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Unveiling the impact of STEC infecting phages on the colon microbiota using an *in vitro* fermentation model

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(Bacterio)phages are considered safe for humans' consumption, being regard as excellent biocontrol tools to prevent foodborne pathogens spread. Phages' major advantage is their inherent specificity towards a bacterial species, yet some reports have shown phages ability to evolve to infect different hosts when transiting the gastrointestinal tract (GIT). And so, it is of extreme importance to understand the safety outcome of using phages as biocontrol agents in food, with particular interest in the ones that target species from *Enterobacteriaceae* family, commonly found in the human GIT microbiota. In this study, the impact of a phage infecting Shiga toxin-producing *Escherichia coli* (STEC), named *E. coli* phage vB_EcoS_Ace (Ace), towards the colon microbiota was investigated. An *in vitro* batch fermentation model was used, and the inoculum was the fecal material of three healthy donors. Fermentations' metabolome was analyzed through GC and HPLC, and the concentration of both phage Ace and STEC strain were monitored along time (up to 24h). The interference with the gut microbiota composition and functional potential was assessed by shot gun metagenomics.

We observed an increase in phage titre only when the host was present, suggesting that there was no other suitable host within the different microbiotas used. Also, the microbiotas' composition did not alter when phage Ace was added. Nevertheless, the attenuated version of STEC strain did indeed create some perturbation in the microbiota, which led to different functional potential. This was corroborated by the differences observed for both gas and short chain fatty acid acids dynamics. The microbiotas' individuality was an important factor for the observed perturbations. Moreover, phage Ace revealed to be a safe phage when intended to be used as a biocontrol agent for food products. Also, we concluded that the *in vitro* fermentation model is a reliable, easy, and non-expensive safety screening methodology for phages.