GM Microorganism Risk Assessment

Full Assessment, New Format

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Extn:
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Final Classification of Project: **Class 1**

Title: **Production of a Salmonella Pdu Microcompartment in E coli**

Lab: Floor 2, north
Bldg: Wellcome Trust Biocentre

1: Brief Description of Project

Over millennia, eukaryotic cells have evolved sophisticated organelles, which enabled them to partition their cytoplasmic contents into functional sectors (e.g. the nucleus for storage of genetic material). Such compartmentalisation allows greater efficiency of cellular processes, where each organelle is allocated a set of specific metabolic tasks. Some prokaryotes, such as Salmonella enterica, have also developed a method of forming intracellular subdivisions called bacterial microcompartments (BMCs) by expressing a set of proteins that 'cage in' a reaction pathway to make it more efficient. One such set of proteins is expressed from the propanediol utilisation (pdu) operon, which is normally involved in the breakdown of the organic compound propanediol.

To begin with, our team aims to produce a BMC comprised of the seven Salmonella Pdu proteins (Pdu-A, -B, -J, -K, -N, -T, -U) in Escherichia coli, which itself is a close relative of Salmonella. Our intention is to add a poly-histidine affinity tag onto each of the compartment proteins. This will aid in determining whether the proteins have been expressed and may allow us to isolate complete compartments from the cell by affinity purification.

Once the BMC is expressed and characterised, we would like to take the project in a number of different directions as we feel the microcompartment has broad potential. For example, we would like to explore the idea of making the compartments magnetic by targeting iron-rich proteins to them. This would allow us to collect the bacteria and/or compartments using a magnet after they have carried out their task, which could be a very useful attribute (e.g. in bioremediation).

Note: The pdu proteins are derived from the genomic DNA of severely attenuated Salmonella enterica serovar Typhimurium LT2 cells. This project does not involve culture of Salmonella but begins with genomic DNA extraction from a Salmonella cell pellet. The production of, and all work with, the attenuated Salmonella LT2 strain is covered by the previously approved Class 2 risk assessment USOGMM1117.

2: Hazards to Human Health

(a) Associated with recipient micro-organism

The recipient microorganism is a widely used, non pathogenic, non colonising, laboratory adapted strain of Escherichia coli, E coli K12, presenting no hazard to human health.

(b) Arising directly from the inserted genetic material (toxin, oncogene)

The pdu proteins are structural proteins that form the Salmonella propanediol-utilising (Pdu) microcompartment. 1,2-propanediol degradation is implicated in pathogenesis [1] but the individual structural proteins or assembled microcompartment itself are not known to have any harmful properties.

The genes of interest will be cloned into the E coli host using the well characterised vector pT7.5, which has a history of safe use.

(c) Arising indirectly from the inserted genetic material (eg alteration of pathogenicity, host range, tissue

tropism, mode of transmission or host range)

Neither the individual pdu proteins nor the assembled microcompartment will alter the host E coli K12 cells in any way that will make them more hazardous to human health.

(d) Arising from transfer of genetic material to a related micro-organism

The genes encoding BMCs are frequently laterally transferred and already widespread among the bacterial phyla.

3: Assign a provisional Containment Level

◉ Class 1/Level 1 Containment ○ Class 2/Level 2 Containment ○ Class 3/Level 3 Containment

4: Hazards to the Environment

(a) Associated with recipient micro-organism

E coli K12 been shown to survive poorly in the environment, has a history of safe use, and is not known to have adverse effects on microorganisms or plants.

(b) Arising from genetic material

Genes encoding BMCs are frequently laterally transferred and already widespread among the bacterial phyla.

5: Nature of Work

(a) Brief description of nature of work (include maximum culture volumes)

Culture volumes will not exceed 5000ml.
Work will be carried out in accordance with the CLS Containment Level 1 General Lab SOP number 61.

(b) Is a microbiological safety cabinet or isolator required to protect against aerosol transmission?

○ Yes ◉ No

(c) Waste Disposal

Liquid waste is collected in a plastic aspirator flask or other sealable, robust, autoclavable container, autoclaved then disposed of to drains.

Solid waste is collected in a large, lined, cardboard biohazard bin, autoclaved then disposed of as normal refuse.

Large, plastic pipettes are collected in a small, lined cardboard, biohazard bin, autoclaved then disposed of as normal refuse.

Agar plates are collected in a small, lined cardboard, biohazard bin, autoclaved then disposed of as normal refuse.

Large, glass pipettes are fully submerged in 1% Virkon solution overnight, drained then washed.

(d) Are sharps required? YES or NO - if yes justify use.

Yes, when excising DNA bands from agarose gels, but not while working with the live cultures.

(e) If the work involves experimental infection of animals is it known if the animal will shed the genetically modified micro-organism? If YES give details and measures to prevent exposure.

Not applicable.

(f) If the work involves the experimental infection of plants what is known about the likely route of transmission of the genetically modified micro-organism?

Not applicable.

(g) Where will the genetically modified micro-organisms be stored?

Glycerol stocks will be stored in -80 degree freezers in MSI2 and/or WTB2.
All samples must be stored securely and clearly labelled (with the nature of the sample and a contact name) so as to prevent spillage, loss, theft, access by unauthorised personnel or accidental removal. All samples should be stored in appropriate robust, leak-proof containers. An effective mechanism for identifying, tracking and auditing stored samples of micro-organisms must be in place, e.g. up-to-date, detailed inventory of -80°C freezer or liquid nitrogen

cryo-store racks. Records must be available for inspection upon request.

(h) How will the genetically modified micro-organisms be transported within/between buildings to minimise risk of spillage/escape?

Transport Within the Building
It is preferable to transport samples in robust, leak-proof containers. Containers should be labelled with the nature of the sample and a contact name. Containers that do not fulfil these criteria, e.g. glass flasks/bottles, must be transported in a plastic tub, on a trolley. Do not overfill vessels that cannot be sealed for transport, e.g. glass conical flasks.

Transport Out With the Building
Samples must be doubly contained during transport. The inner container/tube must be robust and leak-proof. The outer container must be robust, leak-proof and contain enough absorbent material to absorb the total volume of sample should the inner container leak. The outer container must be sealed during transport and must clearly display the nature of the sample, a contact name, work address and telephone number in case of loss in transit. The individual transporting the package must be trained in how to deal with spillage or loss.

(i) Will staff and students receive any vaccinations or health surveillance?

Not applicable.

(j) Emergency Plan

Not required.

(k) Monitoring

Autoclave Testing and Maintenance
During the first four years after installation an annual 12-point validation test, employing independent thermocouples, is used to demonstrate that the autoclave holds the specified temperature and pressure for the required period of time. Thereafter, autoclaves are serviced every 6 months by a reputable service provider and calibrated annually to ensure the validation criteria are met. During normal, daily operation indicator tape and, in the case of liquid waste, a temperature probe placed at the centre of the load, are used to ensure the required conditions are achieved. Servicing and testing is arranged and test reports are kept by the CLS Health & Safety Coordinator.

Maintaining PPE
Users are required to routinely check their PPE (e.g. lab coat, safety glasses) and keep it in good order. Defective PPE must be repaired or replaced immediately. Laboratory Managers are required to ensure the appropriate PPE is readily available and keep an inspection record for non-standard PPE, e.g. that used in Liquid Nitrogen facilities.

Inspections, Audits and Continual Monitoring
Safety Inspections are carried out regularly to ensure health & safety policy & procedures are being followed and that the required risk assessments and training records are complete and up to date. Inspections will be timetabled and inspection teams selected by the CLS Health & Safety Working Group. Inspection team members will be selected from CLS Health & Safety personnel and senior management. Inspection reports will be submitted to the CLS Health and Safety Management Committee for review. Audits performed by an external, independent body will be are arranged by the CLS Health & Safety Working Group when deemed necessary by the CLS Health & Safety Management Committee. Lab Managers and BIological Safety Advisers are required to continually monitor safety standards and compliance with Health & Safety Policy & Procedures, within their designated area, and report problems and non-compliance to the CLS Health and Safety Working Group.

6: Final classification of project

◉ Class 1 ○ Class 2 ○ Class 3

7: Additional information and comments

1. Shouqiang Cheng, Yu Liu, Christopher S. Crowley, Todd O. Yeates, and Thomas A. Bobik. 2008. Bacterial microcompartments: their properties and paradoxes. BioEssays 30:1084–1095.