Exploiting different strategies for the recombinant production of antimicrobial peptides

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Antimicrobial peptides (AMPs) are broad-spectrum host defense molecules, considered as promising candidates to face the crisis of antimicrobial resistance. The human cathelicidin LL37 and its shorter fragments are of great interest for their antibacterial and anti-biofilm properties, also against multi-drug resistant pathogens. Since huge amounts of AMPs are required for clinical applications, the optimization of their production is needed. In this field, recombinant expression represents the most cost-effective option, previously published in: Pennone V et al. (2024) Front Microbiol, Submitted. *E. coli* is the most used prokaryotic expression system, despite two challenges have to be addressed: potential toxicity and proteolytic degradation of AMPs. We applied a fusion strategy to express LL37 peptide in *E. coli* cells linked to the small metal-binding protein (SmbP) carrier to reduce the AMP toxicity and improve solubility. The SmbP-LL37 protein was purified from the crude extract by affinity chromatography with a volumetric yield of 2.4 mg/L and digestion with enterokinase was performed to isolate the LL37 peptide. Since obtaining the peptide without its fusion partner is challenging, an alternative production strategy was established. The FK16 peptide, a promising shorter version of LL37, was expressed in *E. coli* in the form of inclusion bodies. The peptide was solubilized upon treatment of the cell lysate with Triton X-100 and purified by affinity chromatography, obtaining ≈5 mg of peptide per liter of cell culture. The latter expression strategy seems well suited for producing additional LL37 fragments and thus to evaluate their antibacterial properties, assess their conformational changes and investigate bacterial membrane degradation using fluorescent dyes.
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