# PhD Project Advertisement

**Project title:** Plant pathogen control with engineered cysteine rich antimicrobial peptides
**Project No:** FBS2023-27-Kevei-cr

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## Project description:

Tomato is one of the most important horticultural crops worldwide: 187 million tonnes of fruit were produced on 5 million Ha in 2020. However, the production is limited by various abiotic and biotic stresses leading to significant crop losses; especially important are the fungal and bacterial diseases. Fortunately, there are crop lines possessing specific resistance or tolerance against these pathogens, but most of the diseases are difficult to manage. Moreover, where resistance to these pathogens was observed in breeding lines, it is often a far too complex quantitative trait, and it has proved complicated to find simple genetic markers for breeding purposes and eliminate the negative effects of linkage drag during selection process. On the other hand, with modern reverse genetic methods, these breeding challenges can be circumvented, and more targeted genetic changes can be applied.

**Figure 1.** Bacterial speck disease on the tomato.

**Figure 2.** Bacterial wilt damage on a tomato field.

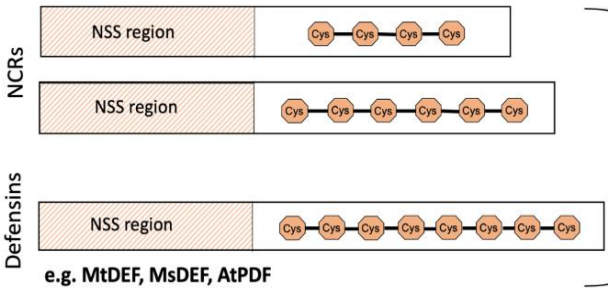


**Figure 3.** The structure of NCRs. They consist of an N-terminal signal sequence (NSS) and four or six cysteines at conserved positions in the mature peptide. They are similar to defensins. Adapted from Roy et al. 2020.

During the symbiotic interaction between Rhizobium and legume plants, a new organ, the root nodule develops, where the bacteria transform into nitrogen-fixing bacteroids. The bacteroid differentiation is a highly specific molecular process which is mediated by a large family of plant peptides produced exclusively in the symbiotic cells. Surprisingly, in the legume plant Medicago truncatula hundreds of Nodule-specific Cysteine-Rich (NCR) peptides are involved in the nodule development and these peptides can be only found in few, particular species of legume plants. The principal role of the NCR peptides is to convert bacteria into large polyploid, terminally differentiated nitrogen-fixing bacteroids in the nodule cells. The typical NCR peptides are small, consisting of ~30 to 50 amino acids, and contain 4 or 6 cysteine residues at conserved positions. They have similar molecular structure to antimicrobial peptides (AMPs), especially to plant defensins (widely distributed in plants). Two of these NCR peptides were extensively studied, NCR247 and NCR335 showed very strong antimicrobial activity against various human "ESKAPE" pathogens, such as *Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Listeria monocytogenes* and *Salmonella enterica*. Other investigated NCRs with increased isoelectric point were also highly active against the pathogenic yeast, *Candida albicans*. Despite the potential importance of NCRs as green antibiotics, there has been limited studies carried out on applying against plant pathogens. So far only a few fungal plant pathogens were positively tested against a selected NCR peptide, NCR044. The numerous NCRs provide a great opportunity to test

selected ones against plant pathogens, hence we seek to extend the availability of green antibiotics for plant protection, to address critical disease challenges impacting on agricultural production.

The overall aim of the project is to select or engineer peptides that are toxic to bacterial and fungal plant pathogens and confer disease resistance in crop plants. Firstly, the student will learn about the exciting field of plant AMPs and select the suitable NCR candidates for the successive studies. Those peptides will be produced or synthetised, and test against plant pathogens using modern microbiological tools and methods. The selected peptides with effective antimicrobial activity will be used in transgenic studies, where tomato lines will be modified with traditional gene fusions and more recent, state of art gene editing technologies. Finally, these modified plants will be tested against key tomato pathogens to demonstrate the anticipated antimicrobial activity for biotic stress alleviation. The project offers an excellent opportunity to investigate the application of "green antibiotics" which is the desired way of producing future antimicrobial agents.

### Training opportunities:

The student will be trained in the Cranfield molecular laboratories which will include general plant molecular biology techniques, tissue culture works to generate transgenic plant lines The student will be also skilled in various techniques of microbiology, working with pathogenic bacteria and fungi, employing biochemical screens of synthetised peptides with biological activity, carry out photometric tests for antimicrobial capacity. Suitable bioinformatics training will also be given to the student at Cranfield to execute the sequence analyses of NCR peptides and pathogen associated tomato genes (including attendance on relevant modules in the Bioinformatics MSc course).

The student will also make visits in year 2 and 3 to Aberystwyth with the aim of testing the transgenic plant lines for pathogenic resistance. This will include lab works to perform leaf assays, and also plant growth experiments in control environment applying pathogen infection on transgenic lines.

The supervisory team provides internationally recognized expertise in tomato genetics, microbiology, and bioinformatics (Cranfield) and in plant pathogen studies (Aberystwyth).

### Student profile:

This project would be suitable for students with a degree in plant sciences, with knowledge of molecular biology, genetics, showing interest in plant breeding and plant pathogens. General practical experience in a laboratory environment would be an advantage although comprehensive training will be given.

### Stipend (Salary):

FoodBioSystems DTP students receive an annual tax free stipend (salary) that is paid in instalments throughout the year. For 2022/23 this will be £17,668 and this will increase slightly each year at rate set by UKRI.

### Equality Diversity and Inclusion:

The FoodBioSystems DTP is committed to equality, diversity and inclusion (EDI), to building a doctoral researcher(DR) and staff body that reflects the diversity of society, and to encourage applications from under-represented and disadvantaged groups. Our actions to promote diversity and inclusion are detailed on the FoodBioSystems DTP website.

In accordance with UKRI guidelines, our studentships are offered on a part time basis in addition to full time registration. The minimum registration is 50% FT and the studentship end date will be extended to reflect the part-time registration.

### References:


1. Van de Velde et al. Science. 2010;327:1122-1126.
2. Jenei et al. Front Microbiol. 2020;11:270.
3. Lima et al. Front Mol Biosci. 2022;9:870460.
4. Velivelli et al. PNAS 2020;117:16043-16054.
5. Lima et al. Front Microbiol. 2020;11:1307.
6. Chisholm et al. Cell. 2006;124:803-814.
7. Nikoloudakis et al. Int J Mol Sci. 2020;21:9380.
8. Vu et al. Plant Biotechnol J. 2020;18:2133-2143.
9. Roy et al. Genes (Basel). 2020;11(4):348.


**For up to date information on funding eligibility, studentship rates and part time registration, please visit the FoodBioSystems website.**