

# FUNCTIONAL CHARACTERIZATION OF SEPTICOLYSIN FROM THE OPPORTUNISTIC PATHOGEN *ACINETOBACTER BAUMANNII*

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One of the biggest threats in health care settings is the Gram negative opportunistic pathogen *Acinetobacter baumannii* which causes a variety of infections in immunosuppressed patients. Its ability to persist in clinical environment and multidrug resistance profile makes *A. baumannii* one of the most challenging nosocomial pathogens [1]. Therefore, identification of the virulent features is for understanding of the pathogenesis mechanisms displayed by *A. baumannii*.

Frequently virulence genes are acquired during horizontal gene transfer, e.g. plasmids or transposable elements [2]. *spl* gene is located in *A. baumannii* plasmid pAB120 [3] and possibly could be described as one of the putative virulence genes, since the product of this gene -septicolysin- has homology to the pore-forming toxins in other pathogenic bacteria [4]. The aim of this work was to assess the importance of septicolysin in *A. baumannii* pathogenesis.

In order to determine, whether septicolysin could be a pore-forming virulence factor, *spl* gene from plasmid pAB120 was cloned into pUC\_gm\_AcORI and pUC\_gm\_AcORI\_Ptac vectors. These constructs were further transformed into septicolysin-lacking *A. baumannii* strain. Resulting strains were tested for the hemolytic activity. Cytotoxicity on the mice lung epithelial cells LLC1 was determined by measuring cell viability by trypan blue uptake. *C. elegans* fertility assay was performed to identify if septicolysin increases *A. baumannii* virulence *in vivo*.

According to our results, we did not observe any differences in hemolysis and cytotoxicity comparing *A. baumannii* wild type strain and the strain with introduced *spl* gene. Also, *spl* gene did not have an impact on nematodes fertility.

In conclusion, septicolysin didn't demonstrate the expected properties of pore-forming toxins in the tested models. However, further investigations using purified septicolysin are needed in order to determine its virulence.

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[2] R. W. Jackson et al., The influence of the accessory genome on bacterial pathogen evolution, *Mobile Genetic Elements* 1:1, 55-65; May/June (2011).

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[4] S. K. B. Cassidy et al., More Than a Pore: The Cellular Response to Cholesterol-Dependent Cytolysins, *Toxins* 2013, 5, 618-636 (2013).