To ‘display’ or not to ‘display’- that is the peptide

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INTRODUCTION

Microbial cell surface display is the anchoring of a heterologous protein or peptide (passenger) to the outside of the cell wall as a fusion to a cell surface associated protein (carrier). This technology has been used extensively for both eukaryotic and prokaryotic systems but has mainly focused around phages (Etz et al 2001), yeast (Kondo and Ueda, 2004) and bacteria (Lee et al 2003).

Flagella are the organelles associated with locomotion in bacteria. The flagellum are composed of up to 20 000 copies of the major flagellin protein (FliC). The central variable domain of the FliC protein is dispensable and can be used for the insertion and display of numerous peptides and proteins (Kuwajima, 1988; Crampton et al 2007). The alkaliphilic, thermotolerant, Gram-positive isolate, *Bacillus halodurans* Alk36 was shown to produce high levels of flagellin (**Figure 1**) (Crampton et al 2007). This has been exploited by fusing the FliC protein to a number of different peptides and shown to be functional (Crampton et al 2007). Two advantages of developing the Gram-positive flagellin display system are that Gram-positive bacteria are more robust than their Gram-negative counterparts and the chimeric flagella are easily isolated from the cell surface.

In this study we wanted to determine limitations of the display system and evaluate amino acid content on yields and display of selected peptides.

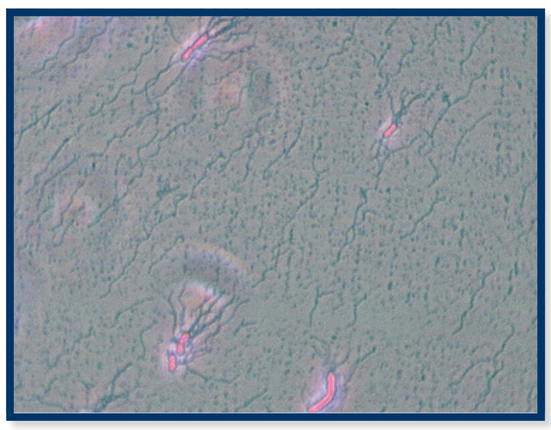

Figure 1: Micro-graph photo showing B. halodurans Alk36 flagella

METHODS

Ten constructs were made using the temperature sensitive expression vector pSECNC6 (Crampton et al 2007). Chimeric *hag* genes were constructed carrying peptides of various sizes and aromatic amino acid content as in-frame fusions into the central variable domain of the flagellin protein (**Figure 2**). These constructs were transformed into *B. halodurans* BhFC01 (Δ *hag*), and the cell surface proteins isolated using the method described by Crampton et al (2007). Chimeric proteins were analysed on a 10% SDS-PAGE gel and Western blot was done using rabbit anti-flagellin antibodies.

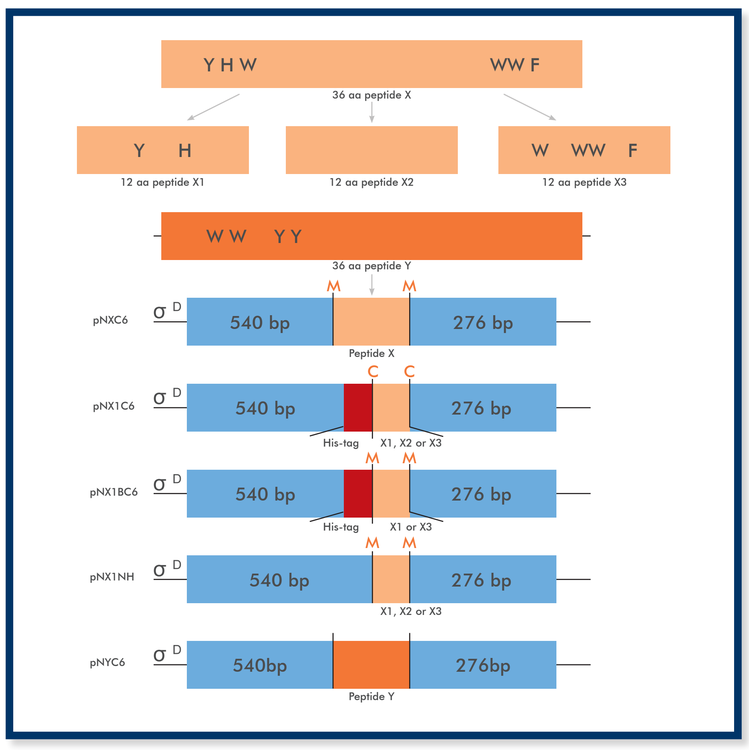

Figure 2: Schematic presentation of the peptides, aromatic amino acid content and subsequent cloning of the different chimeric flagellin genes into pSECNC6. Only peptide X, Y and X1 are shown. Lines represent untranslated DNA regions including the σ^D promoter, filled blue bars the coding region of the *hag* gene, filled tan and light orange bars the peptides and inserted peptides, and the filled red bars the His affinity tag. The numbers in the blocks denote the size in nucleotides of the *hag* gene and inserted peptides. M and C represent the methione and cysteine cleavage sites respectively. Y,H.W and F represent the aromatic peptides and their relative location

RESULTS

Surface-displayed peptides can be seen in **Figure 3** and **4**. Relative display levels can be seen in **Table 1**. Sequence analysis confirmed that all the constructs were correct, thereby confirming that the lack of chimeric flagellin production is due to the expression cassette itself. Western blot analysis confirmed the above results (**Figure 3**).

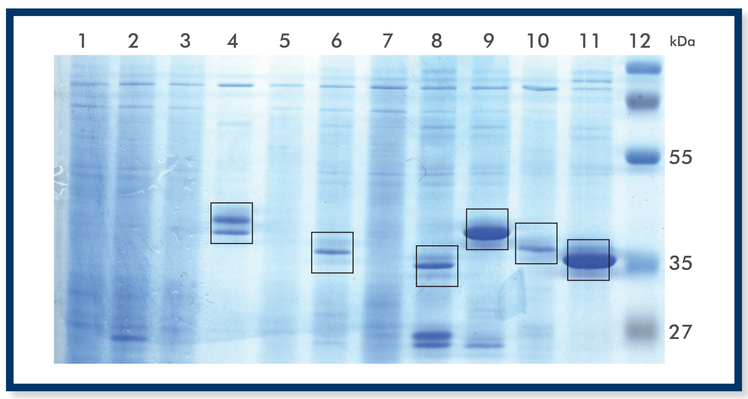

Figure 3: SDS-PAGE of the cell surface protein fractions from the 10 expression cassettes in *B. halodurans* BhFC01. Lanes 1 and 2, pSECNXC6 and pSECNYC6 respectively; Lanes 3 to 5, pNX1C6, pNX2C6 and pNX3C6 respectively; lanes 6 and 7, pNX1BC6 and pNB3XBC6; lanes 8 to 10, pNX1NH, pNX2NH, and pnX3NH respectively; lane 11, *B. halodurans* Alk36; and lane 12, low molecular mass marker (Fermentas). All bands blocked are the expressed and displayed chimeric flagellin proteins

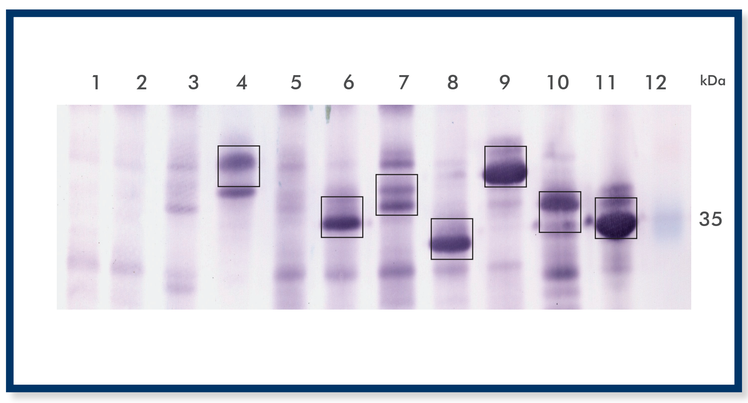

Figure 4: Western blot analysis using rabbit anti-flagellin antibodies of the cell surface protein fractions from the 10 expression cassettes in *B. halodurans* BhFC01 and the wild type *B. halodurans* Alk36 strains. Lanes 1 and 2, pSECNXC6 and pSECNYC6 respectively; Lanes 3 to 5, pNX1C6, pNX2C6 and pNX3C6 respectively; lanes 6 and 7, pNX1BC6 and pNB3XBC6; lanes 8 to 10, pNX1NH, pNX2NH, and pnX3NH respectively; lane 11, *B. halodurans* Alk36; and lane 12, low molecular mass marker (Fermentas). All bands blocked are the expressed and displayed chimeric flagellin proteins

Table 1: Relative display levels of chimeric flagellin gene products to *B. halodurans* Alk36

Vector name	Protein production
pNXC6	-
pNX1C6	-
pNX2C6	++
pNX3C6	-
pNX1BC6	++
pNX3BC6	-
pNX1NH	+++
pNX2NH	++++
pNX3NH	+
pNYC6	-
B. haloduranus	+++++

DISCUSSION AND CONCLUSION

- Display of peptide X and Y was unsuccessful
- Cysteine residues to be used as cleavage sites inhibit the display of peptides within the flagellin display system
- Replacing the cysteine cleavage sites with methione cleavage sites resulted in the successful display of peptide X1
- Removing the His affinity tag resulted in improved yields and successful display of the peptide X3. It is hypothesised that the combination of high aromatic content of peptide X3 and the His tag inhibited flagellin display
- In successful surface display designing an expression cassette is as critical as the sequence of the peptide to be displayed
- In conclusion we were able to display certain peptides to high levels while other peptides required replacement of cleavage sites and the removal of affinity tags to enhance the level of display (**Table 1**).

CSIR researchers have proved it's possible to manipulate bacterial cell surfaces for inexpensive production of valuable proteins for medical and environmental applications.

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