Production and purification of recombinant antimicrobial peptides by exploiting the termoresponsiveness of the elastin-like polymer based on the VPAVG sequence

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The extensive and incorrect use of chemical antibiotics led to a resistance development by pathogens. The antimicrobial peptides (AMPs) arise as a good alternative to traditional pharmaceutical agents. They exhibit a broad range of antimicrobial activity but antitumoral and antiviral activities have also been found. AMPs are usually small, cationic molecules that occur as part of the innate defense mechanism in many organisms, even in microbes and virus [1]. With the increasing interest in AMPs, several new strategies are emerging for their recombinant production.In this work we describe a new strategy for the production of two soluble recombinant antimicrobial peptides in *E. coli*: ABP-CM4 and BMAP-28, from *Bombyx mori*(silkworm), and *Bos Taurus* (cow), respectively. ABP-CM4 is an amphipatic á-helical, 35 amino acid sequence peptide, belonging to the cecropin family and has been described as possessing antibacterial, antifungal and antitumoral activities. BMAP-28 belongs to the cathelicidyn family and it consists on a helical structure with 28 amino acids. The DNA coding sequences were chemically synthesized with the inclusion of an ATG start codon in the N-terminus and a formic acid chemical-cleavage site in the C-terminus. These sequences were fused in frame with the N-terminus of the gene coding for the elastin-like polymer (ELP), consisting of 220 repeats of the main pentamer VPAVG. This ELP has been described as exhibiting thermoresponsive properties that were exploited as a purification method [2]. Both recombinant constructions were cloned in a modified pTE25 expression vector and were expressed in *E. coli* BL21(DE3) (Novagen). The culture conditions for high levels of recombinant proteins expression were attained. Purification was based on the use of the inverse transition cycling method [3]. The global aim of this work involves the full physico-chemical characterization as well as the evaluation of the biological activity of the recombinant ABP-CM4::VPAVG220 and BMAP-28::VPAVG220 fusion proteins produced.

References:
(1) Brogden K. A. (2005) *Nature Reviews Microbiology* **3**:238-250.
(2) Machado R. *et al.* (2009) *Journal of Nano Research* **6**:133-145.
(3) Daniell H. *et al.* (1997) *Methods in Molecular Biology* **63**:359-371.