

Poster Presentation

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Recombinant lipase immobilised in the cell wall of *Bacillus halodurans* Alk 36 exploiting the FliC protein

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Background

There are a number of methods in which heterologous peptides and proteins can be displayed on the cell surface of bacteria. The first use of the FliC system for display was carried out by Kuwajima *et al.* [1] where an eleven amino acid epitope from the egg-white lysozyme was displayed on the surface of *E. coli*. Ezaki *et al* [2] and Tanskanen *et al* [3] both demonstrated that large polypeptides could also be displayed successfully using *E. coli* flagellin. These proteins included an alkaline phosphatase (471 aa) and the collagen binding region of YadA of *Yersinia enterocolitica* (302 aa) respectively. To our knowledge, the display of heterologous proteins using the FliC protein from flagella has not previously been demonstrated in Gram-positive bacteria. *Bacillus halodurans* Alk36 is an alkalophilic, Gram-positive bacterial strain which has the inherent capability of over-expressing the FliC protein. This was harnessed as an opportunity for the development of a novel surface display system using this protein. The *B. halodurans* flagellin is a lot smaller (30 kDa) than that of *E. coli* (~52 kDa) with a small variable region. A Δ hag mutant strain of *B. halodurans* (BhFC04) was developed and two sites were identified in the flagellin variable domain for insertion of heterologous proteins/peptides.

In order to investigate the possibility of using the FliC display system for biotransformation in *B. halodurans*, construct pSECNLipC harbouring the *LipA* gene from *Geobacillus thermoleovorans* was designed and evaluated in shake flasks and batch fermentations. Lipases have been

well characterised and play a role in a number of processes which include detergents, glycerolysis of fats and oils, direct esterification, chiral resolution and acylate synthesis [4]. Bacterial and fungal lipases have been immobilized on the cell surface of *Bacillus subtilis* using the CWB_c cell-wall binding domain. However, instability of fusion proteins was found to be a serious problem [5].

Results

Zymogram of lipase activity (Figure 1) in the extracellular (EX), cell surface (CS), cell wall (CW), and intracellular (I) fractions of BhFC04 harbouring the pSECNLipC construct.

Conclusion

Heterologous lipase expression has been successfully achieved using the *Bacillus halodurans* host-vector system. Lipase activity was localised primarily in the cell wall fraction. However, whole cell assays showed that the enzyme was exposed to the extracellular environment and therefore accessible to the substrate. Lipase production levels attained in shake flasks and batch fermentations exploiting this host-vector system have demonstrated it to be of commercial potential for whole cell biocatalysis.

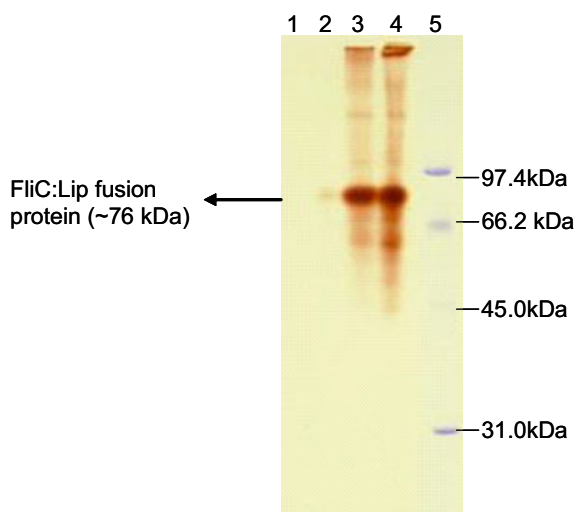


Figure 1

Lane 1-EX fraction.

Lane 2 -CS fraction

Lane 3- CW fraction

Lane 4- I fraction

Lane 5 -Molecular weight marker.

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