ESCHERICHIA COLI EXPRESSION AND PURIFICATION OF LL37 FUSED TO A FAMILY III CBM FROM *CLOSTRIDIUM THERMOCELLUM*

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Introduction

LL-37 (LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES) is a very promising human cationic peptide with 37 aminoacids and α-helix structure. It has been shown to exhibit a broad spectrum of antimicrobial activity and to have additional defensive roles such as regulating the inflammatory response and chemo-attracting cells of the adaptative immune system to wound or infection sites, binding and neutralizing lipopolysaccharides (LPS), promoting angiogenesis, re-epithelialization and wound closure.

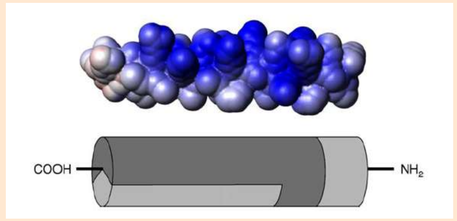

Figure 1 – α-helical structure of LL-37

Objectives

The main objective of this work is the expression and purification of the LL-37 fused to the CBM3 of *Clostridium thermocellum* in *Escherichia coli* for antibacterial and immunological activities. The fusion protein is purified on cellulose exploiting the CBM3 cellulose-binding properties. The chemical cleavage of LL37 from the CBM3 is performed with formic acid and LL37 is finally purified by RP-HPLC.

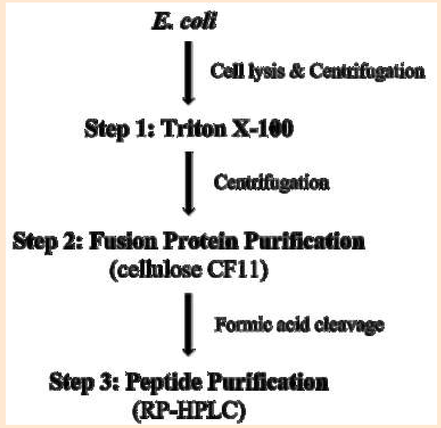

Figure 2 – Flow chart for the expression and purification of LL37 from *E. coli*

Results

Expression and Purification

- The cloning and expression of the recombinant protein (~26 kDa) was successful. The recombinant protein was solubilized with Triton X-100 (figure 3)
- LL37 (~4.5 kDa) was efficiently cleaved from the CBM3 using formic acid 50% (figure 4)
- LL37 was finally purified by RP-HPLC. The purity was confirmed by SDS-PAGE and MALDI-TOF (figure 5)

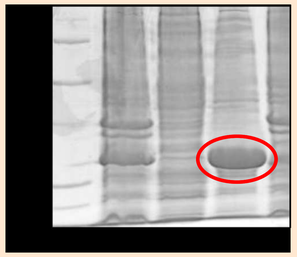

Figure 3 – Expression of recombinant protein CBM3-LL37. Mw: protein molecular weight marker – 1: insoluble fraction – 2: soluble fraction – 3: Triton X-100 solubilized fraction.

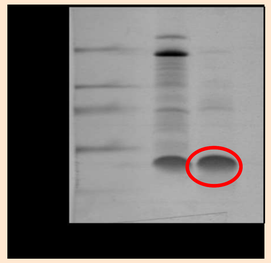

Figure 4 – Formic acid cleavage of LL37. Mw: protein molecular weight marker – 3: insoluble fraction – 4: supernatant of cleaved CBM3-LL37.

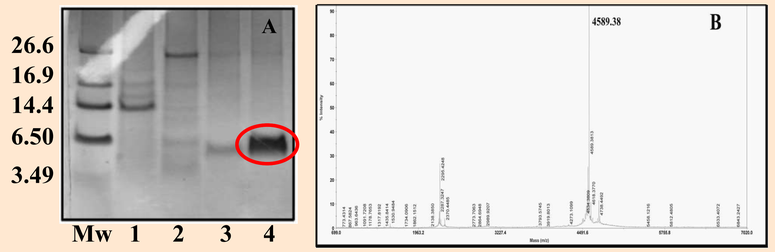

Figure 5 – **A:** RP-HPLC of eluted fractions. Mw: protein molecular weight marker; lanes 1-4: eluted fractions of cleaved LK-CBM3-LL37. **B:** MALDI-TOF MS analysis of purified LL37

Antimicrobial activity

- Recombinant LL37 was tested against *E. coli* K12 and showed antibacterial actiivity with a MIC of 40 μM – 180 μg/ml (figure 6)

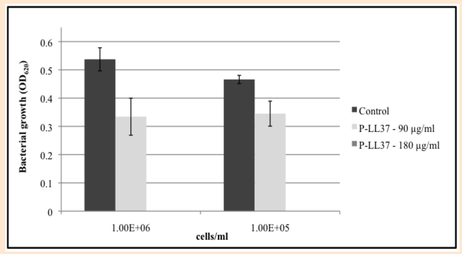

Figure 6 - Antibacterial activity of P-LL37 against *E. coli* K12

Immunological activities

- Recombinant LL37 inhibited LPS-induced production of inflammatory cytokine TNF-α in murine macrophages (figure 7)
- Recombinant LL37 induced angiogenesis (growth of new blood vessels) of endothelial cells (figure 8)

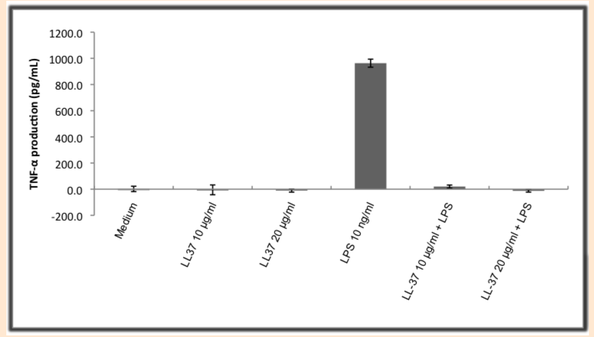

Figure 7 – Inhibition of TNF-α production by murine macrophages stimulated with 10 ng/ml LPS

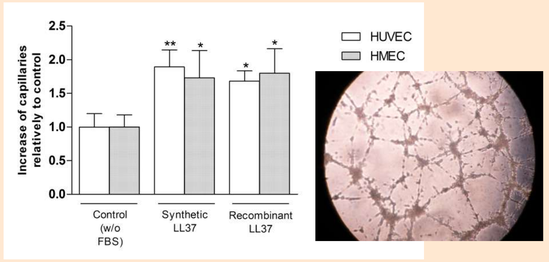

Figure 8 – Angiogenic activity of recombinant LL37 on human umbilical vein endothelial cells (HUVEC) and human mammary epithelial cells (HMEC)

Conclusions

- LL37 has successfully been cloned, expressed and purified in *E. coli*
- The LL37 production and purification methodology developed in this work presents significant advantages towards the previously described.
- The recombinant peptide LL37 is functionally active

Acknowledgments

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