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Exploiting the biotechnological potential of bacteriophages towards food safety

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Bacteriophages are naturally occurring predators of bacteria, ubiquitous in the environment, with high host specificity and capacity to evolve to overcome bacterial resistance. These features make phages appealing options for the control of pathogens occurring in animals and/or food products in order to prevent transmission of foodborne pathogens to humans. Furthermore phages encode murein hydrolases (endolysins) that break down bacterial peptidoglycan at the terminal stage of the phage reproduction cycle. These endolysins can be very attractive tools to control bacterial pathogens, however the use of these enzymes externally in Gram-negative bacteria is challenging.

We have isolated several phages from sewage water and poultry products with the ability to infect and kill strains of *Salmonella enterica* serovar Enteritidis, *Campylobacter coli* and *Campylobacter jejuni* and tested the efficacy of these phages in young chickens. Furthermore, the isolated phages were fully characterized in terms of morphology (Transmission Electron Microscopy), genome size (RFLP), structural proteome (SDS-PAGE) and infection parameters (burst size and latency period). Two phages (PVP-SE1 and CcoM-IBB_35) were sequenced and their sequences deposited in the GenBank. *In vivo* trials demonstrated that phages administered orally and incorporated in food dramatically reduce the numbers of colonising campylobacters and salmonellas [1]. An average 3.1 log and 1.98 log reductions in *Salmonella* and *Campylobacter* numbers were achieved, respectively.

Endolysin genes from the *Salmonella* phages 68 and PVP-SE1 [2]. were cloned and overproduced in *Escherichia coli* and were shown to display antimicrobial activity against *Pseudomonas* sp., in particular when pretreated EDTA. In addition, methods to overcome the outer membrane barrier of Gram-negatives are being developed.

References

- [1] Carvalho CM, Gannon BW, Halfhide DE, Santos S, Hayes CM, Roe JM, Azeredo J, "The in vivo efficacy of two administration routes of a phage cocktail to reduce numbers of *Campylobacter coli* and *Campylobacter jejuni* in chickens", *BMC Microbiology* (2010) 10:1-11.
- [2] Santos S, Kropinski AM, Ceyssens P-J, Ackermann HW, Villegas A, Carvalho CM, Lavigne R, Krylov VN, Ferreira EC, Azeredo J, "Genomic and proteomic characterization of the broad host range *Salmonella* phage PVP-SE1 - The creation of a new phage genus", *Journal of Virology* (2011), 185:11265-11273.