As of 2024.3

This report should be submitted within 2 weeks after you return to Japan. Please do not change the formatting

（Abroad・Domestic）Internship report form（Student） 2024/07/19

(Year/Month/Day)

| | |
|---|---|
| Name | KAMBOYI Harvey Kakoma |
| Laboratory | Division of Infection and Immunity, International Institute for Zoonosis Control |
| Year (Grade) | D4 |
| Internship institution | Hudson Institute of Medical Research |
| Internship period | Internship period: 05/15/2024 - 07/07/2024<br>Departure Date from Sapporo: 05/13/2024, Arrival Date in Sapporo: 07/08/2024) |

- Purpose and the reason why you chose this institute

My internship aimed to acquire training and conduct collaborative research on detailed molecular analysis of host-pathogenic bacteria interactions. In my future work, I will be involved in zoonosis research and control. Therefore, it is essential to acquire knowledge at a molecular level of the interaction of hosts (humans and animals) and pathogens. In addition, collaborative efforts are necessary to control infectious diseases; thus, the Hudson Institute of Medical Research (HIMR) was an ideal place for my internship. The HIMR is a leading Australian medical research institute recognized internationally for discovery science and translational research. The institute strives to improve human health through groundbreaking, collaborative medical research discoveries and the translation of these to real-world impact. Further, the HIMR chief executive officer, Prof. Elizabeth Hartland, who also leads the Innate Immune Responses to Infection research group at CIIID, is an international leader in the fields of microbiology and immunology. Most notably, she has contributed significantly to understanding underlying host and bacterial molecular mechanisms that determine the outcome of bacterial interactions with human cells and disease development. Therefore, combining the research profile of Prof. E. Hartland and the scope of work being done at the CIIID made me choose HIMR as a suitable site for an internship.

- Result of the activity （about 800 words, provide photos, tables and figures that clearly show the activities during the period）

**1. Orientation and general induction at HIMR**

The HIMR is partnered with Monash University and Monash Health and is co-located at

both organizations at the Monash Health Translation Precinct. Therefore, orientation was conducted at Monash University and Health (Hospital) (Fig. 1A). I was introduced to the twelve (12) specialist research groups and their respective laboratories at the Centre for Innate Immunity and Infectious Diseases (CIIID). I was stationed in the Innate Immune Response to Infection (Hartland Laboratory). After that, I went through the Occupational Health, Safety and Environment (OHSE) and local area induction. The OHSE assists with orienting new staff and students into the institute, providing familiarity with the local area, explaining procedures, and providing details of whom to contact for more information. Induction forms were signed after completing the OHSE and local area induction (Fig. 1B, C). Further, in view of the collaborative research I was going to be involved in, I also signed the intellectual property deed poll (Fig. 1D). To access spaces and facilities at HIMR, I was given access keys to Monash University facilities as voluntary staff and Monash Health as a research student (Fig. 1E, F). In addition, I was given an official HIMR email, which I used to access online resources provided by Monash University and Monash Health.


**Figure 1.** Orientation and induction activities. **A.** Orientation at Monash Health.
**B.** Completed and signed OHSE induction form, Local area (Hartland laboratory) induction form **(C)** and intellectual property deed pool form **(D).**

This activity made me appreciate the collaborative network at HIMR and their contributions towards improving public health through groundbreaking, collaborative medical research discoveries and the translation of these to real-world impact.

## 2. Training on handling and caring for mammalian cell lines for infection

In the Hartland laboratory, their work is aimed at investigating the manipulation of host cell signaling by effector protein families and understanding their influence on host cell function, inflammatory signaling, and the innate immune response. In this way, effector proteins can be used as tools to understand the innate responses that are important for the control of pathogens. The research involves infecting various mammalian cell lines with pathogenic organisms or their isogenic mutants, including *Burkholderia, Shigella, E. coli, Salmonella, Legionella,* and *Lentivirus*. To get the insights and skills needed to conduct these research activities, first, I had to familiarize myself with and understand the standard operating procedures (SOPs) for each mammalian cell line and pathogenic organism. After showing adequate knowledge and competency as judged by my supervisors, I was allowed to have hands-on experience through experimentation (Fig. 2A). I was involved in THP1 monocytes resuscitation from liquid nitrogen, growing them to the point of splitting for the next passage and seeding them for infection experiments. I learned how to stain and enumerate THP1 monocytes using a Neubauer hemocytometer, which is required to split or seed a fixed number of THP1 monocytes. THP1 monocytes can be chronically infected with *Mycoplasma,* which interferes with planned infection experiments. Therefore, resuscitated THP1 monocytes had to be routinely screened for *Mycoplasma*. I conducted *Mycoplasma* testing using the MycoStrip™ - Mycoplasma detection kit on the THP1 monocytes assigned to me; fortunately, they tested negative (Fig. 2B and 2C).


**Figure 2.** Some of the laboratory activities. **A.** Preparation for splitting and seeding THP1 monocytes in a PC level 2 clean room. **B.** Conducting a MycoStripTM assay. **C.** Results of the MycoStripTM assay.

To seed THP1 monocytes, we added phorbol 12-myristate 13-acetate which halted division of cells but induced their differentiation into mature macrophages and increased cell adherence to the floor of the bottom of the 24-well plate (Fig. 3A). The adherent monolayer of THP1 macrophages were easily studied for morphological changes that resulted from infection with a pathogenic organism (Fig. 3B). Morphological changes were observed using the EVOS™ M5000 Cell Imaging System. This imaging system was also used to track fluorescent-tagged effector proteins produced by recombinant pathogenic organisms in the host cellular compartment (Fig. 3C). This gave insights into the subcellular level of interaction between the pathogen and the host cells. This level of training prepared me adequately for my planned *Bacillus cereus* infection in my PhD experiments.

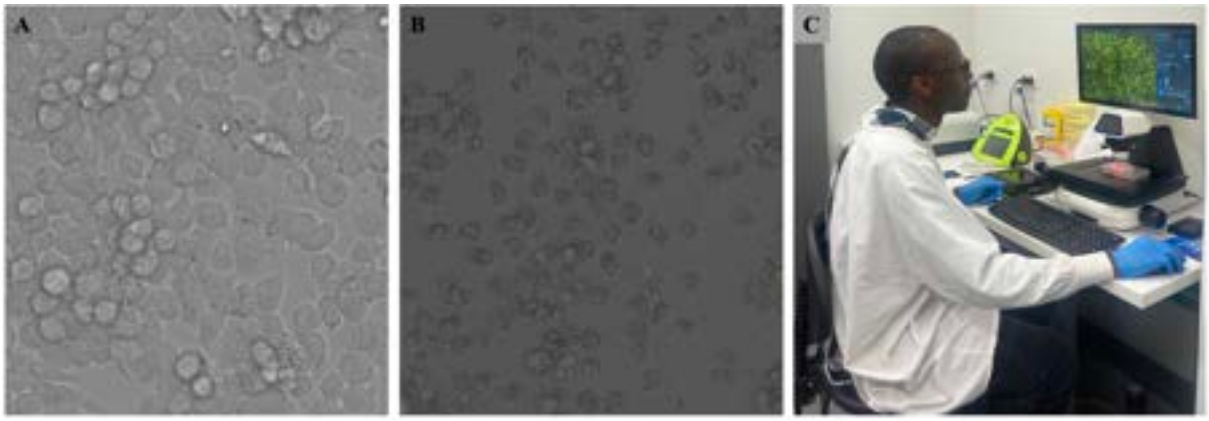


**Figure 3.** Viewing THP1 cells using the EVOS™ M5000 Cell Imaging System. **A.** Uninfected THP1 cells. **B.** Infected THP1 cells. **C.** Viewing of cells infected with a GFP-tagged effector protein.

### 3. Conducting pathogen-specific risk assessment and developing SOPs

In fulfillment of the HIMR guidelines on OHSE, potential risks of any laboratory procedure must be identified and evaluated to determine risk controls. Thereafter, SOPs must be developed for everyone working with or within the laboratory to follow after prior training. As part of the induction process, my supervisors ensured I acquainted myself with relevant SOPs within the Hartland laboratory before I could be involved in any laboratory work. Therefore, given the SFDA to be conducted at the same site, I was required to conduct a risk assessment and develop an SOP for *B. cereus* experiments in a PC2 laboratory. The CIIID had no prior research on *B. cereus*, therefore, its risk assessment had not been done, and SOPs were not in place. Given my long experience working with *Bacillus* species, I led the team from among laboratory members to conduct the risk assessment (Fig. 4A) and develop the SOP (Fig. 4B) for *B. cereus*. Beyond my internship and SFDA at CIIID, we identified a key person within the Hartland laboratory who would oversee all future collaborative research activities on *B. cereus*. This activity demonstrated the importance of public health preparedness and collaboration in achieving

research goals. I believe this mindset is essential in my future career as a zoonosis control expert.


**Figure 4.** Documents developed during my internship. **A.** *B. cereus* risk assessment. **B.** *B. cereus* SOP for infection of THP1 macrophages.

## 4. Other activities

I attended meetings and research seminars hosted by HIMR almost every Thursday from 12:00 to 13:00. The presenters included local and international researchers with an international perspective on current global advances in research. The seminars gave me an opportunity that significantly enriched my understanding of global health problems and solutions for effective pathogen control and prevention (Fig. 4A). I also attended the Hartland laboratory research (Thursday 10:00 to 12:00) (Fig. 4B) and social meetings (Tuesday 14:00) (Fig. 4D. In research meetings, PhD students, postdocs, and research scientists presented their scheduled research progress. I was also given an opportunity to present my internship progress (Fig. 4B). I also participated in a laboratory field trip in the countryside of central Victoria state (Fig. 4C) and Philip Island.


**Figure 4.** Hartland laboratory activities. **A.** Attending an HIMR seminar with lab members and Prof. E. Hartland. **B.** My progress presentation during the lab meeting. **C.** Field trip to the countryside in Victoria state. **D.** Social lab meeting.

What do you think the positive impact of the activity will have on your further career path?

My career path will involve researching infectious diseases and their control in Zambia and internationally, dealing with laboratory and field work. I believe the research network I have been exposed to at HIMR will play a crucial part in actualizing my career plan. The HIMR values that include innovation and partnerships have led the institute to bring together 457 scientists from 29 countries, providing me an opportunity to establish researcher links from Australia, German, Nepal, China, and Ethiopia. In addition, my internship activities have led to a research partnership and collaboration between HIMR and my laboratory, the Division of Infection and Immunity, on *B. cereus* research for the next five years. This partnership will significantly help me develop and advance my research career, working at the international level.

- Report how your activity could link to One Health Approach (If applicable)

If you also conducted OH onsite training (Ally Module 4), please describe some of the examples of One Health approach you implemented in your activity. Or explain the possibility(ies) how you could link this activity to One Health approach for your future.

The activity conducted during the internship training significantly aligns with the principles of the One Health approach, which emphasizes the need for collaborative research and networking among professionals from human, animal, and environmental sectors. For Ally Module 4, I conducted collaborative research on *B. cereus* with individuals from the biomedical sciences and human health sectors originating from different countries, given my background in veterinary medicine. *B. cereus* is an environmental pathogen that causes infection in humans and animals; hence, it is a

zoonotic pathogen and a One Health problem.

- Advice for your junior fellows
- We are fortunate to receive financial support from WISE and other sources, however, we have to pay accommodation, visa, and travel insurance fees upfront and wait for reimbursement later on. Therefore, you need to save enough to cover these fees. If you plan to conduct an internship and SFDA simultaneously at the same site, the accommodation fee may be too much to be up front by yourself. In this case, please engage your laboratory to assist you well in advance.

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| Approval of supervisor | Institution・Official title・Name<br>International Institute for Zoonosis Control, Division of Infection and Immunity. Professor. Hideaki HIGASHI |

※1 A certification form from the host should also be submitted.
※2 The Career Path Committee will first confirm the content of this report and report will be forwarded to the Educational Affairs Committee for credits evaluation.

北海道大学
One Health フロンティア卓越大学院プログラム
One Health Allyコース
Student Free Design Activities報告書

Hokkaido University
WISE Program for
"One Health Frontier Graduate School of Excellence"
One Health Ally Course
Student Free Design Activities Report form

# Student Free Design Activities (One Health on-site Training)
# 報告書 Report

報告者 [Reporter]

| | | | |
|---|---|---|---|
| 氏名 [Full Name] | KAMBOYI Harvey Kakoma | | |
| 学年 [Year] | D4 | E-mail | |
| 所属 [Affiliation] | Graduate School of Infectious Diseases | | |

担当教員 [Instructor]

| | | | |
|---|---|---|---|
| 氏名 [Full Name] | Elizabeth Hartland | | |
| 署名 [Signature] |  | | |
| 所属 [Affiliation] | Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Melbourne, Australia | | |
| E-mail | | | |

活動報告 [Activity Report]
※活動内容が判る様な写真や図表を加えて下さい。/ Provide photos, tables and figures that clearly show the activities during the period.

| | |
|---|---|
| タイトル [Course Title] | Analysis of *Bacillus cereus* T7SS-dependent host interactions |
| 実施期間 [Periods] | 10/06/2024 – 05/07/2024 |
| 共同実施者 [Other participants] | Nil |
| 言語 [Language] | English |
| 実施場所 [Location] | Centre for Innate Immunity and Infectious Diseases, Hudson Institute of Medical Research, Melbourne, Australia |

申請時計画の実施報告 [Report how you carried out your plan in the application form]

I followed the schedule of activities according to the plan with minor modifications based on the initial results of the planned collaborative research. Hence, the transcriptome component of the activity could not be completed within the Student Free-Design Activity (SFDA) period, but our collective efforts will continue in Australia and Japan. This collaborative research will augment my PhD research project on *Bacillus cereus*.

**Prior preparation**

To conduct this collaborative research on *B. cereus*, research permission was first sought from the Monash University Institutional Biosafety Committee. The approval certificate was granted (IBC Reference 34934), and permission to import *B. cereus* strains from Japan was also given by the Department of Agriculture, Fisheries and Forestry of the Australian Government (Permit: 0007003103). Further, Hokkaido University approved the transfer (Approval: 2022-027) of *B. cereus* strains transfer to Monash University for research. The strains were successfully shipped from Hokkaido University to Monash University, Hudson Institute of Medical Research (HIMR). The *B. cereus* strains were resuscitated upon arrival, and glycerol stocks were prepared for long-term storage.

**Experiments on *B. cereus* infection of THP1 cells**

The Centre for Innate Immunity and Infectious Diseases (CIIID) at HIMR had no prior research on *B. cereus*. Therefore, we drafted the SOP for *B. cereus* infection of THP1 macrophages and revised it as we optimized the

conditions for infection experiments. In addition, we conducted the risk assessment for the Occupational Health, Safety, and Environmental hazards for working with *B. cereus* in the PC 2 laboratory.

In setting up the conditions for infection, we used the *B. cereus* wild-type strain and its isogenic type VII secretion system knockout strain *(ΔessC)*. The overall objective of these experiments was to obtain THP1 macrophage response from *B. cereus* infection that can be measured cytometrically and allow for transcriptome analysis. In summary, we titrated the *B. cereus* multiplicity of infection (MOI) from 5 to 0.1 following the protocol illustrated in Figure 1.

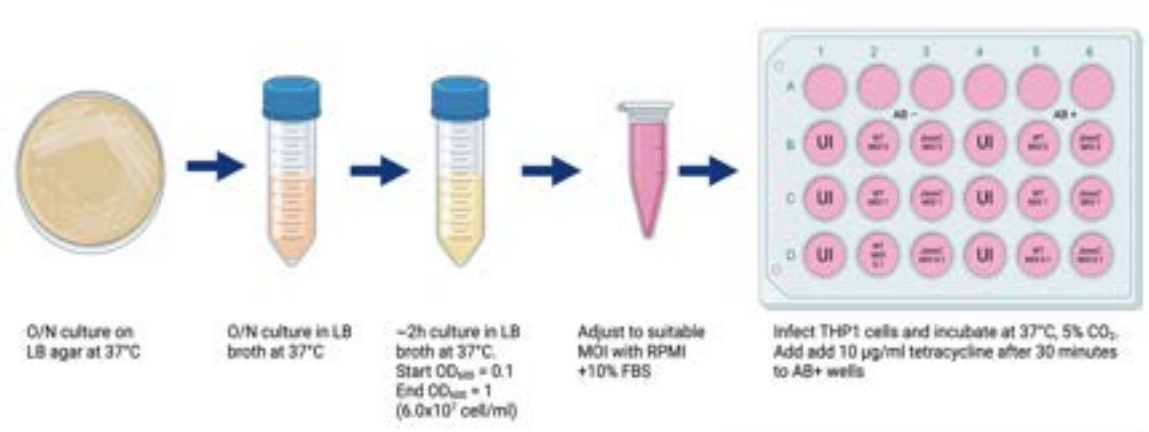


**Figure 1**. Schematic representation of the protocol for infecting THP1 macrophages with *B. cereus* strains.

Unfortunately, almost 90% of THP1 macrophages died within 4 hours post-infection at the lowest possible dose of MOI = 0.1 that would produce a consistent cellular response (Fig. 2a). We observed that *B. cereus* was capable of multiplying too fast in mammalian cell culture media (Fig. 2b). The quick death rate of THP1 macrophages would affect downstream analysis, especially transcriptome analysis. Therefore, we changed the approach of infecting the THP1 macrophages from using actual bacteria to bacterial culture supernatant. We have already established that the T7SS-dependent effectors are secreted into the culture medium.

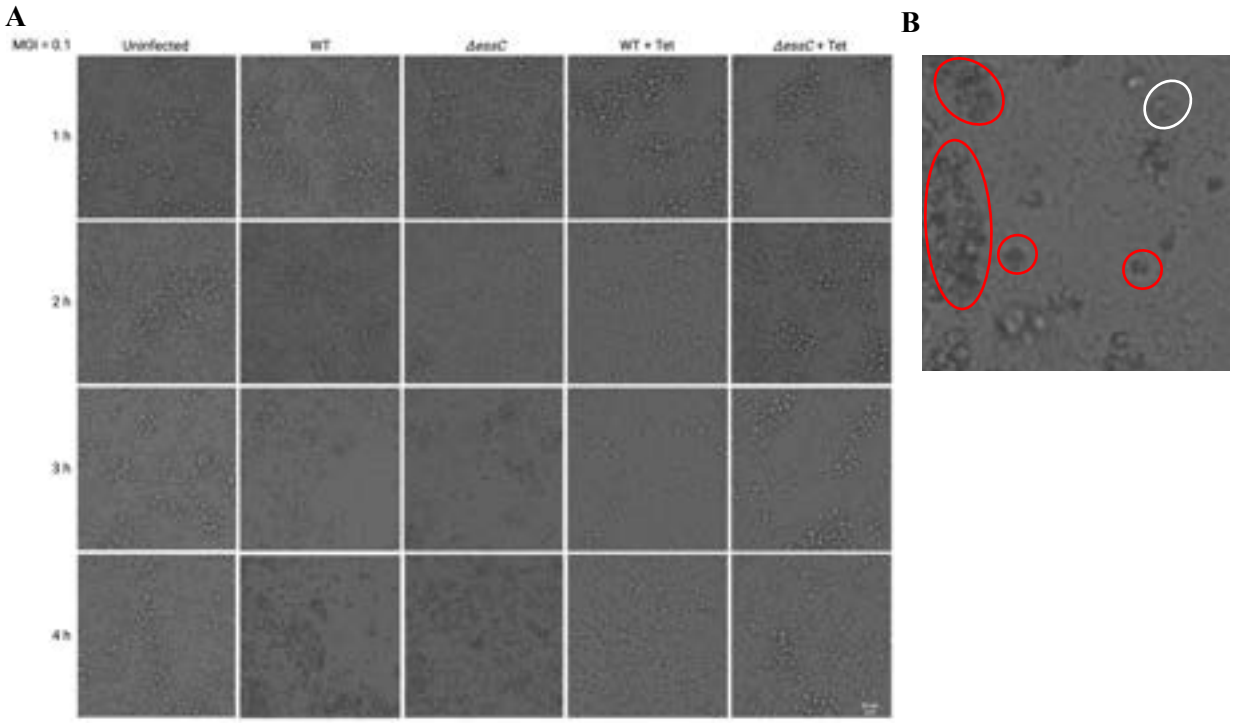


**Figure 2**. *B. cereus* strains caused rapid death of THP1 macrophages. **A**. Representative brightfield images of THP1 macrophages at MOI = 0.1 with and without tetracycline. **B**. Dead and live THP1 cells are encircled in red and white rings. *B. cereus* can be seen as numerous rod-shaped features in the entire image.

THP1 macrophages were consequently exposed to different doses of *B. cereus* WT and *ΔessC* filtered culture

supernatants and then observed for observable responses microscopically (Fig. 3a).

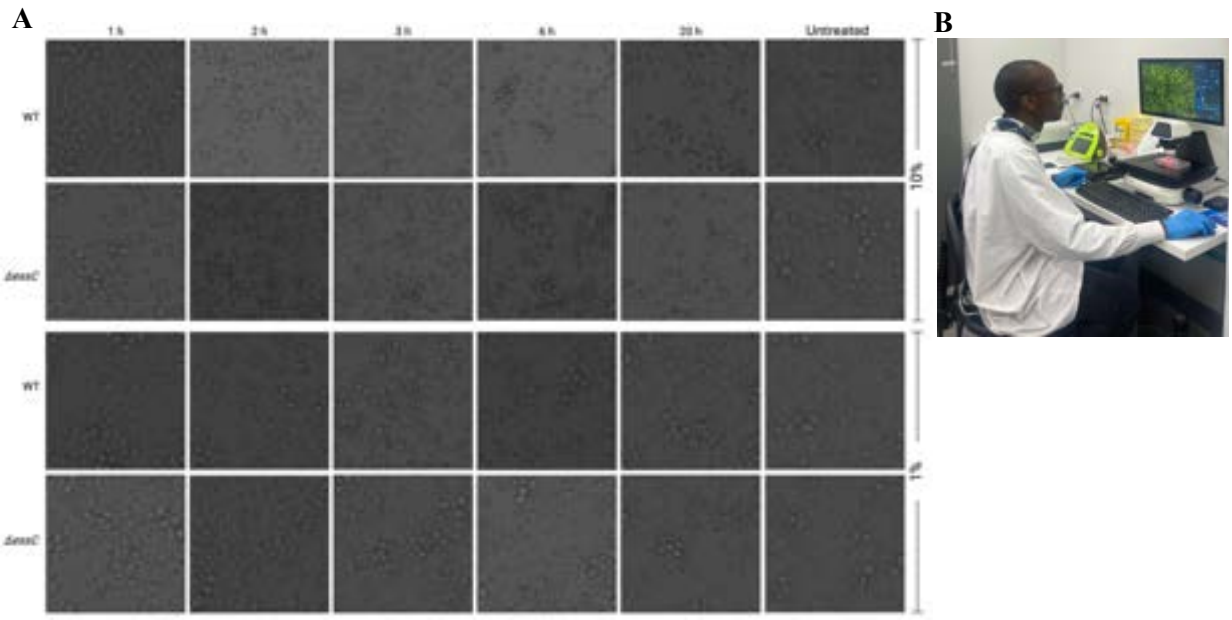


**Figure 3**. *B. cereus* supernatants caused death of THP1 macrophages in a dose-dependent manner. **A**. Representative time-lapse images of THP1 macrophages dosed with 10% and 1% of WT and *ΔessC* culture supernatant. Affected misshaped cells can be seen from 1 h post infection at 10% dose **B**. Viewing cells using the Invitrogen™ EVOS™ M5000 Cell Imaging System.

Further, we assessed cell death using the lactate dehydrogenase assay, which measures lactate dehydrogenase concentration in the cell culture supernatant released by dying THP1 macrophages (Fig 4). The results showed a dose-dependent response of THP1 macrophages to *B. cereus* WT and *ΔessC* supernatants extended up to 20 hours of incubation.

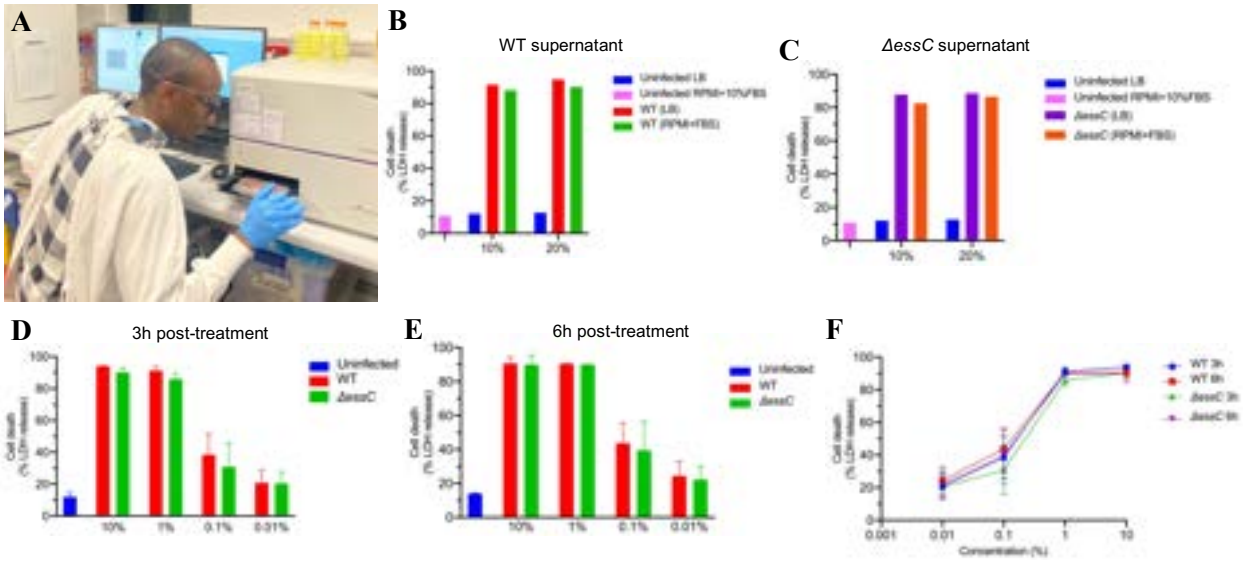


**Figure 5**. Measurement of cell death represented by LDH release. **A**. Spectrophotometric measurement of samples using the CLARIOstar Plus™ Microplate Reader. **B, C**. LDH release in response to LB media or RPMI + 10% FBS culture supernatants from the WT and *ΔessC* mutant after 6 h. **D**, **E** and **F.** Time course assessment of LDH release after treatment with varying doses of RPMI + 10% FBS *B. cereus* culture supernatant.

In summary, preliminary results for *B. cereus* WT and *ΔessC* culture supernatants' effect on THP1 macrophages were similar. At this stage, we could not rule out the influence of other toxins not dependent on T7SS that, in the same way, kill THP1 macrophages, which are present in the two strains we used in our experiments. From the experiments conducted so far, we have created an initial library of cell culture supernatants that will be used to treat cells for cytometric and transcriptome analysis.

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One Health Allyコース
Student Free Design Activities報告書

Hokkaido University
WISE Program for
"One Health Frontier Graduate School of Excellence"
One Health Ally Course
Student Free Design Activities Report form

This collaborative research is continuing with activities being implemented in Australia and Japan.

目的達成状況報告 [Report how you achieved your goal/objectives listed in the application form]

The first objective was achieved, while the second was partially completed. We successfully implemented collaborative research on *B. cereus,* a zoonotic pathogen. This objective will continue to be implemented till we establish the role that the T7SS plays in host infection and possibly further. The second objective to establish the role that T7SS plays in host infection and the nature of innate immune response mounted could not be achieved within the four weeks because we had to establish and optimize the infection conditions first as explained above. Fortunately, this objective will be achieved since the collaborative research is still ongoing.

One Health Approach実践報告 [Report how your activity could link to One Health Approach]

Firstly, *B. cereus* is an environmental pathogen that causes infection in humans and animals; hence, it is a zoonotic pathogen and a One Health problem. In addition, T7SS among Gram-positive bacteria is necessary for niche adaptation and ultimately crucial for survival on hospital and equipment surfaces that serve as sources of nosocomial infections that are challenging to treat because such bacteria, including *B. cereus*, become resistant to antimicrobials that would otherwise kill them. Thus, *B. cereus* links the three sectors of One Health: human, animal, and environmental health sectors. Secondly, I had the privilege of working with and collaborating with professionals from different backgrounds on a One Health-related pathogen. My background is in the veterinary sector, and I conduct collaborative research on *B. cereus* with individuals from the biomedical sciences and human health sectors and different nationalities. One Health calls for collaborative research of multiple disciplines, and I am happy that I have implemented that at the international level.

備考 [Remarks]

※ 報告書を作成後、担当教員に確認をお願いし署名をもらってください。PDFファイルとしてVetlogから提出してください。
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