

# USING A FAMILY 3 CBM TO DIRECT ANTIMICROBIAL PEPTIDES INTO CELLULOSIC SUPPORTS: EXPRESSION AND PURIFICATION OF FUSION PROTEINS IN *Escherichia coli*

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Antimicrobial peptides (AMPs) are cationic molecules with a wide range of physiological defensive mechanisms developed to counteract bacteria, fungi, parasites and viruses. Several hundreds of AMPs have been identified and characterized and they are today recognized as important components of non-specific host defense system and innate immunity in all animal classes. AMPs are gaining increasing importance, as a consequence of their remarkable resistance to microorganism adaptation. The interests on AMPs are enormous and research is much based on whether they could, in the future, be used as novel drugs to fight infections, and to stimulate the patient's immune system. Carbohydrate-binding modules (CBMs) are non-catalytic domains that anchor glycoside hydrolases into complex carbohydrates. *Clostridium thermocellum* produces a multi-enzyme complex of cellulases and hemicellulases, termed the cellulosome, which is organized by the scaffoldin protein CipA. Binding of the cellulosome to the plant cell wall results from the action of CipA family 3 CBM (CBM3), which presents a high affinity for crystalline cellulose. The development of mechanisms for targeting bio-molecules to a diversity of matrixes is an emerging theme in biochemistry and biotechnology. Fusing AMPs to a CBM with high binding capacity for cellulose would allow its future use as a fixing tag for cellulosic supports. In this study CipA family 3 CBM was fused to four different AMPs using recombinant DNA technology and the fusion recombinant proteins were expressed at high levels in *Escherichia coli* cells. Results showed that CBM3 alone does not present antibacterial activity and does not bind to the bacterial surface. Also the four recombinant proteins retained the ability to bind cellulose, suggesting that CBM3 is a good candidate polypeptide to direct the binding of AMPs into cellulosic supports. A comprehensive characterization of the antimicrobial activity of the recombinant fusion proteins is currently under evaluation.

## References

1. Catarina I.P.D. Guerreiro, Carlos M.G.A. Fontes, Miguel Gama, and Lucília Domingues. *Protein Expression and Purification*, **2008**, 59, 161-168.