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Development of a bacteriophage technology to control *Campylobacter* in poultry

Key external stakeholders:

Poultry farmers, poultry processors, food safety authorities, microbiologists

Practical implications for stakeholders:

The recommendation for the application of phages in poultry is;

- Intestinal *Campylobacter* should be reduced in broilers at slaughter to decrease the contamination levels on carcasses during processing.
- Encapsulated *Campylobacter* phages should be administered orally in water 24 hours before slaughter

Main results:

- Phages are isolatable from areas where their hosts are present, ie. *Campylobacter* phages were isolated from poultry faeces and pig farm effluent.
- The phages do not seem to lyse a high percentage of *Campylobacter* strains within species and so a cocktail of phages should be used in applications to control intestinal *Campylobacter* in broilers.
- Encapsulation of phages is necessary to avoid the inactivation of phages during gut transit.
- *Campylobacter* phage genomes do not appear to harbor any virulence genes that may increase pathogenecitiy of *Campylobacter* species.

Opportunity / Benefit:

This project has looked into the feasibility of using *Campylobacter* phages as biocontrol agents in poultry to ultimately improve food safety. Phgaes are easily isolatable from the environment and their production is cost efficient. As they are organic and biodegradable, there is little environmental impact following their use, unlike antibiotics which may persist in water and soil. As a natural and organic entity, phage use for biosanitation is acceptable to the majority of poultry consumers above other decontamination procedures as long as there is transparency (product labelling).

Collaborating Institutions:

Cork Institute of Technology, Bishopstown

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Teagasc project team: Dr. Declan Bolton
Dr. Olivia McAuliffe
External collaborators: Prof. Aidan Coffey

1. Project background:

Campylobacter is the most common food-borne gastrointestinal pathogen globally. The EU notification rate of confirmed cases has shown a significant increasing trend in the last five years, a cause for considerable public health concern. In 2011 the notification rate in Ireland increased by an unprecedented 50%. Poultry are the primary source. Bacteriophage/phage are viruses that infect bacteria. Phage therapy is a natural, sustainable and potentially effective strategy to control *Campylobacter* in poultry. However, the long term efficacy of phages to control *Campylobacter* in poultry requires research to develop the most effective application method and investigate potential negative consequences of phage therapy.

2. Questions addressed by the project:

Are *Campylobacter* phages isolatable from a variety of poultry, human and environmental samples?
Are these phages successful in infecting and lysing *Campylobacter* poultry strains?
What is the best method of phage application to reduce *Campylobacter* numbers in broilers/poultry?
What is the acceptability of phages in poultry products?
Is it possible to clone and express phage enzymes for potential external use for *Campylobacter* lysis?

3. The experimental studies:

Screening for phages was performed on approximately 250 samples ranging from poultry faeces to human stool samples and environmental samples. Four phages were isolated from poultry samples, however the plaquing ability of two didn't allow for characterisations regarding stability and bacterial host range
The two isolated phages were characterised, vB_CjeM_Los1 and vB_CjeM_SOS, regarding their host range against *C. jejuni* and *C.coli* strains, their pH stability, temperature stability and growth rates.
DNA was extracted from phage Los1 and sequencing was performed. In-depth bioinformatics analysis was performed on Los1. Some brief studies were performed assessing the ability of Los1 to reduce the numbers of its host on poultry skin. Novel lytic enzymes from Los1 were identified and cloned into *E. coli* cells. Expression was not possible with *E. coli* in soluble form for any of the enzymes and so *S. cerevisiae* was investigated as an alternative host.

4. Main results:

The phage isolation strain *C. jejuni* PT14 was used primarily for phage isolation due to its previous success in isolating Campylobacter phages. No susceptible phages were found in *Campylobacter*-positive human fecal samples, or environmental samples, but phages were found in poultry faeces, and effluent from a pig farm. Phages Los1 and SOS produced countable plaques in agar overlays and therefore were chosen for further analysis. Both phages were proven to be stable at temperatures encountered in a broiler house, stable upon mechanical manipulations such as vortexing and spraying, and phages were sensitive to pH values encountered in environments such as gastric acids. Phage SOS was isolated with a host range greater than that of Los1, lysing both *C. jejuni* and *C. coli* hosts, whereas Los1 lyses *C. jejuni* only. The host range of each phage within each species is limited, and so a cocktail of phages should be used in applications to ensure a wide array of poultry *Campylobacter* strains are susceptible to at least one phage in the mix.
When Los1 was sprayed onto artificially contaminated chicien skin, an approximate reduction in *C. jejuni* of 1 log unit was observed, in comparison to controls. However post slaughter biocontrol in foodstuffs disagrees with the EU's ''farm to Fork'' mantra. A Literature review was conducted on the findings of previous broiler house trials for intestinal *Campylobacter* elimination using phages. Previous attempts were found to have many drawbacks. One of these was inactivation of phages in the chicken gut, and so encapsulation of phages was investigated, looking for suitable materials to use. This will commence in 2018. It was also determined that administering phages no more than 24 hours before slaughter should maximally decrease intestinal Campylobacter, but if feed withdrawal is practiced, the water supply may be a viable phage administration route. A survey was also disseminated to understand the consumer attitudes towards the

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prospect of phage use in foods. Approximately 370 responses have been received to date with an overall majority favouring the improvement of food safety using natural and organic means. The survey is still open and the results will be fully analysed when all demographics regarding age, sex and educational backgrounds have been addressed.

Los1 was fully sequenced and it was determined to be a lytic phage similar to others in its genus (Cp8viruses). A paper been submitted to Archives of virology detailing the genome features of Los1 while also utlining genomic comparisons between all Cp8viruses sequenced to date. The phages were found to be highly conserved regarding overall genome sequence, open reading frames and gene order, despite their geographical origin. No antimicrobial resistance genes were identified, and the putative endolysin and holin were also identified. The phage endolysin was cloned into *E. coli* (BL21 and Xl1 Blue) as expression vectors and expression attempts were performed, however the lysin was only expressed in insoluble form and only when the sequence was truncated to the enxymatic domain (excluding a signal peptide). Resolubilisation and purification of the protein was possible, however the activity of the protein could not be detected. *S. cerevisiae* was investigated as an alternative host as the production of recombinant endolysin was proving toxic to the *E. coli* cells, despite attempts to optimise the overexpression process. The protein was cloned into the yeast cells with correct plasmid sequence and expression attempts are so far unsuccessful, however a new method is to be tried.

5. Opportunity/Benefit:

The project has investigated the feasibility of utilizing *Campylobacter* phages for control of *Campylobacter* in poultry, and also possibly their hydrolytic enzymes. Recombinant enzyme production proved difficult in both prokaryotic and eukaryotic hosts and further studies are necessary to optimize yields of active protein. It was postulated that the best course of phage administration to broilers should be dissemination in encapsulated form through the broiler house water system no more than 24 hours before slaughter for maximum Campylobacter reduction in poultry. The use of whole *Campylobacter* phages is also not likely to cause transduction of undesirable genes to *Campylobacter* hosts as these were not identified in the sequenced genome of Los1.

6. Dissemination:

Poster presented ‘’Bioinformatic analysis of an Irish lytic *Campylobacter jejuni* Bacteriophage, vB_CjeM_Los1’’, Phages In Interaction, KU Leuven, Sept 2015.

Poster presented ‘’Genome Features of a Lytic *Campylobacter jejuni* Bacteriophage, vB_CjeM_Los1, Isolated in the Republic of Ireland’’, EMBO Viruses of Microbes, Liverpool July 18 – 22 2016

Poster presented ‘’Comparative genomics of fully sequenced Cp8viruses, with reference to the Irish *Campylobacter* phage, vB_CjeM_Los1’’. Evergreen Phage Meeting, Olympia, Washington, Aug 6 – 11th 2017

Oral Presentation ‘’Bacteriophages to control Campylobacter in poultry; administration optimisation and consumer attitudes’’. 46th Annual Food Science and Technology Conference, Ashtown. Dec 6th 2017

Main publications:

O’Sullivan L, Buttimer C, McAuliffe O, Bolton D, Coffey A. Bacteriophage-based tools: recent advances and novel applications. F1000Research. 2016;5:2782. doi:10.12688/f1000research.9705.1.

Accepted manuscript:
Comparative genomics of Cp8viruses with special reference to Campylobacter phage, vB_CjeM_los1, isolated from a slaughterhouse in Ireland (Archives of Virology)

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