Project Title	Development of phage biopesticide to control *Xanthomonas campestris* pv. *campestris* causing black rot in Brassicas
University (where student will register)	University of Warwick
Which institution will the student be based at?	As above
Theme (Max. 2 selections)	Climate & Environmental Sustainability ☒ Organisms & Ecosystems ☐ Dynamic Earth ☐
Key words	Biological control, Bacterial diseases, Phage therapy
Supervisory team (including institution & email address	**PI:** Dr Mojgan Rabiey, School of Life Sciences, University of Warwick, Mojgan.rabiey@warwick.ac.uk **Co-I:** Prof Murray Grant, School of Life Sciences, University of Warwick, m.grant@warwick.ac.uk

Project Highlights:

- Isolation of phages against *Xanthomonas* species
- Characterisation of phages most effective as biocontrol agents
- Development of phage cocktail to treat brassica black rot

Overview (including 1 high quality image or figure):

The diverse gram-negative bacterial genus *Xanthomonas* causes disease on more than 400 plant species, including trees, and often causes economically significant losses in agriculture. *Xanthomonas campestris* pv. *campestris* (*Xcc*) causes disease in a large number of economically important vegetable Brassica crops, such as cauliflower, cabbage, and broccoli and a number of other cruciferous crops, ornamentals and weeds, including the model plant *Arabidopsis thaliana* (Vicente and Holub, 2013). *Xcc* comprises eleven races, of which Race 1 and Race 4 are considered the most common and destructive for Brassica crops (Lu et al., 2021). *Xcc* is a vascular pathogen, colonising via hydathodes or wounds on the plant and forms biofilms in the vascular system, where it releases degradative extracellular enzymes and virulence factors (Papaianni et al., 2020).

The control of black rot is difficult and currently relies on the use of pathogen-free planting material and the elimination of other potential inoculum sources (infected crop debris and cruciferous weeds) (Vicente and Holub, 2013). One potential alternative method to control bacterial diseases is through bacteriophage biocontrol. Bacteriophages (phages) are viruses that infect bacteria and represent one of the most abundant organisms in the biosphere. Phage are almost always highly specific to their target bacterial host, hijacking its replication machinery to complete their replication, leading to lysis of their bacterial host, without directly causing harm to plants or animals. The potential of phage against *Pseudomonas syringae* pathovars *actinidiae*, *aesculi* and *syringae* have been explored as potential biocontrol agents (Di Lallo et al., 2014, James et al., 2020, Rabiey et al., 2020).

We aim to develop a novel phage-based biocontrol for use in Brassicas (cabbage, broccoli, cauliflower, kale) affected by *Xcc* black rot disease.

Methodology:

- ***Collection of bacteria and phages from* brassicas field**. Wellesbourne curates a well-characterised set of *Xcc* isolates (>600). Phages will be isolated following our established

methods (Rabiey et al., 2020). Phage characterization will be done (electron microscopy and genome sequencing). Host specificity of isolated phages will be tested, initially on an *Xcc* diversity set based on whole genomic sequence of 600 *Xcc* isolates.

- ***Bacteria-phage interaction coevolution study***. Phages will be co-evolved with *Xcc* to determine whether bacterial resistance emerges. Both single phage isolates and cocktails of natural isolates and evolved phages will be tested, monitoring any antagonistic or synergistic effects that emerge from these studies and prioritising phage for efficacy studies.
- ***Testing the efficacy of phage for controlling disease***. These selected phages will be optimized in terms of production environment (e.g. temperature, MOI, time) with the aim of producing maximal titres for commercial applications. The best phage mixtures will be assessed for *Xcc* control in controlled environment containment facilities across a wider spectrum of the Wellesbourne collection, including other *Xantghomonas* species.

Training and skills:

Students will be awarded CENTA2 Training Credits (CTCs) for participation in CENTA2-provided and 'free choice' external training. One CTC equates to 1⁄2 day session and students must accrue 100 CTCs across the three years of their PhD.

The student will receive specialist training for this multidisciplinary project, encompassing fieldwork, microbiology, electron microscopy, genomics, data management and statistical analysis and interpretation of large and complex data sets.

The student will be supported to develop these skills within the SLS at Warwick, allowing the student to excel in these key aspects of data acquisition, analysis and dissemination and to build important networks.

The supervisory team is multi-disciplinary and highly experienced, based in excellent, well-equipped institution, and will provide comprehensive support for the student across all aspects of the project. S/he will also have access to unique imaging tools and the PhytoBac Explorer bacterial genomics database to undertake comparative studies between effective and tolerant phage to identify potential toxin/antitoxin components.

Partners and collaboration (including CASE):
Further information on partners and collaboration (including CASE):

Possible timeline:

Year 1: Field experimental design and field data/sample collection training will be done. Samples will be collected from Brassicas fields. Phages will be isolated from the samples. Phage characterisation will be done, including killing curve assay, host range assay, growth rate assay, whole genome sequencing, and temperature assay.

In parallel develop the initial diversity screening panel that maximises isolates with geographic and genomic diversity across the key Races, 1 and 4.

Year 2: Isolated phages will be coevolved with the pathogen and the genetic mechanism of phage-bacteria interaction and evolution will be tested by whole genome sequencing. The phages will be tested *in planta* on brassicas species.

Year 3: The impact of phages on other microbiota will be tested *in planta*, by DNA extraction and 16S and ITS sequencing. Data analysis of the sequencing data and year 1 and 2 experiments undertaken.

Year 3.5: Final analysis of the data, explore stakeholder interest and possibility of dual pahge/bacteriocin treatments. Writing up of manuscripts and thesis completion.

Further reading:

DI LALLO, G., EVANGELISTI, M., MANCUSO, F., FERRANTE, P., MARCELLETTI, S., TINARI, A., SUPERTI, F., MIGLIORE, L., D'ADDABBO, P., FREZZA, D., SCORTICHINI, M. & THALLER, M. C. 2014. Isolation and partial characterization of bacteriophages infecting Pseudomonas syringae pv. actinidiae, causal agent of kiwifruit bacterial canker. *J Basic Microbiol,* 54**,** 1210-21.

JAMES, S. L., RABIEY, M., NEUMAN, B. W., PERCIVAL, G. & JACKSON, R. W. 2020. Isolation, Characterisation and Experimental Evolution of Phage that Infect the Horse Chestnut Tree Pathogen, Pseudomonas syringae pv. aesculi. *Curr Microbiol,* 77**,** 1438-1447.

LU, L., MONAKHOS, S. G., LIM, Y. P. & YI, S. Y. 2021. Early Defense Mechanisms of Brassica oleracea in Response to Attack by Xanthomonas campestris pv. campestris. *Plants (Basel),* 10.

PAPAIANNI, M., PARIS, D., WOO, S. L., FULGIONE, A., RIGANO, M. M., PARRILLI, E., TUTINO, M. L., MARRA, R., MANGANIELLO, G., CASILLO, A., LIMONE, A., ZOINA, A., MOTTA, A., LORITO, M. & CAPPARELLI, R. 2020. Plant Dynamic Metabolic Response to Bacteriophage Treatment After Xanthomonas campestris pv. campestris Infection. *Front Microbiol,* 11**,** 732.

RABIEY, M., ROY, S. R., HOLTAPPELS, D., FRANCESCHETTI, L., QUILTY, B. J., CREETH, R., SUNDIN, G. W., WAGEMANS, J., LAVIGNE, R. & JACKSON, R. W. 2020. Phage biocontrol to combat Pseudomonas syringae pathogens causing disease in cherry. *Microbial Biotechnology,* 13**,** 1428-1445.

VICENTE, J. G. & HOLUB, E. B. 2013. Xanthomonas campestris pv. campestris (cause of black rot of crucifers) in the genomic era is still a worldwide threat to brassica crops. *Mol Plant Pathol,* 14**,** 2-18.

Further details:

Please add project/institutional contact details including a link to the application website if applicable