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436-1 DEVELOPING PHAGE THERAPY FOR THE REDUCTION OF NONTYPHOIDAL SALMONELLA IN BROILER CHICKENS: CHARACTERIZATION OF WILD-TYPE BACTERIOPHAGES

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Resumo:

Bacteriophages products have been proposed as alternative approach to reduce intestinal colonization of foodborne bacteria in broiler chickens at farms. The success or failure of poultry phage therapy depends on the choice of bacteriophages sufficiently virulent against the target bacteria, the number of viable phages produced in the cell host, a broader field strains host range and their ability to overcome bacteria-mediated protection mechanisms. Furthermore, therapeutic phages must be lytic and free of genes encoding for antimicrobial resistance (ARG) and virulence factors. Bacteriophage increase population varies according to adsorption rates to the host bacterium, including the time from infection until the first new infective phages particle have formed inside the infected cell (eclipse time), the duration of the infection starting with adsorption and ending with lysis (latent period), and the number of phage progeny produced per cell upon lysis (burst size). We have previously reported the cecal concentration reduction of Salmonella Enteritidis in broiler chickens after oral delivery of a cocktail with three wild-type lytic phages. Here we used one-step growth curve analysis to quantify the ability of such phages to lyse Salmonella field strains in vitro. Complete genome sequence and comparative genomic analysis were also conducted. Eclipse time, latent period and burst size of each phage at 42 °C were determined against S. Enteritidis and S. Heidelberg isolated from broiler litter, in a MOI of 0.1. Two aliquots of each co-incubation were taken every 5 min for a total duration of 75 min. To measure viable phages, one sample was immediately 10-fold serially diluted and enumerated by double layer agar assay. The other sample was lysed with 1% chloroform to release intracellular phages before serial dilution and enumeration. Genomic DNA of phages was shotgun-sequenced using the Illumina MiSeq platform. Sequences were assembled from the paired-end reads and ORFs were identified and annotated. Prediction of ARGs and virulence genes was carried out using public databases. Available integrase genes sequences were downloaded and aligned against the phages genes. Lytic growth kinetics showed that phages had relatively similar eclipse time against both evaluated Salmonella serotypes. The lowest latent period and higher burst size found against S. Heidelberg was 45 min and 54 PFU/cell, respectively. Shorter latent period (15 to 35 min) and larger burst sizes (up to 103 PFU/cell) in S. Enteritidis indicated faster in vitro infection and proliferation against such a serotype. DNA sequencing analysis revealed genomes containing 156,612 to 159,072 bp with overall GC content of 44.4%. Between 199 and 201 coding sequences (CDS) were predicted, none of them containing ARG or virulence factor genes. CDS alignment against 88,034 integrase amino acids sequences revealed absence of integrase genes in the phages genomes. Phages genomes comparison showed similarities from 92.59% to 96.27% between each other. Phages were taxonomically assigned to the family Ackermannviridae as predicted by in silico analysis. In conclusion, studied phages are lytic and able to propagate in different levels inside the infected target bacterium, according to the Salmonella serotype. Genome sequencing ensured the safety of studied phages making them candidates for further studies on therapy applications in broilers.

Palavras-chave:

poultry, salmonellosis, phage therapy, genomic analysis

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