## Isolation, characterization and *in vivo* performance evaluation of a coliphage.

<u>A. Oliveira</u><sup>1,2</sup>, A. Nicolau<sup>2</sup>, R. Sereno<sup>1</sup>, and J. Azeredo<sup>2\*</sup>. \*jazeredo@deb.uminho.pt

*Escherichia coli* (*E. coli*) infections, colibacillosis, are responsible for significant economic losses in poultry industries. The increased resistance of *E. coli* strains to antibiotics has encouraged the search of new solutions to control severe infections ensuring good meat quality and minimising environmental impact. Bacteriophages (phages) have been pointed out as a valuable alternative to antibiotics in animal production.

Studies intending the development of an efficient, safe and competitive phage product to treat colibacillosis in commercial birds are presented. F78E was a phage isolated from poultry sewage and the lytic spectra showed that 35.14% of 148 avian pathogenic E.coli strains were sensitive to it. This phage exhibits strictly lytic characteristics as demonstrated through the prophage release induction test. TEM observations of this phage showed that it is a 16-19 phage, *Myoviridae*, roughly like T4, belonging to Caudovirales order. The RFLP pattern was defined with 3 restriction enzymes. Three different In vivo tests were performed according to FELASA principles of animal welfare, in order to evaluate the phage suspension toxicity, the phage dynamics in chicken's organisms, and the phage efficiency. Phage toxicity was evaluated by injecting 1 ml of a phage crude lysate containing 1.67x10<sup>8</sup> pfu/ml, intramuscularly in chickens. The results revealed that this phage suspension did not promote any decrease in feed and water intake, or body weight lost during the *in vivo* trial and the post mortem necropsies did not show any macroscopic lesions in the internal organs. Phage dissemination in the chickens' organs was assessed by administering intramuscularly, orally or by spray, different concentrations of the phage. The presence of F78E in the chicken's lungs and air sacs was detected after 3, 10 and 24 hours of challenging with 1x10<sup>8</sup> pfu/ml by spray and orally, and 3 and 10 hours after administer 1x10<sup>7</sup> pfu/ml by the same way, suggesting that these two routes of administration are promising to treat respiratory *E.coli* infections. Therefore the *in vivo* efficacy of this phage was initially tested through the oral administration or by spray of 1ml of a phage concentration of 1x10<sup>9</sup> pfu/ml, just after injecting chickens intra-sacs, with 1.4x10<sup>8</sup> cfu of an avian pathogenic *E. coli*, F78E sensitive. A decrease of 25% of the chickens' mortality and a reduction of 43% of morbidity was obtained.

The results of these studies suggest that the oral or spray administration of a suspension of phage F78E can safely and efficiently treat severe *E. coli* infections in poultry.

Notes:

<sup>1.</sup> Controlvet, Segurança Alimentar, S.A., Zona Industrial de Tondela, ZIM II, L 6, 3460-070Tondela, Portugal.

IBB, Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar 4710-057, Braga, Portugal.