Project Title	**The potential of natural phage communities in biofilm degradation**
Host University	**University of Warwick**
Theme	**Organisms & Ecosystems**
Key words	**Phage, microbiology, biofilms, antimicrobial**
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Project Highlights

- Examine the structure of phage communities from a range of environments with metagenome sequencing.
- Construct a synthetic model biofilm in the laboratory, which is representative of waste water treatment biofouling.
- Determine the efficacy of phage communities from the different environments in degrading the synthetic biofilm.

Overview

Biofilms are the cause of costly biofouling in industrial processes and the shipping industry and this project aims to reduce biofilms with the use of phages. Biofilms are caused when microorganisms such as bacteria adhere to any solid surface, leading to a community of microorganisms colonising the surface that are associated with an extracellular matrix and DNA. Microbial biofilms are more resistant to microbial agents than planktonic microbes and therefore cause problems and can be very expensive to clear up. In industrial processes biofilms can cause blockages which can require shutdowns and cleaning regimes, by reducing biofouling this project aims to increase productivity and sustainability.

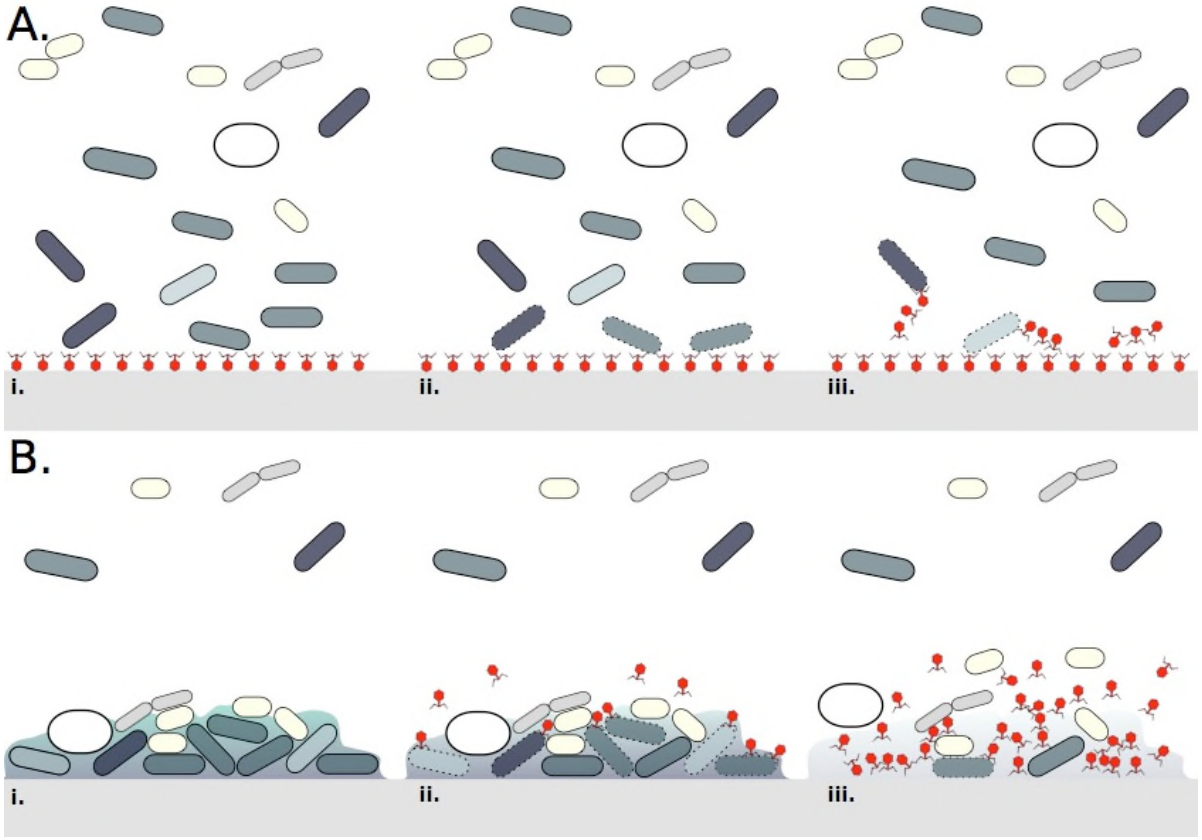
This project will investigate the potential for using natural viral (particularly bacteriophage; viruses that infect bacteria) communities as an antimicrobial to prevent biofilm formation (Fig. 1A) and break up established biofilms (Fig. 1B). Bacteriophage (or phage) are the most abundant biological entities on earth. Lytic phage have the capacity to kill their host bacteria while some break down extracellular polymers that can form the adhesive that holds biofilms together, without entering their hosts. Anywhere bacteria are found, phage that infect them are also found.

The project will compare the efficacy of phage communities from different environments at preventing and degrading biofilms. These environments will include compost heaps, ponds, rivers, estuaries, marine systems and sewage works. To this end the student will develop a synthetic mixed species biofilm as a model to test the efficacy of different phage communities. The establishment of a multi-species biofilm (Fig. 1B.i) will help to understand the roles of environmental phage in microbial community dynamics and how different elements of the biofilm react to them. Developing

a biofilm model will involve selecting eukaryotic (i.e. fungi) and bacterial partners that co-exist to form a stable biofilm.

Specific phage that infect members of the synthetic biofilm will be compared to the natural phage communities in terms of efficacy of biofilm prevention and removal. A variety of phage that have previously been isolated at the University of Warwick are available and the isolation of other specific phage to the biofilm members will be undertaken.

Figure 1



Methodology

Develop a high through-put biofilm assessment method, such as using 96 well microtitre plates with peg lids or polycarbonate filters. Cells can be detached by sonication. Peg plates or filters will be used to establish a biofilm and assess different phage populations for reducing biofilms. A synthetic biofilm of dominant biofouling species (3-6 species i.e. representative Bacteroidetes, Proteobacteria and yeast) will be selected based on the literature. Biofilm species structure will be characterised by live/dead Q-PCR and confocal microscopy.

Phage isolation and characterisation will be carried out on individual biofilm species using plaque assays to isolate and enumerate phage. Phage latency period, burst size and virulence will be determined through plate reader assays and flow cytometry.

Natural phage communities will be separated from bacteria and eukaryotes by 0.2um filtration, then concentrated using protein concentrating centrifuge tubes. Concentrated phages will have Illumina metagenomics sequencing carried out to examine different phage communities.

Training and skills
CENTA students are required to complete 45 days training throughout their PhD including a 10 day placement. In the first year, students will be trained as a single cohort on environmental science, research methods and core skills. Throughout the PhD, training will progress from core skills sets to master classes specific to CENTA research themes.

Phage isolation from water samples and characterisation of isolated phage. Including separation of phage DNA and extraction. Microbiological methods to ascertain whether mixed communities are present – Q-PCR and confocal imaging. These methods will reveal not only which species are present, but potentially the effects of phage on biofilm physical structure (thinner, flatter, amount of ESP etc).

Partners and collaboration
Severn Trent will be involved in training students and supplying samples.

Possible timeline
Year 1: Select organisms for building a synthetic biofilm. Based on the dominant species observed in biofilm literature. Establish a robust biofilm in the laboratory, with trials of peg plates, filters and hydrogels. Isolate phage against the species in the synthetic biofilm mix.

Year 2: Develop methods for assessing biofilm formation, persistence and dispersal in the laboratory, such as crystal violet, biomass assessment, confocal microscopy, QPCR, etc. Explore the biofilm assessment measures, using phage isolates applied before and during biofilm formation. Purify natural phage communities from different environmental niches. Assess the effect of these natural phage on biofilm formation and degradation.

Year 3: Characterise phage communities before and after application to the biofilm model. Through TEM and metagenomic sequencing.

Further reading
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