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Poster Presentation

Microarray-based analysis of recombinant protein production in *E. coli*

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Background

The production of heterologous proteins in *E. coli* is a powerful tool in the generation of many important biotechnological and medical products. Despite its widespread use as an expression host, however, yields of correctly folded, functional protein are frequently low in *E. coli*. This is due largely to the formation of insoluble protein aggregates and to premature lysis of the bacterial cells. We, and others, have previously shown that the cell lysis phenomenon associated with recombinant protein production in *E. coli* is not a direct result of synthesis of heterologous proteins [1], [2]. Instead, protein production triggers a global stress response in the bacterium, but the mechanism by which cell lysis subsequently occurs remains unclear [3].

We have carried out a microarray-based study of the response of *E. coli* to production of two recombinant proteins. In this analysis, a murine scFv antibody fragment and a human renal enzyme were produced in the *E. coli* periplasm, followed by co-production in turn of the cation efflux protein CzrB from *Thermus thermophilus* and *E. coli* disulfide bond isomerase DsbC. These latter proteins had previously been demonstrated in our group to delay lysis of the host *E. coli* cells and increase yields of the two proteins [1], [4].

Results

Growth and functional yields of the two recombinant proteins were studied using standard techniques. Co-expression of *czrB* and *dsbC* led to delayed lysis of host *E. coli* cells and to improvements in functional yields of recombinant proteins (see Figure 1).

Subsequent to mRNA purification and microarray analysis, data mining identified a number of genes whose expression was significantly altered upon recombinant protein production. Phage shock proteins and numerous chaperones were significantly upregulated, while OmpF was the main downregulated protein. Genes whose expression reverted towards pre-induction levels upon co-production of CzrB and/or DsbC were also identified. We report results of manipulation of expression of a number of these genes in an attempt to increase functional yields of the two recombinant proteins *in vivo*.

Conclusion

A microarray-based analysis of recombinant protein production was utilised to identify changes in gene expression in *E. coli* upon induction. Manipulation of expression of a number of these genes has been used to increase functional protein yields *in vivo*.

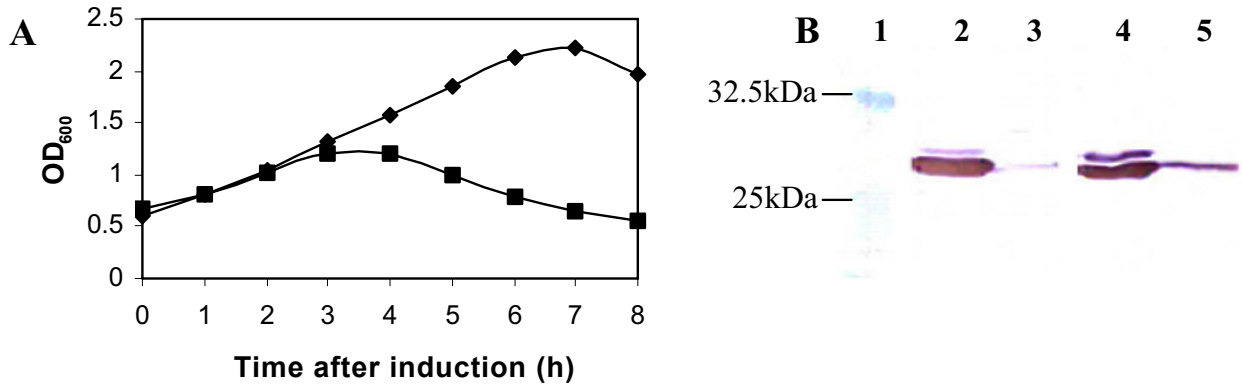

Figure 1
A Growth of *E. coli* cells producing the murine2H12 scFv fragment with (diamonds) and without (squares) co-production of DsbC. **B** Immunoblot detection of 2H12 scFv produced in the presence and absence of DsbC overproduction. Lane **1**. Molecular weight marker; lanes **2–3**. no DsbC; lanes **4–5**. + DsbC; lanes **2,4**. insoluble scFv; lanes **3,5**. soluble scFv.

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