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CHARACTERIZATION AND *IN VIVO* EFFICACY OF A T4 BACTERIOPHAGE TO REDUCE NUMBERS OF *CAMPYLOBACTER* IN CHICKENS

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KEYWORDS

Bacteriophage, phagetherapy, *Campylobacter*

EXTENDED ABSTRACT

Campylobacter is recognized worldwide as the major etiologic agent in human diarrheoal disease, with *Campylobacter jejuni* and *Campylobacter coli* being the most prevalent species. The reason for this high incidence is that once *Campylobacter* is introduced into a flock, infection spreads rapidly by environmental contamination and coprophagy. The problem of *Campylobacter* contamination of poultry is exacerbated following slaughter by cross-contamination from *Campylobacter*-positive to *Campylobacter*-negative carcasses during processing in the abattoir, showing that standard biosecurity measures on the processing plant are ineffective. Even if it were possible to reduce the level of carcass contamination, such measures would be costly, difficult to maintain and restrictive. Consequently, another strategy is to operate control measures on the farm and thus significantly reduce colonization with *Campylobacter* prior to slaughter.

Recent legislation restricting the use of antibiotics as growth promoters in animal production, together with the risk of antibiotic-resistant bacteria entering the human food chain, has produced a requirement for alternatives to the use of antibiotics, as methods to control and treat animal infections.

Bacteriophages (phages) are naturally occurring predators of bacteria and they are ubiquitous in the environment. Their high host-specificity and their capacity to evolve to overcome bacterial resistance make them potentially important biocontrol agents of foodborne diseases. Therefore their use as therapy is one possible way to control *Campylobacter* colonization of poultry and prevent *Campylobacter* entering the human food chain.

This study exploits phages as biocontrol agents to reduce the levels of *Campylobacter* in broilers. There are only few reports on *Campylobacter* bacteriophages, probably due to the fastidious nature of the host bacterium which makes the isolation of these phages challenging. Moreover the refractory nature to restriction enzymes digestion of their DNA causes difficulties in characterizing *Campylobacter* phage genomes by common methods such as restriction fragment length polymorphism.

Figure 1 – Phage halos and phage plaques

In the present study 47 *Campylobacter* phages were isolated from poultry intestines. The phages were screened against a panel of food and clinical isolates of *Campylobacter coli* and *Campylobacter jejuni*. Three phages(phiCcoIBB_12;phiCcoIBB_35; phiCcoIBB_37) showed the broadest lytic spectra and therefore were further characterized. These 3 phages were investigated by transmission electron microscopy (TEM), pulsed-field gel electrophoresis, restriction endonuclease analysis and by SDS-PAGE. The three phages had icosahedral heads and long contractile tails and were classified as members of the family *Myoviridae*.

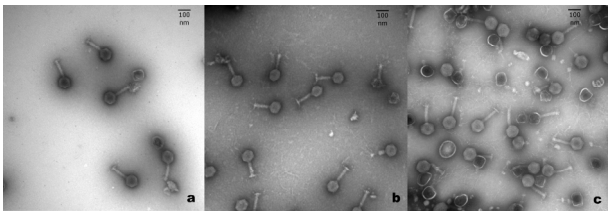

Figure 2 – Electron micrographs of the *Campylobacter* phages that composed the cocktail: (a) Phage phiCcoIBB12; (b) Phage phiCcoIBB35; (c) Phage phiCcoIBB37

Restriction endonuclease analysis demonstrated that DNA from the phages was refractory to digestion by a number of restriction enzymes. The three phages showed similar genome sizes and a similar protein

profile which indicates that although they have different lytic profiles and different latent period/burst sizes they must be very similar. Restriction endonuclease analysis demonstrated that DNA from the phages was refractory to digestion by a number of restriction enzymes. The three phages showed similar genome sizes and a similar protein profile which indicates that although they have different lytic profiles and different latent period/burst sizes they must be very similar.The genome of one of the three phages (phiCcoIBB35) was further studied. The PFGE analysis indicates that the genome of this phage is approximately 204 kb. The DNA sequence data consists of five DNA contigs in a total of 172 kb. Attempts to close the gaps were unsuccessful since the DNA preparations appeared to contain substances that inhibit Taq and ϕ29 polymerases.

The sequence of this phage showed significant homology only to the 178 kb *C.jejuni* phage CP220 (http://www.sanger.ac.uk/Projects/Phage/). Annotation indicates that most of the ORFs are unique and that homology exists with members of the *Teequatrovirinae* namely for all T4 tail proteins, one head protein (gp23), neck protein (gp20); and baseplate proteins (gp6, gp25, gp48). Moreover homologs were found to T4 proteins involved in morphogenesis, nucleotide metabolism, transcription, DNA replication and recombination.

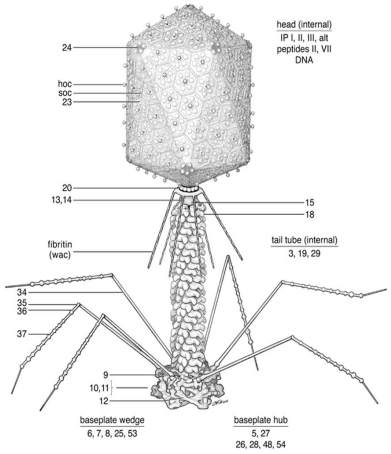

Figure 3 – Structure of phage T4

Unique genes involved in the carbohydrate metabolism, pathogenesis and amino acid metabolism were also observed. Several incidences of gene duplications, split genes with intein and introns and "insertion-like sequences" were present. To our knowledge this study represents the first report of the genomic sequence of a lytic *C.coli* phage and therefore is of extreme importance for further comparisons with other phage sequences. The present study aimed to test the efficacy of a phage cocktail composed by the three previously characterized phages for the control of *C.coli* and *C.jejuni* in infected poultry. Moreover, it evaluated the effectiveness of two routes of phage administration (by oral gavage and in feed) in order to provide additional information regarding their future use in a poultry unit.

The phage cocktail was able to reduce the titre of both *C.coli* and *C.jejuni* in faeces by approximately 2 $\log_{10}$ cfu/g when administered by oral gavage and in feed. This reduction persisted throughout the experimental period and neither pathogen regained their former numbers. The reduction in *Campylobacter* titre was achieved earlier (2 days post-phage administration) when the phage cocktail was incorporated in the birds' feed. *Campylobacter* strains resistant to phage infection were recovered from phage-treated chickens at a frequency of 13%. These resistant phenotypes did not exhibit a reduced ability to colonize the chicken guts and did not revert to sensitive types.

Our findings provide further evidence of the efficacy of phage therapy for the control of *Campylobacter* in poultry. The broad host range of the novel phage cocktail enabled it to target both *C.jejuni* and *C.coli* strains. Moreover the reduction of *Campylobacter* by approximately 2 $\log_{10}$cfu/g, as occurred in our study, could lead to a 30-fold reduction in the incidence of campylobacteriosis associated with consumption of chicken meals (according to mathematical models).

To our knowledge this is the first report of phage being administered in feed to *Campylobacter*-infected chicks and our results show that it lead to an earlier and more sustainable reduction of *Campylobacter* than administration by oral gavage. Therefore the study described herein is a proof of principle that *Campylobacter* phages given orally or administered in feed can effectively reduce the *Campylobacter* colonization levels. Further studies need to be undertaken in order to test phage effectiveness in older chickens, their use as prophylactic agents and longer time course trials in order to reflect the production cycle.

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