Cloning and purifying a novel endolysin from a lytic *Pseudomonas aeruginosa* bacteriophage

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Introduction: The successful results of phage therapy have led to novel research involving bacteriophages and enzymes encoded by the viruses that can be applied in the control of multidrug resistant bacteria and healthcare-associated infections. **Objective:** This study cloned, expressed and purified novel recombinant bacteriophage-encoded endolysins to further application as antibacterial and antibiofilm agents. **Methods**: The gene that code for endolysin in the bacteriophage vB_PaeM_USP_2 was cloned [pET-28(+) and used to transform *Escherichia coli* BL21 (DE3) strain. One transformed clones was isolated and cultivated in LB broth supplemented with 50 µg/mL of kanamycin to produce the novel recombinant endolysin. When the culture achieved the exponential growth phase, 0.5 mM of IPTG was added and the cells were further cultured for another 16 h at 18°C. Subsequently, the cells were collected and lysed by sonication. The suspension was centrifuged, filtered and subjected to nickel affinity chromatography. The novel recombinant endolysin was eluted with 250 mM imidazole, 20 mM NaH_2PO_4, 300 mM NaCl, and 30% (v/v) glycerol. The lytic activity of the novel purified endolysin was measured through peptidoglycan layer degradation and decrease in turbidity of permeabilized gram-negative bacteria. **Results**: The novel recombinant endolysin showed clear muralytic activity and decrease turbidity of permeabilized *Pseudomonas aeruginosa, Klebsiella pneumonie*, *Salmonella enterica* and *E. coli*. **Conclusion**: Although the results are promising, additional studies are necessary to evaluate the antimicrobial and antibiofilm activities of the novel recombinant endolysin.

Key words: Endolysin, bacteriophage, *Pseudomonas aeruginosa.*

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