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**Antibacterial activity on opportunistic *Pseudomonas aeruginosa* pathogen by a novel *Salmonella* phage endolysin****Hugo Oliveira<sup>1</sup>, Maarten Walmagh<sup>2</sup>, Leon Kluskens<sup>1</sup>, Rob Lavigne<sup>2</sup>, Joana Azeredo<sup>1</sup>**

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The Gram-negative pathogen *Pseudomonas aeruginosa* can cause severe infections of burn wound or cystic fibrosis on patients. Bacteriophage endolysin based strategy can offer a new alternative antimicrobial therapy. Endolysins are lytic enzymes that break down the peptidoglycan of bacterial cell wall at the late phage lytic cycle, however they are inactive on their own against Gram-negative bacteria when applied exogenously as recombinant proteins due to the peptidoglycan (endolysin substrate) protective outer membrane. We propose an innovative strategy to target Gram-negative *Ps. aeruginosa* based on the combination of endolysin enzymes and an outer membrane permeabilizing agent - ethylenediamine tetraacetic acid (EDTA). To validate this approach, we have isolated a novel *Salmonella* phage endolysin (68gpLys). Cloning this gene into *E. coli* expression system and subsequent large scale protein expression led to a high soluble yield of 14,3 mg/L of expression culture. In order to characterize it, muralytic assays on chloroform/Tris-HCl pretreated *Ps. aeruginosa* strain PAO1k (to remove the outer membrane) were made to check activity levels on substrate (398.05 Units/mM). The pH range was also determined with pH 7 being the optimum for the endolysin activity. For antimicrobial test, *in vitro* assays showed that incubation of  $10^6$  *Ps. aeruginosa* cells/mL with 0.5 mM EDTA and 5000 nM of 68gpLys, led to a strain inactivation of  $3.42 \pm 0.02$  logarithmic reduction units in a time-frame of 30 min. Here we prove that the synergistic effect of endolysin 68gpLys with EDTA can significantly reduce *Ps. aeruginosa* contamination. These results suggest the great potential of this strategy for prevention and/or control of other Gram-negative pathogens. Current work has been also development to engineer new endolysins with incorporated cell penetrating peptides (CPP), employing site- and random-mutagenesis molecular techniques, to further enhance outer membrane permeabilization.

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