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

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INTRODUCTION: The requirement for novel antimicrobial drugs to treat multidrug-resistant bacteria has driven numerous research groups to search for alternative molecules with novel mechanisms of action, including antimicrobial peptides (AMPs). However, high costs in chemical synthesis are a limitation in AMPs production for clinical approach. 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 promotes aggregation after the expression and inteins stimulate a controlled AMP release. In summary, this work aims to create a cost-effective production system for Pa-MAP 1.5.

MATERIAL AND METHODS: The expression vectors pET21a were used as backbone to expression of Pa-MAP 1.5 fused to GyrA intein at N-termini region followed by an ELP (60 repetitions). For these, transformed BL21 \textit{E. coli} cells were overnight grown and further inoculated in selective TB media (1:100). The expression was induced by IPTG for 4 h. Target protein was precipitated by inverse transition cycle (ITC) at 50 °C and isolated from the lysate by centrifugation. Furthermore, the intein was temperature activated (19 °C / 48 h), releasing the peptide, which was separated from ELP by a new ITC.

DISCUSSION AND RESULTS: SDS-PAGE analysis of total cellular extract showed a proteinaceous band after induction, with approximately 47 kDa. 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 activity of recombinant PaMAP showing a MIC of 25 μM against \textit{E. coli}.

CONCLUSION: Data here reported correspond to the development of an expression system that can be possible used as a cost-effective AMP method of production, a future alternative to conventional antibiotics.

Keywords: ELP tag, intein, antimicrobial peptide, production system
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