GM Microorganism Risk Assessment

Full Assessment, New Format

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Assessment Complete: ◉ Yes

Assessment Reviewed by BSA: ◉ Yes

Permission Granted by HoD: ◉ Yes

Final Classification of Project: **Class 2**

Title: **Creating a new type of synthetic E. coli expressing a type VI secretion system incorporating a C. difficile specific endolysin**

Lab: Floor 2

Bldg: Medical Sciences Institute

1: Brief Description of Project

Many bacterial species have evolved various types of secretion system. Type VI secretion systems are naturally found in gram negative organisms, including Serratia species, Vibrio cholerae and Pseudomonas aeruginosa. A type VI secretion system has also been found in Salmonella typhimurium, which is closely related to Escherichia coli (E. coli). The proteins for Salmonella type VI secretion systems are encoded by more than 13 genes, including Hcp, which encodes for the main structural component of the needle. This projects through the periplasm and outer membrane and can inject directly into competing cells, via the tip protein which is encoded by the gene VgrG. In this way, the type VI secretion system punctures other cells. Hcp and VgrG are largely conserved across all species expressing these systems. TypeVi secretion systems can also be associated with secreted effector molecules, these are thought to play a role in the pathogenesis of higher organisms and could help facilitate interactions with other bacteria.

The aim of our project is to create a new type of synthetic E. coli, expressing a type VI secretion system that will incorporate a C. diff specific endolysin fused with the needle. It is hoped that the needle will be able to puncture the C. diff cells and secrete the endolysin directly into the organism. We hope to prove that this engineered strain of E. coli will be able to produce enough needles on the cell surface to interact with and kill C. diff cells and that enough endolysin is secreted to optimise this killing. We hope to carry out in vitro experiments to test this hypothesis.

In addition to this main experiment, we hope to do some proof of theory experiments which will involve fusing endolysin with Hcp and VgrG in Salmonella typhimurium.

2: Hazards to Human Health

(a) Associated with recipient micro-organism

i) Type VI secretion system (T6SS) genes, PCR'd up from S. typhimurium genomic DNA, will be introduced into disabled, lab adapted strains of E. coli, e.g. DH5 alpha, in stages to produce a combinatorial clone. Such E. coli hosts are incapable of colonising or infecting humans.

ii) The 'proof of theory' experiments utilise the ACDP Hazard Group 2 pathogen S. typhimurium as the host. This serovar does not express verocytotoxin and does not cause typhoid fever. It can give rise to a form of gastroenteritis called ‘salmonellosis’ if contaminated food is ingested. Note that symptoms are mild in healthy adults and most severe in infants and elderly people. Infection requires antibiotic treatment commonly with ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, or ciprofloxacin, ie. therapeutic interventions are available.

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

i) T6SS could facilitate secretion of harmful molecules into target cells but, in isolation, it is not inherently harmful. Once fused with the C. difficile specific endolysin, it would only be considered hazardous to C. difficile cells.

ii) The Hcp/endolysin and VgrG/endolysin constructs are not inherently harmful.

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

tropism, mode of transmission or host range)

i) If the E. coli host produced toxins, enzymes or other molecules that could have a deleterious effect on human cells and the T6SS facilitated or enhanced their secretion, the GM E. coli could be considered more hazardous than the unmodified parent. However, the E. coli strains being use do not produce such molecules.

ii) In terms of the Hcp/endolysin and VgrG/endolysin constructs, if they integrate into the S. typhimurium T6SS, rather than the non fused native proteins, they may have the desired effect of killing C. difficile but will not make the pathogen any more harmful to humans.

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

i) Considering the argument given above, you could end up with a more harmful microorganism if: the entire construct transferred to a microorganism that produced a toxin or other molecule harmful to humans; all T6SS genes were transcribed and translated; and the harmful substrate was successfully targeted and secreted by the T6SS. The likelihood of all these events occurring is extremely low.

ii) Transfer of the Hcp/endolysin and VgrG/endolysin constructs would not make the recipient any more hazardous to humans.

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

i) None.

ii) Salmonella is already widely prevalent in the environment.

(b) Arising from genetic material

i & ii) Because the endolysin is C. difficile specific, the GM strains only pose a greater risk than the wild-type to this particular microorganism.

5: Nature of Work

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

Standard molecular biology and bacterial culture techniques. Culture volumes below 500ml.

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

○ Yes ◉ No

(c) Waste Disposal

<u>For E. coil work</u>
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.
Sharps waste is collected in an autoclavable sharp-safe container, autoclaved then disposed of as clinical waste. The use of sharps should be avoided if possible.

<u>For S. typhimurium Work</u>
Autoclaving conditions of 124 degrees C for 20 min are adequate. Virkon is effective and will be used for surface disinfection.
Liquid waste is collected in a sealable, robust, autoclavable container, autoclaved then disposed of to drains. If using a microbiological safety cabinet store the container within the cabinet during use and seal before removal. Do not use aspirator set-ups.
Solid waste (including plastic pipettes and agar plates) is collected in a red, lined, biohazard labelled, autoclavable bin, autoclaved then disposed of as normal refuse.
Large glass pipettes are not used.
Sharps waste is collected in an autoclavable sharp-safe container, autoclaved then disposed of as clinical waste. If

using a microbiological safety cabinet store the container within the cabinet during use and engage the temporary closure before removal. The use of sharps must be avoided unless essential.

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

No

(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?

MSI Floor 2

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

For E. coli cultures
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.

For S. typhimurium cultures
Samples must be doubly contained during transport within/between buildings & clearly labelled with a contact name & number, the nature of the sample & the biohazard symbol. Inner container/tube must be robust & leak-proof. Outer container must be robust, leak-proof & contain enough absorbent material to absorb the total volume of sample should the inner container leak.

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

Not required

(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.

Microbiological Safety Cabinet Testing and Maintenance (TC suites only)
MSCs are serviced and operator protection (KI) tested on an annual basis by a reputable service provider. A

certificate of conformity to the required standard is displayed on each cabinet. The Health and Safety Information Officer is responsible for arranging the servicing schedule, ensuring fumigation/decontamination is carried out prior to testing and issuing and keeping a copy of the certificates of conformity. Users are required to perform a visual check on all alarms and indicators before each use and report any defects immediately to their Lab Manager. Note: If an MSC is moved to a new location, or equipment in a room containing a cabinet is significantly re-arranged, to the extent where it may affect the airflows within the room, the cabinet must be KI tested before use to ensure operator protection has not been compromised.

Negative Pressure Testing (TC suites only)
Pressure differentials in TC suites are checked regularly to ensure the suite is at an air pressure negative to the immediate surroundings. Checks are arranged by the CLS Health & Safety Information Officer.

6: Final classification of project

○ Class 1 ◉ Class 2 ○ Class 3

7: Additional information and comments

Although the project, due to the genetic modification of viable S. typhimurium, is Class 2 overall, experiments involving only disabled, lab adapted strains of E. coli can be carried out at Containment Level 1.

The following SOPs must be adhered to:
SOP 47 - Containment Level 2: General Laboratory- for S. typhimurium work
SOP 61 - Containment Level 1: General Laboratory - for E. coli work

Note: C. difficile is classified by the ACDP as a Hazard Group 2 pathogen. Therefore, the in vitro experiments referred to in the project description must also be carried out at Containment Level 2.