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ESCHERICHIA COLI EXPRESSION AND PURIFICATION OF LL37 FUSED TO A FAMILY III CARBOHYDRATE-BINDING MODULE FROM *CLOSTRIDIUM THERMOCELLUM*

Reinaldo Ramos, Lucília Domingues and Miguel Gama
Department of Biological Engineering
E-mail: reinaldo_ramos@deb.uminho.pt

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INTRODUCTION

Antimicrobial peptides (AMPs) are part of the innate immune system and have a large spectrum of antimicrobial activities against Gram-positive and Gram-negative bacteria, fungi (Ajesh and Sreejith 2009) and viruses (Hancock and Diamond 2000).
In mammals, defensins and cathelicidins represent the two major types of AMPs. The hCAP-18/LL37 is the only human cathelicidin. The antimicrobial peptide is referred to as LL37, since it has a 37 aminoacids sequence starting with two leucines. It is a 4.5 kDa, cationic (+6), amphipathic α-helical peptide, with a broad spectrum of antimicrobial activity. Besides its protective effect against infections, a variety of other biological activities have been described.
Family-III CBDs normally comprise ~150 amino acids residues and have been identified in many different bacterial enzymes, and also in non-hydrolytic proteins (Tormo, Lamed et al. 1996). *Clostridium thermocellum* produces a multi-enzyme complex of cellulases and hemicellulases, termed the cellulosome, which is assembled by the scaffoldin protein CipA. Binding of the cellulosome to the plant cell wall is driven by the action of the CipA family 3 CBM (CBM3), which presents high affinity for crystalline cellulose (Guerreiro, Fontes et al. 2008).
In this work, we describe the successful cloning, expression and purification of LL37 using the CBM3 from *Clostridium thermocellum* as fusion partner. The CBM3 is overexpressed in *E. coli* and it is possible to take advantage of its affinity properties to purify recombinant proteins on cellulose fibers, reducing significantly the costs of purification.

RESULTS AND DISCUSSION

Expression and Purification of Recombinant Proteins

The protein LL37 was successfully cloned at the N-terminal of the LK-CBM3 in the expression vector pET21-a. After transformation of the recombinant plasmid into *E. coli* BL21 (DE3), , the production of the protein (~27 kDa) in the soluble form was only achieved using the nonionic detergent, Triton X-100 (figure 1).

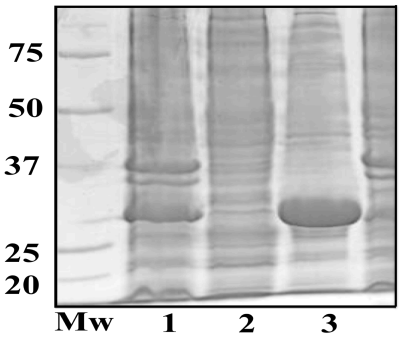

Figure 1 – Expression of recombinant protein CBM3-LL37. Mw: protein molecular weight marker – 1: insoluble fraction – 2: soluble fraction – 3: Triton X-100 solubilized fraction.

Chemical Cleavage and Purification of LL37

The chemical cleavage of the recombinant protein was performed, in this work, with formic acid 50%, at 50ºC for 24h. LK-CBM3-LL37 protein was first purified on cellulose.

Then, formic acid was applied directly on the CF11 fibres with the bound protein.

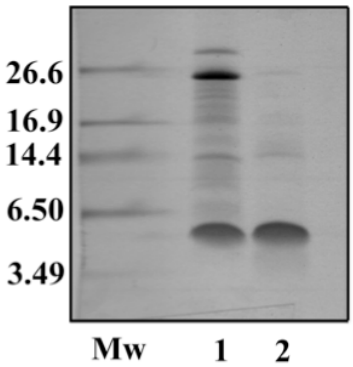

Figure 2 – Formic acid cleavage of LL37. Mw: protein molecular weight marker – 3: insoluble fraction – 4: supernatant of cleaved CBM3-LL37.

The soluble supernatant, with a large proportion of LL37, was then subjected to RP-HPLC for improved purification. The purification of LL37 was successfully achieved. The fractions corresponding to the major peaks were collected and the purity of the peptide was confirmed on 16.5% Tris-tricine gel and MALDI-TOF.
Figure 3 presents eluted fractions from RP-HPLC. The lane 4 demonstrates the purity of the peptide.

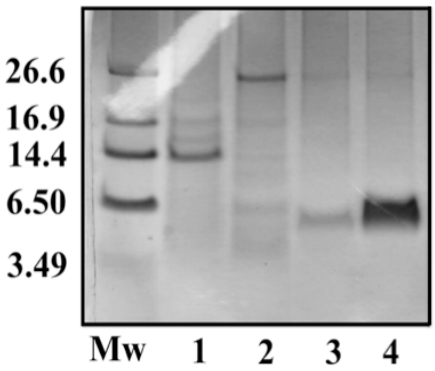

Figure 3 – RP-HPLC of eluted fractions. Mw: protein molecular weight marker; lanes 1-4: eluted fractions of cleaved LK-CBM3-LL37.

Antibacterial Activity

P-LL37 antibacterial activity was tested against *E. coli* K12. The concentration of the peptide was quantified by UV spectroscopy using the Waddell's method (Waddell 1956). Figure 4 demonstrates that P-LL37 has antimicrobial activity, with a MIC of about 180 μg/ml (40 μM).

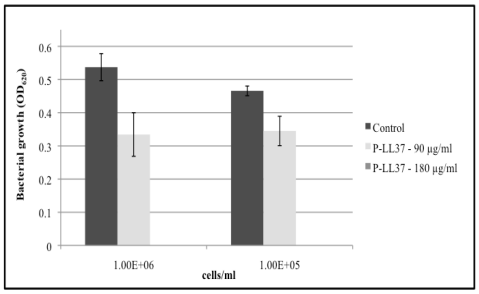

Figure 4 - Antibacterial activity of P-LL37 against *E. coli* K12

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AUTHOR BIOGRAPHY

REINALDO RAMOS

2006- : Biochemistry and Biotechnology PhD at the department of Biological Engineering, Univ. Minho

2003-2005: Biotechnology Master degree at the department of Biological Engineering, Univ. Minho

1997-2002: Biological Engineering degree, Univ. Minho

reinaldo_ramos@deb.uminho.pt