OP4.1 - CONTROLLING DIARRHEAGENIC *E. COLI* WITH BACTERIOPHAGES: FACTS AND CHALLENGES

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ABSTRACT

Enterotoxigenic *Escherichia coli* (ETEC) colonizes the intestine, causing severe diarrhoea in humans and animals. The rise of antibiotic resistances and limitation on their use demands news strategies to tackle this pathology.

Bacteriophages (phages), viruses specifically infecting bacteria and harmless to animals and plants, are a promising antibacterial tool. Although studies support their ability to efficiently overcome ETEC infections, they have been shown to be highly strain-specific. If this can be associated with the presence of anti-phage defense systems (APDS) in ETEC genomes, it is also true that phages can counter-evolve to escape APDS.

This work aimed to define phage cocktail with broader lytic spectra, capable of overcoming APDS of ETEC, enhancing phage efficacy.

We firstly sequenced 29 ETEC strains from our collection to search for the presence of APDS in their genomes. Then, we performed phage isolation and the subsequent *in vitro* and genomic characterization: i) evaluation of lytic spectra against ETEC collection; ii) whole-genome sequencing and phage safety evaluation (absence of undesirable genes) iii)presence of proteins responsible for escaping the main APDS.

We were able to identify distinct mechanisms supporting APDS. Bacterial proteins that prevent the entry of DNA from phages (CRISPR-Cas-related proteins) or that enable the cut of phage nucleic acids (restriction-modification enzymes) were detected, however, most of them were related with the induction of “abortive infection” events (e.g. toxin-antitoxin systems).

We also isolated 3 phages, SUS35, SUS42 and SUS65, which proved to be safe for therapy and to encode proteins enabling to escape APDS, inclusively against “abortive infection”.

Phage-host interaction mechanisms must be considered when preparing phage- based products for therapy. This work clearly indicates that a strict selection of phages, or the construction of synthetic phages with desired traits will be a turning point in their versatility to fight against ETEC infections.