FoodBioSystems DTP - PhD Project Advertisement Text

Project Title: FOODBIOSYSTEMS - A novel approach for reducing multiple-drug resistance in foodborne bacteria: application of CRISPR technology

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Research Group: FOODBIOSYSTEMS BBSRC DTP
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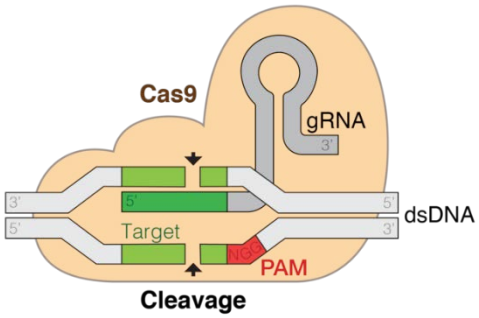
Project Description: Antibiotic resistance is a major threat to both global health and food security with increasing numbers of bacterial infections, (including food-borne diseases, that are becoming more difficult to treat as new resistance mechanisms emerge and spread and our portfolio of antibiotic options becomes less effective. Recent estimates indicate death dues to antibiotic treatment failure could exceed those caused by cancer by 2050 and we are now on the threshold of entering a 'post-antibiotic era' where infectious disease is once again the major cause of death in the developed world. There is a pressing need to develop new strategies to counter the threat of antibiotic resistance.

Our approach is to utilise the power of CRISPR-Cas technology to remove antibiotic resistance from the food production chain and thus eliminate one of the major sources of human health risk. Animal production contributes to human health issues due to the presence of multidrug resistant (MDR) bacteria and there is a drive to remove antibiotics from the food chain including EU directives on antibiotic reduction in animal production and the "No Antibiotics Ever" campaign in the USA. The objective of this significant and socially responsible study is to re-enable the efficacy of our antibiotics by developing a novel strategy for removal of resistance genes. This a highly innovative approach has high potential impact to provide front line and last resort antibiotics an extended lifespan through treatments that remove the resistance genes in animals and thus reduce their presence in humans.

The testable hypothesis is that ***the CRISPR-Cas systems can be exploited to reduce the burden of AMR in bacterial populations such that:***

a) ***the risk of resistance passing into the human food chain is dramatically reduced and***
b) ***the treatment permits use of the antibiotic, thus expanding the potential utility of current antibiotics***.



The CRISPR-Cas system consists of a sequence-specific nuclease (Cas9) and a guide RNA (gRNA) that provides sequence specificity to its DNA cleavage activity (see **Fig**). CRISPR-Cas9 is a versatile and highly specific next-generation antimicrobial that allows targeting of specific pathogenic bacteria while leaving the remaining microbiome intact. The preliminary data generated by the commercial partner, Folium Science, has demonstrated the application of CRISPR technology to reduce the burden of *Salmonella* in poultry (Cogan et al., in press). Conjugative delivery of CRISPR-Cas9 and target-specific gRNA by a mobilisable plasmid from a donor bacterium (a 'probiotic') to *Salmonella* caused degradation of the DNA of the targeted bacterium and subsequent bacterial cell death. Folium Science is providing this know-how and access to its patent portfolio to enable our consortium to tackle one of the most pressing needs currently - countering MDR bacteria in animal production.

Our recent studies demonstrated very high prevalence of MDR bacteria in commercial poultry. Avian Pathogenic *E. coli* (APEC) have been linked with Urinary Tract Infection (UTI) in humans and resistance means treatment failure. The overall goal is to devise a CRISPR-Cas system that effectively reduces the incidence of selected test antibiotic resistance genes (e.g. CTX-M 15) for use in poultry in the first instance.

The four objectives are:

(1) *In silico* analysis of WGS data from commercial poultry microbiomes to identify antibiotic resistance genes, their prevalence and vectors. Targets (guides with relevant PAM motif for Cas activity) will then be designed.

(2) Synthetic target sequences will be cloned by Golden Gate synthesis approaches onto Folium vectors constitutively expressing Cas9 or Cas cascade enzymes. The constructs will be mobilised into test strains and loss of the targeted resistance monitored both phenotypically and genotypically.

(3) *In vitro* gut models will be utilised to determine the dynamics of delivery of the CRISPR-Cas system to the poultry gut microbial community and to assess reduction of the target resistance gene and its associated vector.

(4) The CRISPR-Cas probiotic strain developed above will then be used in commercial broiler chicks. Microbial molecular profiling by NGS will be undertaken to examine dissemination of the CRISPR-Cas system, loss of the targeted AMR gene and any population shifts in the gut microbiome.

Funding Notes: This project is part of the FoodBioSystems BBSRC Doctoral Training Partnership (DTP), it will be funded subject to a competition to identify the strongest applicants. Due to restrictions on the funding, this studentship is only open to UK students and EU students who have lived in the UK for the past three years.

This is a CASE project and is thus strongly supported by the co-sponsor, Folium Science, through provision of significant research funding, CRISPR technology and scientific expertise.

The FoodBioSystems DTP is a collaboration between the University of Reading, Cranfield University, Queen's University Belfast, Aberystwyth University, Surrey University and Brunel University London. Our vision is to develop the next generation of highly skilled UK Agri-Food bioscientists with expertise spanning the entire food value chain. We have over 60 Associate and Affiliate partners. To find out more about us and the training programme we offer all our postgraduate researchers please visit https://research.reading.ac.uk/foodbiosystems/.

Training opportunities: The training opportunities are extensive and will provide a skill set for the next generation of synthetic molecular biology specialists in human and animal health with interests in gut health and sustainable production. The microbiome of the gut is now regarded as the next frontier, linking nutrition with gut microbial populations and their interaction with the host to generate health benefits. This doctoral training programme will provide very strong experimental design aspects supported by detailed training that covers bioinformatics, molecular epidemiology and microbiota population profiling/metagenomics (using NGS) to support this, linked with relevant *in vitro* and *in vivo* models. Opportunities with the industrial partner to understand skills transfer to product development and ultimately the complete commercial pathway will be gained providing a unique and much needed multidisciplinary skill set. Gaining these insights into synthetic biology and utilization of CRISPR technology approaches will provide understanding and confidence in the latest molecular technology that can be exploited for many goals including for example animal improvement.

University of Surrey will provide training in bioinformatics and molecular epidemiology plus *in vitro* poultry gut models. University of Reading will provide training in synthetic biology and *in vivo* models. The candidate will work at Folium's laboratories and offices in Bristol to gain an understanding of the commercial drivers behind taking fundamental research through development and commercialisation. A broad understanding of the drivers in animal production and its improvement are essential as we move to meet the arising demands of sustainability and reduced environmental impact.

Student profile: The student should have a 2.1 equivalent (or higher) BSc in a subject relevant to the scope of this proposal (e.g. Animal Science, Food Science, Microbiology, Molecular Biology). Relevant qualification at Masters (Merit/Distinction) level would be an advantage. Research skills in molecular genetics, bioinformatics (IT), epidemiology, bacteriology and animal nutrition would also be an advantage. A keen interest and understanding of the field of AMR would be expected. Strong communication and teamwork skills are necessary, along with capability for data handling and critical analysis. The student should display critical thinking, diligence, independence, problem-solving skills, professionalism, good record keeping and the ability to time-manage effectively (e.g. to meet deadlines).

References:

- https://www.agritech-east.co.uk/folium-sciences-new-guided-biotic-triggers-salmonella-self-digestion
- https://www.forbes.com/sites/donaldmarvin/2019/06/11/folium-science-uses-crispr-to-remove-antibiotics-in-livestock/#518ba4d7359a
- Lutful Kabir, SM. (2010). Avian colibacillosis and salmonellosis: A closer look at epidemiology, pathogenesis, diagnosis, control and public health concerns. International Journal of Environmental Research and Public Health, 7(1), 89–114. http://doi.org/10.3390/ijerph7010089
- Yamaji R, Friedman CR, Rubin J, Suh J, Thys E, McDermott P, Hung-Fan M, Riley LW. A Population-Based Surveillance Study of Shared Genotypes of *Escherichia coli* Isolates from Retail Meat and Suspected Cases of Urinary Tract Infections. mSphere. 2018 Aug 15;3(4). pii: e00179-18. doi: 10.1128/mSphere.00179-18