Development of a Production System for an Antimicrobial Peptide PaMAP 1.5 Based on ELP-Intein Tag

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The frequent description of multidrug resistant bacteria lead to search for novel antimicrobial drugs with novel mechanisms of action. Among then, antimicrobial peptides (AMPs) stand out as promising anti-infective molecules. However, high costs associated to isolation from natural sources or chemical synthesis are a limitation in AMPs production for clinical and research proposes. Aiming to solve these problems, the expression of Pa-MAP 1.5, a polyalanine AMP, fused to an ELP-Intein tag was here proposed. The ELP was used to promote aggregation and simple isolation after expression and intein to stimulate a controlled AMP release. In summary, this work aims to create a cost-effective production system for Pa-MAP 1.5. For these, the expression vectors pET21a were used as backbone to production of Pa-MAP 1.5 fused at N-termini region to a modified *Mxe* GyrA intein followed by an ELP (60 repetitions). Competent BL21 *E. coli* cells were transformed with this plasmid, overnight grown and further inoculated in selective TB media (1:100). At mid-log phase, the expression was induced by addition of 1 mM IPTG for 4 h. Cells where recovered by centrifugation and target protein was precipitated by inverse transition cycle (ITC) at 50 ^{o}C and isolated from the lysate, by centrifugation and resuspended in cold PBS. Furthermore, the intein was temperature activated (19 ^{o}C / 48 h), releasing the peptide, which was separated from ELP by a new ITC. SDS-PAGE analysis of total cellular extract showed a proteinaceous band after induction with approximately 47 kDa corresponding to PaMAP 1.5 linked to its ELP-Intein Tag. This same band was the only visualized after ELP-precipitation. After intein activation, a band of 2.5 kDa, correspondent to isolated Pa-MAP 1.5 was also observed. An antimicrobial test proved the bactericidal activity of recombinant PaMAP 1.5 showing a MIC of 25 µM against *E. coli*. Data here reported correspond to the development of an expression system that can be possible used as a cost-effective method of production for PaMAP 1.5, a future alternative to conventional antibiotics.

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