliminating the need to grow organisms for which the amylases are derived. Starch is an abundant agricultural waste product and is more cost effective than glucose, so starch degrading strains are expected to make products created by G. oxydans cheaper.

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[1] Only a UWL faculty or staff member may be listed as the PI on a Biosafety Protocol Application. All other project personnel, including students, must be listed in Section IV. Personnel.

[2] If lab courses involve recombinant materials, they are subject to NIH Guidelines, and a protocol is required. Lab courses not involving other biological materials but not recombinant materials may submit a protocol but are not required to do so.

[3] For revisions to research elements, biological materials used, and/or locations, complete this form. For personnel or award modifications, submit the IBC Personnel & Award Modification Form.

Section II: Biological Materials

A. Recombinant Materials

Complete this section if working with any recombinant or synthetic DNA/RNA materials. Provide the following information, and expand the table if needed:

- Gene name(s) and acronym(s)
- All pertinent biological activities of the encoded protein(s) (e.g., normal biological function, oncogenic potential, toxicity) – If unknown, indicate "unknown" and explain. Address the suspected nature of the gene, if any.
- Biological source/origin (genus and species)
- Risk group (RG) of the source organism(s) – see ABSA Risk Group Database
- Vector(s) (bacterial plasmid, virus, or other vector)
- Host(s) (genus, species, strain, tissue, cell line) that the recombinant material might be inserted into
- Risk group (RG) of the host – see ABSA Risk Group Database

Name of Gene or Gene Fragment	Nature of Gene	Source Organism(s)	RG of Source Organism	Vector(s)	Host Administered to	RG of Host
1. ANI_1_820034	Glucoamylase that breaks down starch	None: Commercial synthetic cDNA derived from the Aspergillus niger genome	2 (synthetic DNA only, non-replicative)	PBBRp452 RL1-3/FL1-3-oprf188; PBBRp264 RL1-3/FL1-3-oprf188; PBBRp0169-RL1-3/FL1-3-oprf188;	Escherichia coli 10b	1
2. ANI_1_820034	Glucoamylase that breaks down starch	Commercial synthetic cDNA derived from the Aspergillus niger genome	2 (synthetic DNA only, non - replicative)	PBBRp452 RL1-3/FL1-3-oprf188; PBBRp264 RL1-3/FL1-3-oprf188; PBBRp0169-RL1-3/FL1-3-oprf188;	Escherichia coli S17-1	1
3. ANI_1_820034	Glucoamylase that breaks down starch	Commercial synthetic cDNA derived from the Aspergillus niger genome	2 (synthetic DNA only, non - replicative)	PBBRp452 RL1-3/FL1-3-oprf188; PBBRp264 RL1-3/FL1-3-oprf188; PBBRp0169-RL1-3/FL1-3-oprf188;	Gluconobacter oxydans 621H	1
4. AmyE	a-amylase that breaks down starch	Commercial synthetic DNA derived from the Bacillus subtilis 168 genome	1 (synthetic DNA only, non-replicative)	PBBRp452 RL1-3/FL1-3-oprf188; PBBRp264 RL1-3/FL1-3-oprf188; PBBRp0169-RL1-3/FL1-3-oprf188;	Escherichia coli 10b	1
5. AmyE	a-amylase that breaks down starch	Commercial synthetic DNA derived from the Bacillus subtilis 168 genome	1 (synthetic DNA only, non-replicative)	PBBRp452 RL1-3/FL1-3-oprf188; PBBRp264 RL1-3/FL1-3-oprf188;	Escherichia coli S17-1	1

				PBBRp0169-RL1-3/FL1-3-oprf188;		
6. AmyE	a-amylase that breaks down starch	Commercial synthetic DNA derived from the Bacillus subtilis 168 genome	1 (synthetic DNA only, non-replicative)	PBBRp452 RL1-3/FL1-3-oprf188; PBBRp264 RL1-3/FL1-3-oprf188; PBBRp0169-RL1-3/FL1-3-oprf188;	Gluconobacter oxydans 621H	1

a. For each material listed in the table above, indicate all categories from the NIH Guidelines, Section III that apply.

Section III-D-2-a: The non-toxic glucoamylase derived from RG2 Aspergillus niger genome will be transferred to nonpathogenic RG1 hosts.

Section III-F-1: The nucleic acid encoding the glucoamylase and a-amylase (listed in section II A #1-6 above) are non-replicative fragments derived from genome sequences. The bacteria that encode these genes will not be grown in the laboratory. The nucleic acid will be obtained from a commercial vendor. If accidental release were to occur, this DNA would be inert and does not encode any toxin or harmful product. These will be inserted into expression vectors for replication in Escherichia coli and Gluconobacter oxydans hosts.

Section III-F-4: The AmyE gene from Bacillus subtilis 168 will be transferred to Escherichia coli 10b / S17-1 and Gluconobacter oxydans 621H by well-established physiological means after cloning, namely conjugation and/or transformation.

Section III-F-6: Escherichia coli 10b will be used for initial cloning and DNA transferred to Escherichia coli S17-1, which are natural exchangers in Appendix A-I sublist A.

Section III-F-8: Escherichia coli 10b / S17-1 and Gluconobacter oxydans 621H are all RG1 organisms and will be used in volumes less than 10 L. All nucleic acid products and gene fragments listed in II.A are non-toxic. As such, they pose no or low risk to the environment.

b. Attach plasmid maps as part of the protocol in Section VI: Attachments.

B. Microorganisms, Viruses, & Prions

Complete this section if working with any prokaryotes, fungi, virus, viral vectors, or prions. Provide the following information, and expand the table if needed:

- Organism name(s) (genus, species, strain), name of virus(es), and/or prion name(s) and natural host(s)
- Whether the agent is a human, animal, and/or plant pathogen (and if a plant pathogen, whether it is indigenous to Wisconsin) – if none apply, enter “n/a”
- Risk group (RG) – see ABSA Risk Group Database
- Biosafety level (BSL) – see UWL Biosafety Manual, Summary of Biosafety Levels
- Any rDNA (plasmid, virus, DNA fragment, or other vector)
- Host (genus, species, strain, cell lines) exposed to the microbial agent

Organism, Name of Virus, or Prion Name & Natural Host	**Human, Animal, or Plant Pathogen**	**RG**	**BSL**	**rDNA Added?** *(If yes, indicate identity from II.A.)*	**Administered to host?** *(If yes, reference protocol section)*
1. Escherichia coli 10b	n/a	1	1	Yes (# 1, 4)	no
2. Escherichia coli S17-1	n/a	1	1	Yes (# 2, 5)	no
3. Gluconobacter oxydans 621H	n/a	1	1	Yes (# 3, 6)	no

a. If the identity of your microbial agents, viruses, or prions is unknown, please explain.

b. If any of the microbial agents, viruses, or prions are pathogenic, indicate the host(s) organism(s) at risk of infection.

c. Will any of the microorganisms be grown in volumes of 10 liters or more? If so, indicate which and the volume.

All volumes will be under 10 L.

d. In protocol section III.A., address the following information for each microorganism, virus, or prion: Describe the safety procedures personnel will use to protect themselves from exposure and appropriate response if accidental exposure occurs. Address both collection and research if applicable.

C. Human and Non-Human Animal Tissues, Cell Lines, & Blood Products

Complete this section if working with any human-derived materials *or* non-human animal-derived materials that are infectious, potentially infectious, or recombinant. Provide the following information, and expand the table if needed:

- Type of material used (species, strain, technical name)
- Source
- Risk group (RG) – see ABSA Risk Group Database
- Biosafety level (BSL) – see UWL Biosafety Manual, Summary of Biosafety Levels
- Any vector (bacterial plasmid, virus, or other vector) that will be delivered into the sample – if applicable, indicate identity from other protocol sections
- Host (genus, species, strain) to which the samples will be applied – if applicable, reference protocol section

Type of Material	Source	RG	BSL	Exposed to biological material or rDNA?	Administered to host?
1.					
2.					

a. Does the material contain a known infectious agent?

☐ Yes ☐ No

b. If administering nucleic acids, toxins, nanoparticles, microbes, viruses, or other biohazardous material to animals, describe the route of delivery.

c. In protocol section III.A., address the following information for each material listed above: Describe the safety procedures personnel will use to protect themselves from exposure and appropriate response if accidental exposure occurs.

d. **All work involving live (non-fixed) human-derived materials (e.g., blood or blood components, tissues, secretions), cell lines, and/or bloodborne pathogens must comply with the OSHA Bloodborne Pathogens Standard** (29 CFR 1910.1030). If this is applicable to your project, contact Environmental Health & Safety to ensure compliance with annual training and other UWL Bloodborne Pathogens Program requirements.

D. Biological Toxins

Complete this section if working with any toxin(s) of biological origin. Provide the following information, and expand the table if needed:

- Biological toxin name(s) and acronym(s) if appropriate, biological source/origin (genus species)
- Median lethal dose (LD_{50}) as ng/kg
- Risk group (RG) – see ABSA Risk Group Database
- Biosafety level (BSL) – see UWL Biosafety Manual, Summary of Biosafety Levels
- Whether toxin is a CDC Select Agent (see CDC Select Agents and Toxins List)
- If the gene encoding the toxin will be cloned into a vector (bacterial plasmid, virus, or other vector), or host (genus, species, strain) that the toxin or recombinant materials containing the toxin gene might be inserted into
- Maximum amount administered to each type of recipient at one time (e.g., 0.1 ng)

Biological Toxin and Source Organism	LD_{50} (ng/kg)	RG	BSL	Select Agent? (Y/N)	Administered to host? (Y/N; if yes, reference protocol section)	Max Amount Administered (at one time)
1.						
2.						

a. In protocol section III.A., address the following information for each biological toxin: Describe the safety procedures personnel will use to protect themselves from exposure and appropriate response if accidental exposure occurs.

b. For CDC Select Agent Toxins, address the following information in protocol section III.A. Include information for both collection and research if applicable.

i. Maximum amount of toxin inventory and how you will document inventory (permissible amounts of select toxins are listed on the CDC website)
ii. How the toxin will be stored securely
iii. For toxins that will be reconstituted from a powder, how select toxins will be reconstituted (should be conducted inside containment, e.g., chemical fume hood, biological safety cabinet)
iv. How each toxin will be inactivated (Appendix H of the BMBL describes inactivation procedures)
v. List aerosol generating activities and how an exposure risk will be mitigated
vi. Indicate if sharps will be used in procedures involving toxins.
vii. If administering the toxin to live animals, describe the route of delivery and maximum dose.

E. Vertebrate & Invertebrate Animals

Complete this section if working with any vertebrate or invertebrate animals administered biological materials. Work with vertebrate animals additionally requires IACUC review and approval before initiating work. Provide the following information, and expand the table if needed:

- Animal common name and genus species
- Risk group (RG) – see ABSA Risk Group Database
- Animal Biosafety Level (ABSL) – see UWL Biosafety Manual; BMBL Section V, and NIH Guidelines, Appendix M
- Whether animal is transgenic
- Biological materials administered to the animals – type of materials, quantity, and method of administration
- Housing – type of housing for animals (e.g., static microisolators, rack system), and building(s) and room number(s) where animals will be housed

Vertebrate or Invertebrate (Common name; Genus species)	**RG**	**ABSL**	**Transgenic?** (Y/N)	**Biological Materials Administered** (Type, Quantity, Method of Administration)	**Housing** (Type, Building & Room Number(s))
1.					
2.					

a. Indicate routes of shedding for any biological material administered (e.g., feces, urine, saliva, respiratory droplets, bites).

b. List PPE used to reduce exposure risk when personnel handle the animal(s) listed.

c. Indicate the period of infectivity and shedding for any biological materials administered.

d. List aerosol generating activities involving biological materials and how an exposure will be mitigated.

e. Address any additional information that would facilitate a complete biosafety review.

F. Plants & Soils

Complete the relevant table(s) in this section if working with any of the following:

- Plants that are recombinant (transgenic), exotic, or grown in association with pathogenic or recombinant microbes or pathogenic or recombinant small animals (insects, etc.)
- Foreign soils or domestic soils from counties listed under federal quarantine by the USDA to another US location (see the USDA APHIS federal domestic soil quarantines map)

For Plant Biosafety Levels, see the UWL Biosafety Manual and NIH Guidelines, Appendix L.

Plant (Common name, Genus species)	**Plant Biosafety Level**	**Transgenic?** (Y/N)	**Biological Materials Administered**	**Growth Location** (e.g., greenhouse, growth chamber location)
1.				
2.				

Soil Source Location	Foreign or Quarantined Domestic?	Destination Location	Estimated Quantity	"Active" or Sterilized Soil?
1.				
2.				

a. If applicable, describe how pathogenic organisms will be stored.
b. If applicable, describe when and how pathogenic organisms will be disposed of at the termination of the study.

Additional permits and approvals:

- The import of foreign plant material requires a permit from the USDA. If applicable, approved permits must be attached to this protocol. See USDA APHIS plant import information.
- The import of foreign soils into the continental US requires a permit from the USDA. If applicable, approved permits must be attached to this protocol. See USDA APHIS soil import and permit information.
- The movement of domestic quarantined soil requires authorization by the local APHIS office. If applicable, approval must be attached to this protocol. Contact USDA APHIS PPQ for information regarding quarantine status, soil regulations, or movement eligibility.

Section III: Safety Precautions & Waste Disposal

A. Safety Precautions

Address the following information as applicable:

- Describe the methods for handling materials and/or organisms addressed in Section II.
- If you will be employing Biosafety Level 2, 3, or 4 materials, provide additional information about investigator experience, adequacy of facility design and containment equipment, personnel practices, decontamination and disposal, staff training, and chemical hygiene considerations.
- Address any additional required information as directed in relevant subsections from Section II.
- All laboratories using hazardous chemicals must take actions to minimize exposure to hazardous chemicals as defined in the UWL Chemical Hygiene Program (CHP) and Hazard Communication Policy. The Chemistry & Biochemistry and Microbiology Departments have department-specific CHPs. Hazardous chemical means any chemical that is classified as a physical hazard or a health hazard, a simple asphyxiant, combustible dust, pyrophoric gas, or hazard not otherwise classified. Contact Environmental Health and Safety for additional information.

All molecular work will be done using standard molecular methods. Handling of bacteria will follow standard BSL1 safety procedures for aseptic technique. We will use quaternary ammonia-based disinfectant or 70% ethanol to disinfect all work surfaces. Bacteria will be grown in designated incubators. All lab personnel will be required to wear PPE (e.g. lab coat, eye protection, closed toed shoes, pants or other coverings of the legs, gloves when appropriate). Any spills of microorganisms will be disinfected using quaternary ammonia or 70% ethanol. Safety showers and eye wash stations are available in the event of accidental exposure. Long-term stocks of any strains resulting from this work will be stored at -80C in a 15-20% glycerol solution. Chemical handling and accidental exposure will follow the UWL and Microbiology CHP.

B. Waste Disposal & Terminal Inactivation

In the table below, describe the method of disposal of hazardous substances, animal wastes and carcasses, and residual human substances (e.g., incineration, autoclaving, chemical disinfection). If chemical disinfectant is used, state kind and concentration. Is autoclave monitored with a biological indicator (e.g., spore strips)?

Substance	Disposal method	Description of procedure
Bacteria: Escherichia coli S17-1 Escherichia coli 10b Gluconobacter oxydans 621H	Autoclave	Collection of solid microbial waste in an autoclavable container. Small waste products will have a tabletop bin that is then combined in a large autoclave bag. Dry waste will be collected in a large autoclavable bag and sealed shut when it becomes ¾ full. The bag will be put into a large plastic tray and transported to the

		autoclave room using a rolling cart and autoclaved for at least 90 min. Liquid waste will be collected separately from solid waste. Capped liquid biological waste will be placed in a plastic tray and transported to the autoclave room using a rolling cart and autoclaved for at least 90 min. Autoclaves are maintained by the Microbiology Prep Room staff and are routinely tested using spore strips.
Bacteria: Escherichia coli S17-1 Escherichia coli 10b Gluconobacter oxydans 621H	Chemical disinfection	70% ethanol (10 min minimum contact time) or quaternary ammonia (300-350 ppm, 15 min minimum contact time) will be used to disinfect work benches and small spills. After disinfected, spills will be absorbed with paper towels and discarded as solid autoclave waste.

Section IV: Personnel

In the table below, identify all personnel, including students, who will be working on the biological materials described in this protocol. Copies of required CITI training completion certificates for the PI and all individuals listed below must be included as an attachment (see Section VI. Attachments). For training requirements, see the IBC website.

Teaching laboratory courses: Students enrolling in laboratory courses do not need to be listed below and are not required to complete CITI training modules unless directed by the instructor. Instead, summarize the training provided to students who will be involved in the course (e.g., hands-on training, instructor-based training, online learning) and include the summary as an attachment (see Section VI. Attachments).

Name	Personnel Type	Project Role (e.g., PI, co-PI, research assistant)
	Choose an item.	
	Choose an item.	
	Choose an item.	
	Choose an item.	
	Choose an item.	
	Choose an item.	
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	Choose an item.	
	Choose an item.	
	Choose an item.	

Section V: PI Assurances

I certify that the information contained in this application is accurate and complete. I am familiar with and agree to abide by the NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (current edition), CDC Biosafety in Microbiological and Biomedical Laboratories (current edition), and University of Wisconsin-La Crosse (UWL) Biosafety Manual. Also, I agree to abide by the following requirements:

a. I will not initiate any biological research subject to the guidance and guidelines mentioned above until that research has been registered, reviewed, and approved by the UW-La Crosse (UWL) Institutional Biosafety Committee (IBC). The purview of the UWL IBC includes biological research involving recombinant or synthetic nucleic acids; biological agents and pathogens; human cells, tissues, materials and embryonic stem cells; non-human animal-derived cells, tissues, materials, or samples that are infectious, potentially infectious, or recombinant; animals or plants that are recombinant, exotic, and/or grown in association with pathogens, biological toxins, and/or recombinant materials; select agents and toxins; biological toxins; dual use research of concern (DURC) agents and toxins; and the use of any of these in animal or plant research.
b. I will assure that personnel, including animal care staff or other laboratory support staff, have received appropriate information, including signage, about the biological hazards of the research outlined in this application by making available copies of approved protocols, Biosafety Manuals, and Biological Research Registrations that describe the potential biohazards and precautions to be taken to prevent exposures or release to the laboratory or the environment.
c. I will ensure that laboratory personnel understand the procedures for dealing with incidents and spills of biological materials and know the appropriate waste management procedures.
d. I will work with appropriate university personnel to comply with all training and shipping requirements for the transport of hazardous biological materials (e.g., export controls regulations, US Department of Transportation (DOT) 49 CFR 171-178, International Civil Aviation Organization (ICAO), International Air Transport Association (IATA), US Department of Agriculture (USDA) 9 CFR 122).
e. I will comply with the OSHA Bloodborne Pathogen Standard 29 CFR 1910.1030 if my research includes human cells, tissues, materials, or embryonic stem cells.
f. I will ensure that all laboratory personnel working with biological materials are listed on this application.
g. I will assure that I along with all laboratory personnel have completed all required biosafety training and that their training records are up to date.
h. I assure that all laboratory spaces associated with the research and/or instruction described in this application are listed.
i. I am familiar with and understand my responsibilities as a Principal Investigator as outlined in Section IV-B-7 of the NIH Guidelines.
j. I will assure adequate supervision of personnel and will correct work errors and conditions that could result in breaches of the guidelines and regulations pertaining to this research as listed above.

I understand that failure to adhere to all related requirements may result in penalties outlined in federal and state regulations, sponsor guidelines, and institutional policies such as the IBC Noncompliance Policy.

[redacted]	
Principal Investigator Signature	**Date**

Section VI: Attachments

A. Training

Attach copies of the required CITI training completion certificates for the PI and all individuals, including students, listed in Section IV. Personnel. For training requirements, see the IBC website.

Teaching laboratory courses: Students enrolling in laboratory courses are not required to complete CITI training modules unless directed by the instructor. Instead, summarize the training provided to students who will be involved in the course (e.g., hands-on training, instructor-based training, online learning) and include the summary as an attachment or in the space below.

B. Supporting Materials

Attach plasmid maps and/or any other supporting materials as instructed by applicable subsections in Section II.

[redacted]