Production, purification and characterization of proteins of the proteobacterial acinetobacter chlorhexidine efflux, PACE family involved in antimicrobial resistance

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Introduction

Drug resistance is an increasing problem in clinical settings with some bacterial pathogens now resistant to virtually all available drugs. Chlorhexidine is a bisbiguanide antimicrobial agent that is extensively used in antiseptics ranging through soaps, mouthwashes and preservatives. Increasing resistance to chlorhexidine is seen in some pathogens such as *Acinetobacter baumannii*, in which a novel efflux resistance was associated with a gene designated *acel* (for Acinetobacter chlorhexidine efflux I). We have cloned genes encoding proteins that are homologues of AceI, with a view to their amplified expression, purification and characterization as a preliminary towards structural studies.

Results

Genes encoding AceI homologues from 23 species of bacteria were transferred to the pTTQ18 plasmid vector, and transformed into *Escherichia coli* BL21(DE3) host cells, where the expression of each cloned gene in membrane fractions was detected in Coomassie stained SDS gels comparing preparations from induced with uninduced cells (nine examples are shown in Figure 1). Western blots detecting the His_6-terminus of each protein were used to verify the extent of expression (Figure 1).

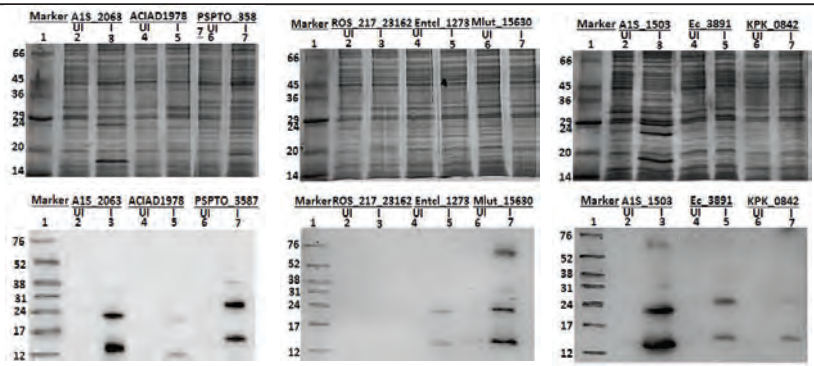

Figure 1: expression screening of PACE family proteins. SDS-PAGE (top) and Western blot (bottom) analysis of membrane preparations from small-scale cultures of *E. coli* expressing each indicated protein. Lane 1 shows molecular weight markers for un-induced (UI) and induced (I) preparations as shown.

Out of twenty three investigated, seven genes were expressed at levels sufficient for production at a 30 litre fermentation scale. Each of these was then purified in mg quantities by IMAC. The appearance of more than one band (cf Figure 2) does not necessarily represent degradation or multimers, but an effect of SDS in partially unfolding the molecules in a preparation. The integrity of the purified proteins was also assessed by assaying binding to known or putative substrates (Figure 3a). Several of the highly expressed AceI homologues conferred resistance to acriflavine, a nucleic acid intercalating biocide. Acriflavine fluorescence is reduced when it is intercalated in nucleic acids, allowing real time measurements of acriflavine transport in *E. coli* cells (Figure 3b), showing activity of the over-expressed proteins.

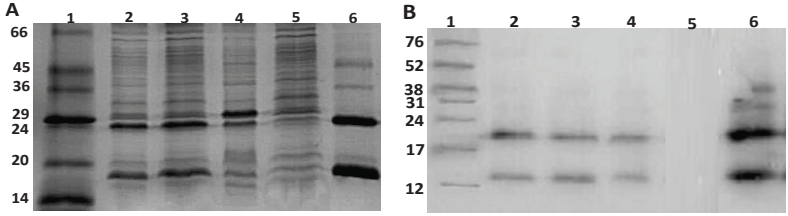

Figure 2: purification of Fbal3166 protein. Fbal_3166 protein was purified from the inner membrane of *E. coli* BL21(DE3) pTTQ18 (Fbal_3166)-His_6. The inner membranes were solubilised in 1% DDM. (A). Coomassie blue stained 15% SDS-PAGE of Fbal_3166 protein (B). Western blot. Samples loaded as follows: (1) mol. Wt. markers (kDa); (2) membranes; (3) supernatant; (4) membrane pellet; (5) unbound flow; (6) purified protein.

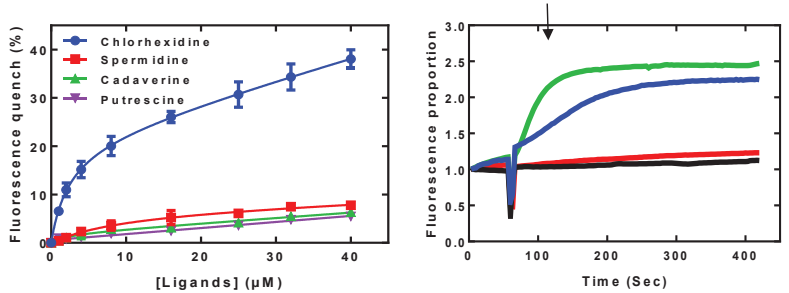

Figure 3: activities of the Fbal_3166 protein. (**A**) (Fbal3166)-His_6 protein was purified from the inner membrane of 30L cultures of induced *E. coli* BL21(DE3) pTTQ18-Fbal3166)-His_6, and solubilised in 1% DDM. The fluorescence change (quench) of tryptophan residues in the protein was monitored at increasing concentrations of the potential ligands: chlorhexidine (blue); spermidine (red); cadaverine (green); putrescine (mauve). (**B**) Small volume cultures were grown of cells expressing the Amva chlorhexidine efflux protein (green), induced cells expressing Fba-His6 protein (blue), uninduced cells not expressing the Fba3166 protein (black), and cells containing pTTQ18 plasmid without the gene insert (red). D-glucose was added at the point indicated by an arrow in order to energise efflux of fluorescent acriflavine. Cells expressing FbaHis6 are competent for efflux of acriflavine.

Current work continues with the seven homologues of the PACE efflux transporter family that have been purified and their ability to bind and transport ligands tested. Structural studies are under way.

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