

(Abroad) • Domestic) Internship report form (Student)

2016/03/30 (Year/Month/Day)

Name	Jeewan Thapa
Laboratory	Division of Bioresources, CZC
Year (Grade)	D3
Internship institution	Prof. Stephen Gordon's laboratory, University College Dublin (UCD) School of Veterinary Medicine, UCD Veterinary Sciences Center, Belfield, Dublin-4, Ireland
Internship period	Internship period: 02/17/2016 - 03/20/2016 (Departure Date from Sapporo: MM/DD/YYYY, Arrival Date in Sapporo: MM/DD/YYYY)
Purpose	To learn macrophage cell infection by <i>Mycobacteria</i> sps and explore research career.

- The reason why you chose this institute

Prof. Gordon presented a wonderful lecture on Tuberculosis at SaSSOH 2015. I was interested by his work. When I talked with him about exploring research career possibilities in one-health issues, he advised that UCD can be one of the institutions to explore. He advised that his lab is specializing on *Mycobacterial invitro* experiments. I also wanted to learn these experiments for my future research. I wish to develop my career as a researcher so wanted to have more research experience and explore career possibilities. Thus, to achieve above goals, I choose to visit Prof Gordon laboratory for internship activity.

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

The main objective of the internship activity was to have research experience on *Mycobacterial invitro* experiments to build foundation for future research and future career as a researcher. I have briefly outlined the activities of my internship activity as followings:

1 Learning experiments

1.1 Training on tissue culture

Before starting the experiment, Prof Gordon arranged a training program with one of the tissue culture technician to learn basic principles and procedures of cell culture. During the training, I worked with TR146 and 4T1 cell lines. I also performed small experiment to culture 4T1 cancerous cells, measure its real time growth and stain the growing cells (Fig 1).

Table 1. Outline of education activities at UCD

Feb 18-19:	Arrival to UCD and introduction
Feb 22-Mar 4:	Training on tissue culture
Feb 29-Mar 4:	Preliminary experiments with <i>M. avium</i> infection of macrophage
Mar 4-11:	Experiment with <i>M. avium</i> infection of macrophage
Mar12-16:	Infection biology module.

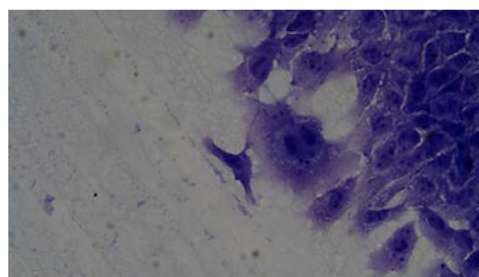


Fig 1. Observation of real time growth of 4T1 cells

1.2 Setting of *M. avium* culture and RAW 264.7 macrophage culture

We cultured *M. avium* in 7H9 middlebrook liquid media to be used for cell infection. Similarly RAW 264.7 macrophage cell culture was set up. Cells were monitored by adding media and creating new passage; numbers counted and prepared them for bacterial infection.

1.3 Fluorescent microscopy and bacterial uptake

Experiment to observe bacterial uptake by macrophage cell was conducted to determine bacterial dose for cell infection (Fig 2).

1.4 *M. avium* infection of RAW macrophages

After determining cell number and suitable dose for bacterial uptake, macrophage cells with 500,000 cells/well were infected with low and high dose of bacteria. Later, mRNA for cytokines expression (ACTB, Interleukin 1 β , IL-6 and IL-12) was determined by qRT-PCR. In our experiment, there was increased expression of IL1 β , IL-6 and IL-12 in high infection than low infection (Fig 3).

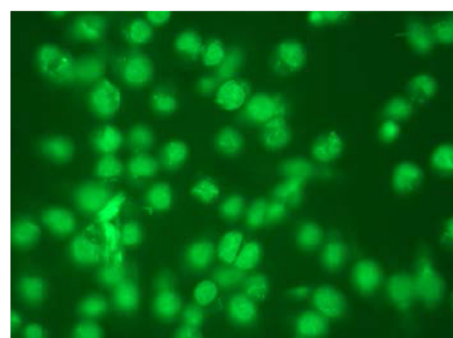


Fig 2

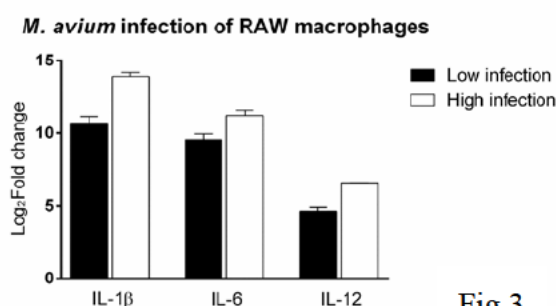


Fig 3

1.5 Colony forming unit (CFU) from *M. avium* infected cells

We conducted this experiment to understand how macrophage cells interact with infected bacteria. We were interested to know if bacterial cells number increased or decreased after infecting the cells (Fig 4 & 5).

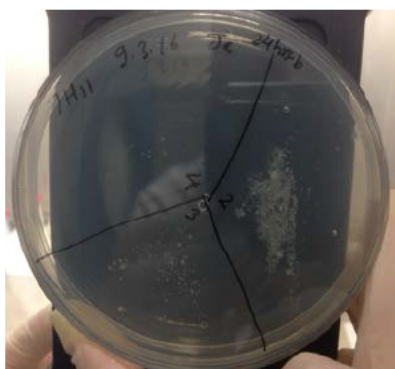


Fig 4. CFU measurement

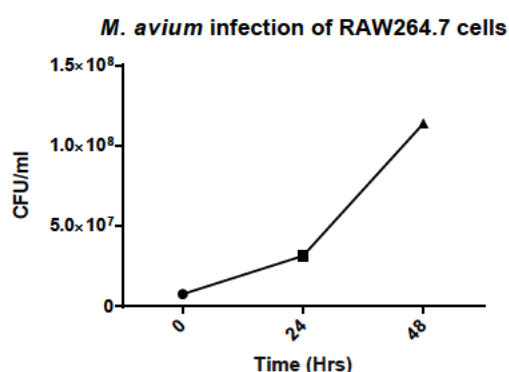


Fig 5. Real time CFU measurement

I performed above experiments as a model to learn *Mycobacteria* infection of macrophage cells. In my PhD research, we have found a relative new member of *Mycobacterium tuberculosis* complex (MTC), namely *M. orygis* from different animals of Nepal and Bangladesh. We have performed genotyping on those isolates. In future, I am interested to explore immune response by *M. orygis* and differentiate why and how *M. orygis* is different from other members of MTC. So, having experience on cell infection and monitoring immune response is important for me.

2. Attending infection biology module course

Along with other fellow students from Hokudai, I attended this infection biology course. In the module, various lectures on bacterial, viral, parasitic, prion diseases and global health issues were discussed. I had opportunity to explore wide variety of research areas and witness latest research in this field.



3. Exploring research career

Another major objective of internship was to explore research career opportunities. I had opportunity to talk with my host supervisor. He advised me to determine what I would like to do in long term and find for career opportunities according to it. He shared his own experience with me. He provided some information about the opportunities from European Union (EU) and thankfully accepted to write a reference letter for me. In UCD there were many researchers including international ones, I talked with them and listened their advice. In addition to research work, I had opportunity to observe laboratory management of UCD. I found there was slight difference between our lab and my working lab in UCD. This experience also helped me to adjust and work in different environment. I think this kind of

experience is also important for a researcher.

4. Exploring Ireland

Although my stay in Dublin was brief, I had some quality time to explore Ireland. I was staying as a paying guest in a home. My house lady was a kind person and used to explain about Ireland history and culture. I also shared my culture and also talked about Japan. Once, I visited outskirts of Dublin and was fascinated by its natural beauty. Like Hokudai, UCD was also a natural paradise. Interestingly Dublin city was filled with ancient heritages. I thoroughly enjoyed staying in Dublin.



UCD campus



Dublin city

Finally I would like to sincerely thank leading office especially Prof. Horiuchi, Maki San and Terashima San and leading steering committee for providing opportunity to conduct internship activity. I would also sincerely thank my supervisors Suzuki sensei and Nakajima sensei for allowing me to conduct internship and guiding in my endeavor. Finally, I would like to sincerely thank Prof Gordon and his laboratory for accepting me as an intern and providing opportunity to learn many things.

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

In this internship period, I conducted some experiments which are potential research area for my future research. I also explored bacterial infection of macrophages and was able to perform small experiments for immune response. I think these research experience will help my future career as a researcher. I also had opportunity to explore UCD and interact with host supervisor, different researchers. During these discussions, I was exploring research career and I hope their advice will be helpful for me.

- Advice for your junior fellows

The most important advice to junior fellow is to identify what they would really like to do in their future career and find suitable place for internship. If these two thing match, then there will be very high chance for productive internship activity.

Beside this, we should do very careful planning. Travel arrangement should be carefully planned. I would strongly advice to carefully check for visa requirements, including transit visas for international colleagues. Finally, I would like to suggest working very hard to fulfill their dream.

Approval of supervisor	Institution • Official title • Name Hokkaido University, Research Center for Zoonosis Control Professor Yasuhiko SUZUKI <div style="text-align: right;">印</div>
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- ※1 Send the electronic file to the Leading School section, International Affairs Office, also submit the original print out with seal of supervisor to the Leading School section, International Affairs Office.
- ※2 Attach a copy certificate of the content of internship activity that is prepared by the counterpart at the internship institution (any form with a signature of the counterpart).
- ※3 The Steering Committee of the Leading Program will first confirm the content of this report and report will be forwarded to the Educational Affairs Committee for credits evaluation.

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