Title of PhD project / theme	**The role of pathogen and host bioenergetics in *Campylobacter jejuni* infection**
Supervisory team	**Nagasaki University** Lead: Dr Daniel Ken Inaoka (danielken@nagasaki-u.ac.jp) **LSHTM** Dr Ozan Gundogdu (Ozan.Gundogdu@lshtm.ac.uk, Faculty of Infectious and Tropical Diseases) Dr Abderrahman Hachani (University of Melbourne)
Brief description of project / theme	All organisms require a continuous source of energy to sustain life. Such energy is supplied as ATP, which can be synthesized by oxidative phosphorylation (OXPHOS) and substrate-level phosphorylation. ATP synthesis by OXPHOS is dependent on electrochemical gradient generated by the electron transport chain (ETC). In general, the ETC is composed by several dehydrogenases which transfers electrons from various substrates to respiratory quinones, such as menaquinone and ubiquinone (Fig. 1). Those electrons are ultimately transferred to terminal electron acceptors by respiratory enzymes. In the ETC, some enzymes such as complex I, III and IV couple electron transport to translocation of protons across the membrane generating an electrochemical gradient, which is in turn used to synthesize ATP by complex V (ATP synthase). In the field of infectious diseases, the ETC from mammals and few pathogens have been individually studied, and shown to use different donor and acceptor of electrons to maintain the electrochemical gradient and to meet the cellular ATP demand. However, in the context of intracellular infection, the bioenergetic consequences caused by interplay between pathogen and host are less understood. Such knowledge is crucial to understand the mechanism of (i) pathogen adaptation to extracellular/intracellular environment, (ii) pathogenesis, (iii) host intracellular response against pathogens, and (iv) persistence of the disease. In addition, it provides opportunities for development of new pathogen-specific drugs.

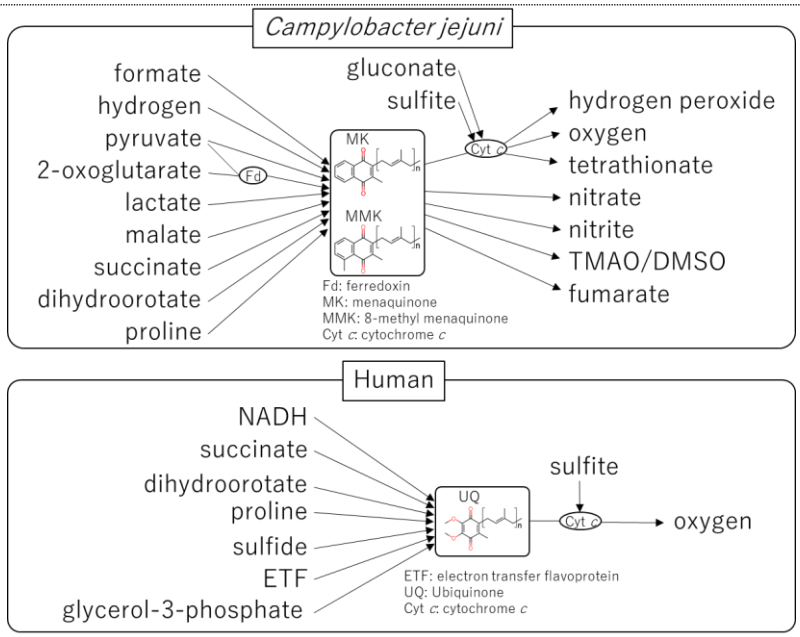

Figure 1. Electron transport chain from *C. jejuni* (top) and human (bottom). The transfer of electrons from each substrate (left) to respiratory quinones (centre) is catalysed by ETC dehydrogenases. Those electrons are transferred to terminal acceptors (right) by respiratory enzymes. Some ETC enzymes can bypass the quinones and transfer the electrons (from sulfite and gluconate) directly to cytochrome *c*.

Campylobacter is the most common bacterial cause of human gastroenteritis in the world, with the species *Campylobacter jejuni* responsible for over 80% of C*ampylobacter* infections (1). *C. jejuni* is abundant within the avian gut and the consumption and handling of poultry is the main route of transmission to humans. In humans, *C. jejuni* infection ranges from asymptomatic carriage to bloody diarrhoea, fever and abdominal pains as well as serious post-infectious sequelae such as the neuromuscular paralysis of Guillain-Barré syndrome. In high resource countries, an estimated 1 in every 100 individuals develop a *Campylobacter*-related illness each year, with elderly people most at risk to serious complications. With an ageing population, the consequences of *Campylobacter* infection are therefore bound to increase.

C. jejuni is known to invade and survive within the host cell and it has been linked to disease persistence. From the genome sequence analysis of *C. jejuni* several ETC dehydrogenases and alternative respiratory enzymes can be identified. Several ETC enzymes such as formate dehydrogenase, sulfite oxidase, succinate dehydrogenase and fumarate reductase has been suggested to be either essential for survival or colonization of host intestine. In *Helicobacter pylori* (that belongs to ε-proteobacteria class as *C. jejuni*), we have shown that dihydroorotate dehydrogenase (DHODH) is essential for survival in animal models, and the target of intervenolin and its derivatives (2). Most recently, we have characterized for the first time malate:quinone oxidoreductase (MQO), an unusual ETC enzyme not conserved in mammals but conserved in apicomplexan parasites (*Plasmodium falciparum* and *Toxoplasma gondii*) and a potential drug target (3, 4). From the sequence analysis, it is evident that those parasites have acquired MQO from ε-proteobacteria by lateral gene transfer, and as expected, displayed high amino acid sequence identity to MQO from *C. jejuni* and *H. pylori*.

Amongst the ETC enzymes from *C. jejuni*, DHODH and MQO have not been characterized and this project will focus on these two enzymes with the final goal to understanding their role(s) in *C. jejuni* biology either by genetic or chemical biology tools.
Following are the specific objectives.

1. **Biochemical studies of recombinant DHODH and MQO.** Inaoka's group have been working extensively on membrane proteins and we have the knowledge necessary to express and purify those proteins in *E. coli*. Preliminary studies conducted at NU have shown that DHODH and MQO from *C. jejuni* can be actively expressed in *E. coli* membranes.
2. **Attempt to generate knockout/knockdown of DHODH and MQO in *C. jejuni* and conduct a phenotypic characterization *in vitro* (extra/intra-cellular) and *in vivo*.** Gene KO/KD studies will be conducted under supervision of LSHTM group.
3. **Determination of crystal structure of DHODH and MQO and identify residues essential for enzyme catalysis by site-directed mutagenesis.** Using purified enzymes, the crystallization condition will be identified using commercial kits. After optimization of crystallization condition, the 3D structure will be solved using synchrotron X-ray from SPring 8 and Photon Factory in Japan.
4. **Development of a high-throughput screening platform for identification of specific inhibitors of DHODH and MQO.** Semi-automated dispenser robots and plate readers are routinely used in our lab and will be used to screen inhibitors of *C. jejuni* DHODH and MQO.
5. **Impact of DHODH and MQO inhibitors on the bioenergetics of extra/intra-cellular *C. jejuni*.** ATP biosensor such as A-TEAM (FRET-based) and MaLion (Fluorescence Intensity-based) are being used by our group to monitor the ATP dynamics in living cells. This system can be adapted to *C. jejuni* and study the impact of inhibitors on its bioenergetics.
6. **Impact of *C. jejuni* infection on host bioenergetics.** Host cells expressing the ATP biosensors can be used to study the impact in the host bioenergetics caused by *C. jejuni* infection.

1. Gundogdu and Wren *et al.*, 2020, ***Microbiology*.**
2. Ohishi *et al.*, 2018, ***Helicobacter*.**
3. Hartuti *et al.*, 2018, ***BBA Bioenergetics*.**
4. Acharjee *et al.*, 2021, ***Int J Mol Sci***.

The role of LSHTM and NU in this collaborative project	The primary supervisor from NU (Inaoka) is currently supervising several Master and PhD students in the field of biochemistry, molecular biology, structural biology and drug development. The co-supervisor from LSHTM (Ozan) is an expert in genomics and molecular microbiology, and currently leading a diverse set of PhD students at the LSHTM investigating *Campylobacter* pathogenesis. The PhD candidate will learn how to culture *C. jejuni* and mammalian cells. The candidate will have the opportunity to develop core skills from molecular biology, recombinant protein expression and purification, and biochemistry. Once the target enzymes are purified, the PhD candidate will learn how to screen and optimize the crystallization condition. X-ray diffraction data from the crystal will be collected using synchrotron facilities from SPring-8 (Harima) and Photon Factory (Tsukuba), and how to solve the three-dimensional structure of proteins. The candidate will also have the opportunity to develop skills to identify specific inhibitors, the first-step in drug development process and to monitor the ATP dynamics in living cells in real-time. Co-supervisor from LSHTM will provide expertise in generation and phenotypic analysis of target gene knockout/knockdown mutants of extra/intra-cellular *C. jejuni* as well as using specific inhibitors.
Particular *prior* educational requirements for a student undertaking this project	The doctoral candidate should have completed an undergraduate and postgraduate degree related to microbiology or biology or biochemistry.
Skills we expect a student to develop/acquire whilst pursuing this project	*Biochemistry* – We expect the student to develop skills in membrane protein purification and determination of enzymatic activities using spectrophotometers and spectrofluorometers. How to evaluate the affinity to electron donors and acceptors, and some biophysics skills such as surface-plasmon resonance and calorimetry. *Molecular Biology* – The student will have the opportunity to develop core molecular microbiology and recombinant DNA skills, learn how to grow *E. coli, Campylobacter*, and mammal cells. How to create *C. jejuni* isogenic mutants, and how to perform several different phenotypic assays depending on the direction the doctoral student wishes to drive the project. *Drug development* – The student will develop skills on how to validate genetically and chemically a drug target. How to develop a screening system to identify true inhibitors against a target protein. How to develop *in vitro* and *in vivo* evaluation systems for the identified inhibitors. In addition to the scientific skills described above, the doctoral student will be provided with world-class training that will lead to them becoming an independent scientist. We will encourage the doctoral student to join national and international conferences/meetings and social events to improve the presentation and questioning skills. Such participation will help the student to know other students and scientist in the field and will improve the communication skill.