**Characterisation and quantification of secreted factors from strains of the honey bee brood pathogen, *Melissococcus plutonius* (EFB)**

**Supervisors: Kirsty Stainton at Fera Science Ltd. and Thorunn Helgason at University of York**

***Background***

European foulbrood (EFB) is caused by the bacterium *Melissococcus plutonius*. It is the most widespread bacterial pathogen of honeybees in the UK. It is a statutory notifiable disease, which in approximately half of cases will result in the destruction of the infected honey bee colony. The bacteria infect developing bee larvae through feeding and colonise the gut causing high levels of brood mortality. The mode of its pathogenesis in the gut is not fully understood; it may outcompete the larva for food in the gut, secrete toxins or biofilms, and/or inhibit beneficial endosymbionts. Fera Science Ltd. and the University of York have a Ph.D. student, Nicola Burns, who is studying the genetic basis of virulence factors of EFB using next generation DNA sequencing. She is due to complete in Summer 2020. She has sequenced the genomes of strains of EFB from the 3 clonal complexes and performed *in-vivo* studies on comparative virulence of different strains of EFB.

The proposed project is to use a proteomic approach to identify specific bacterial effector proteins from *Melissococcus plutonius*. EFB can be grown in culture and the effectors secreted into the extracellular matrix can be separated from the EFB itself, identified and measured by mass spectrometry. This way you can identify proteins directly involved in virulence and toxicity. Id

A similar methodology was used for American foulbrood (AFB) caused by the bacterium *Paenibacillus larvae* and it was discovered that the toxins secreted by the bacteria were not the same as those predicted from the genomic data (Erban *et al*., 2019). Functional characterisation of toxins is required as genomic data alone cannot provide this information. That study identified novel proteins which were not previously implicated in AFB virulence; and showed that a factor previously implicated in virulence was not as important as predicted.

***Techniques:***

The student will be trained in honey-bee rearing and honey bee bioassays, molecular techniques including DNA and RNA extraction, real-time qPCR and conventional PCR, and also in proteomics techniques including protein extraction, western blotting and proteomics based mass-spectrometry. The student will require a biochemistry background with some laboratory experience.

***Proposed research***

There are virulent and avirulent strains of *Melissococcus plutonius*. Strains can be grown in culture and the extracellular matrix processed for mass spectrometry. The supernatant from the cells can be removed and passed through a filter and precipitated for mass spectrometry. New bacterial effectors were identified using this method for the human pathogen, *Vibrio cholerae* (Altindis *et al*., 2015).

1. Comparisons of the secreted proteins can be made between strain types; toxins can be identified that explain virulence and factors involved in persistence.
2. Comparisons for antibiotic resistant EFB strains grown with and without antibiotics to assess potential shifts in secreted proteins where antibiotics are present.
3. In-vivo experiments: comparisons of strains identified through steps 1 + 2 which express molecules of interest. Guts will be dissected from infected larvae for histological and cellular analysis at different timepoints to determine cellular effects/localisation of the toxins/effectors and disease progression. (i.e. electron microscopy, mitochondrial damage, nuclear/chromatin effects, apoptosis stains…).
4. Case study: ST2 is a very persistent strain of EFB, which frequently reappears after treatment. In addition to identifying and quantifying the secreted factors for this strain and comparing it to others, we will perform an in-depth comparison of the effects of this strain on the honey bee gut versus other strains of the same clonal complex to help inform why this strain is so persistent.

This work will define the functional differences between strain types and provide biological information on why some strains are more persistent than others and why strains respond differently to treatments.

***Value***

Identification of the effectors in virulence of the most widespread, notifiable disease of honey bees will help with our further understanding of the pathogen and may help develop future diagnostics tools. i.e. identification of a secreted effector from a particularly virulent strain may inform the choice to destroy the colony rather than perform a shook-swarm or identification of a factor involved in antibiotic resistance would inform the choice not to use OTC in that instance.

***References***

Altindis, E., Dong, T., Catalano, c. and Mekalanos, K. (2015) mBio, 6 (2), e00075-15.

Erban, T., Zitek, J., Bodrinova, M., Talacko, P., Bartos, M. and Hrabak, J. (2019) Virulence. doi: 10.1080/21505594.2019.1603133