Susceptibility to Phage Attack: Comparing Bacterial Success Stories

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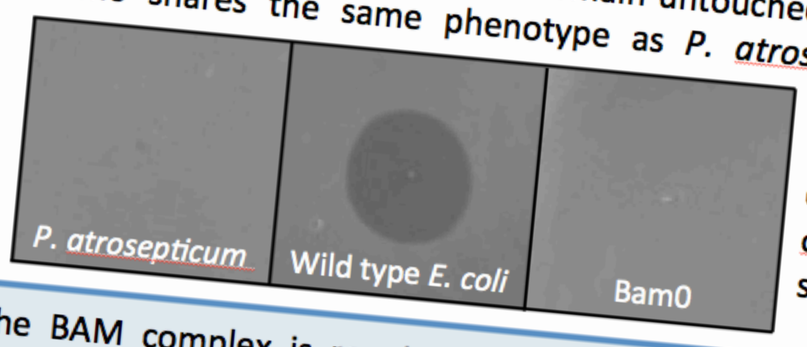
Every year, toxin-producing *Esherichia coli* infect humans worldwide in sporadic cases that can lead to severe outbreaks. Overall, 5-25% of patients develop lifelong and potentially life-threatening cases of Haemolytic Uremic Syndrome (HUS) or Thrombotic Thrombocytopenic Purpura (TTP). These diseases only result from infections of *E. coli* that construct Shiga toxin (Stx). Stx genes are transferred into susceptible hosts by bacteriophages (Stx-phages) via a receptor, BamA. However, not all bacterial species are susceptible to Stx-phages, so differences between the BamA of phage-immune and phage-susceptible species could reveal potential strategies for either preventing infections or reducing the severity of HUS and TTP symptoms.

Removing $\phi24_B$ Susceptibility from *E. coli*

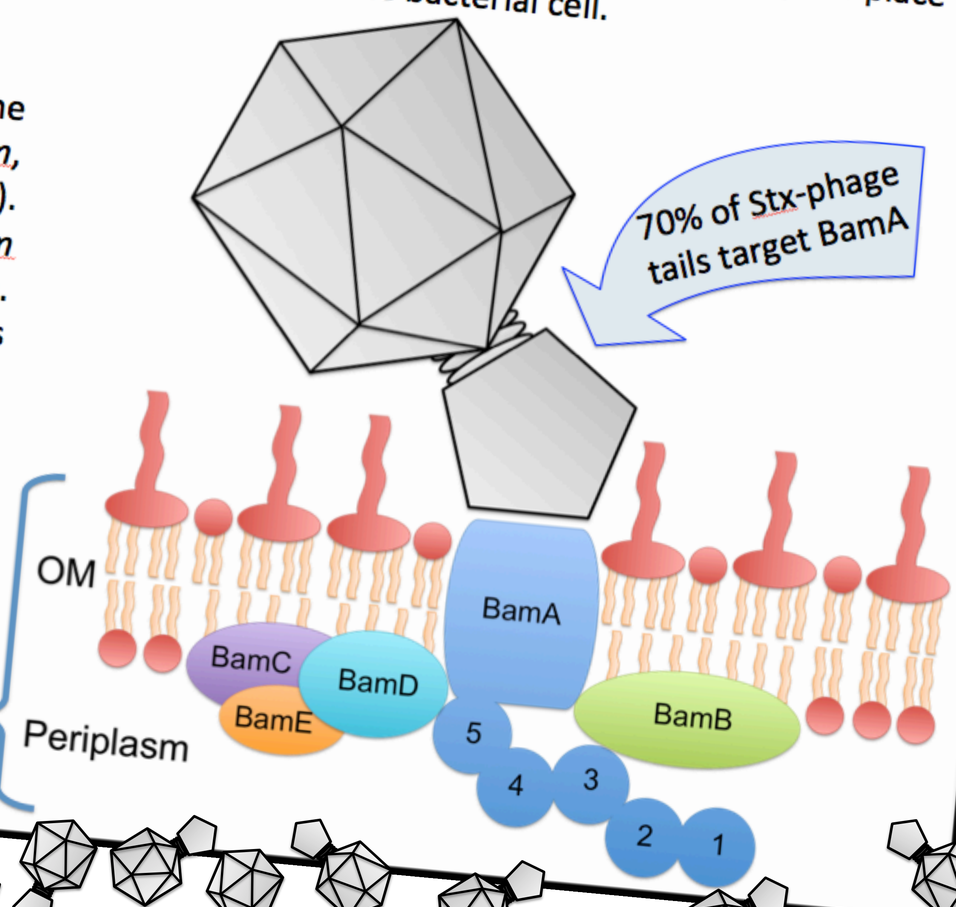
Our model phage ($\phi24_B$) consistently binds to *E. coli* BamA but does not bind to the BamA homologue of a closely related bacterium, *Pectobacterium atrosepticum*, despite a highly conserved protein sequence (78% identical, 87% similar). Substituting the *E. coli* trans-membrane domain with that of the *P. atrosepticum* BamA (leaving the periplasmic domain untouched) created an *E. coli* strain (Bam0). Bam0 shares the same phenotype as *P. atrosepticum* (left – a spot indicates that successful infection occurs), demonstrating that this single change to the receptor prevents $\phi24_B$ from binding to a previously susceptible host.



The BAM complex is required to correctly fold β-barrelled proteins during their insertion into the outer membrane (OM) of bacteria. Therefore, BamA must always be present and is permanently available as an adsorption target to incoming phages.

Stx-phage pre-infection process

1. Recognise host bacterium via the BamA protein (depicted below)
   - This requires the phage to possess a tail with a complimentary surface.
   - 70% of Stx-phages from environmental samples possess such a tail[1]. This is a ***very*** high proportion!
2. Permanently attach (adsorb) to receptor protein.
   - The resulting protein complex likely undergoes a conformational change to "lock" the phage in place on the surface of the bacterial cell.



What is the Difference Between the BamA proteins?

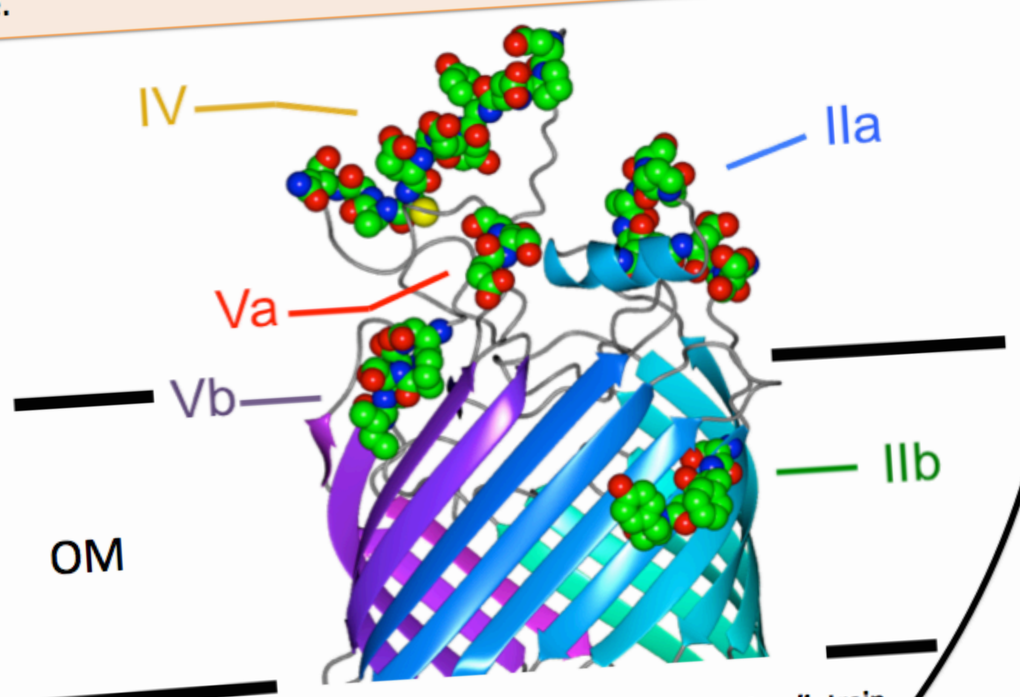
To answer this, BamA proteins from seven species were aligned and compared to identify five epitopes within the proteins as potentially responsible for this change in phenotype (bottom left, within coloured boxes). The locations of these changes are shown using spheres on a 3D model of the BamA protein (bottom right), most of which are extracellular and are accessible to incoming phages.

Sequences from the $\phi24_B$-susceptible *E. coli* are being substituted into the $\phi24_B$-resistant *P. atrosepticum* BamA with the *E. coli* periplasmic domain, which will determine the combination of these epitopes that is required to reintroduce susceptibility to $\phi24_B$ adsorption and subsequent infection.

Long Term Goals

The results of this screening will inform further biochemistry-based attempts to determine the mechanism of adsorption. This potentially leads to developing a competitive inhibitor that acts to limit Stx-phage dissemination within the gut by interfering with adsorption, thereby reducing the severity of disease symptoms and improving prognosis for infected patients.

Separately, as phage-susceptible species can survive in aquatic environments[2], the potential for a modified $\phi24_B$ tail to be used as the base of a simple colour-change test to detect such species in groundwater sources may also be a viable research avenue in the future.



[left] Adapted from Smith *et al*. (2007). Protein sequence alignments of BamA homologues from seven species (top to bottom: *S. flexneri*, *S. sonnei*, *E. coli* strain MC1061, *S. enterica* serovar Cholesaesuis strain SC-B67, *C. rodentium* strain ICC168, *P. atrosepticum* and *P. luminescens* subsp. *Laumondii* strain TTO1). Sequence differences between species that support phage adsorption and those which are resistant are highlighted in grey. Coloured boxes surround the sequences to be substituted between *P. atrosepticum* and *E. coli*. [right] The predicted structure of *E. coli* BamA from Noinaj *et al.* (2013) showing the locations of the identified sequences on the protein structure with labels colour-matched to the boxes from the left.

BBSRC Doctoral Training Partnerships

[1] Smith DL *et al.* (2007) *J. Bacteriol.* **189**(20):7223-33.
[2] Chekabab SM *et al.* (2013) *FEMS Microbiol Lett.* **344**(1):86-93.
[3] Noinaj N *et al.* (2013) Nature **501**(7467):385-90.