Iron Starvation and Satiation in the Biofilm Influence the Dispersal Decision

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A biofilm is defined as an accumulation of microbial cells, adhering to both neighbouring bacteria and an interface, encased in an extracellular polymeric substance (EPS) matrix[1]. Biofilms form on a variety of surfaces including living tissues and indwelling medical devices. Bacterial growth in a biofilm is less susceptible to antibiotic treatment than its planktonic, free floating counterpart and similarly less prone to host defences. The biofilm cycle involves a series of stages which see bacteria transitioning from free-form planktonic cells to sessile aggregations in response to environmental cues, including the availability of nutrients.

The ability of uropathogenic *Escherichia coli* (UPEC) to aggregate and form a biofilm enhances virulence by providing a protective form of growth in a hostile environment. UPEC cells can survive in the urinary tract in the form of a biofilm on urinary tract epithelium and as intracellular biofilm-like pods found in superficial bladder cells, both of which provide protection from host and antimicrobial attacks[2]. The formation and dispersal of such bacterial reservoirs may serve as the source of transition from acute to recurrent infection.

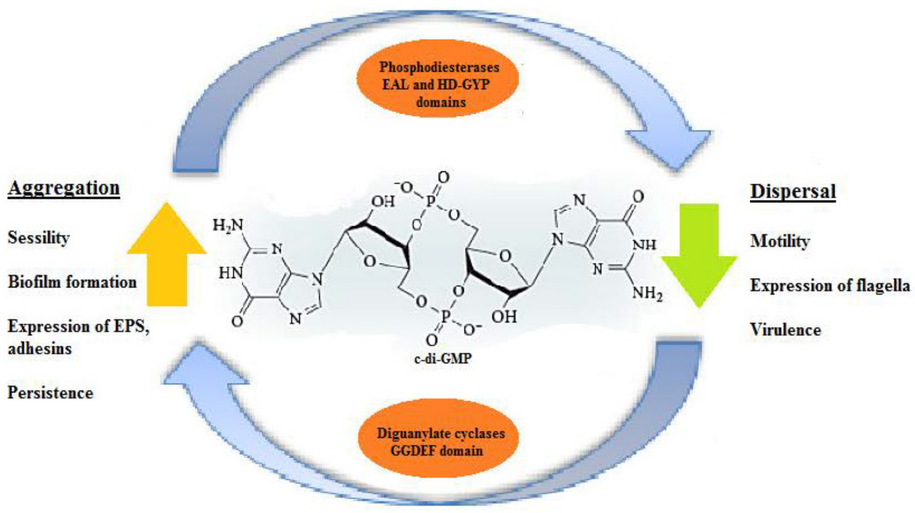
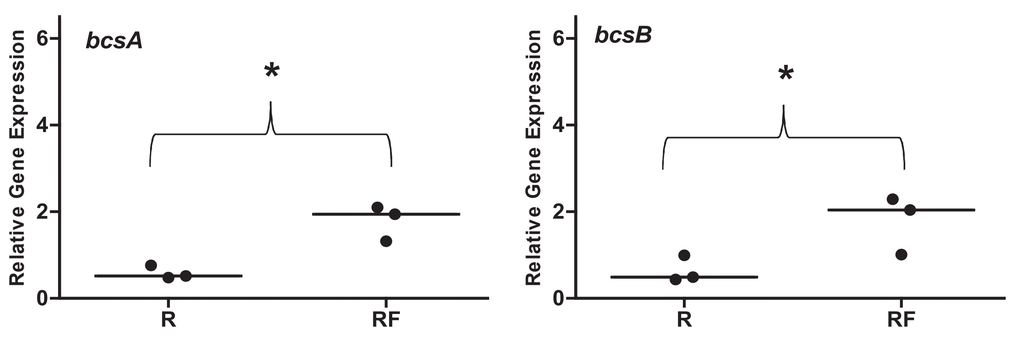


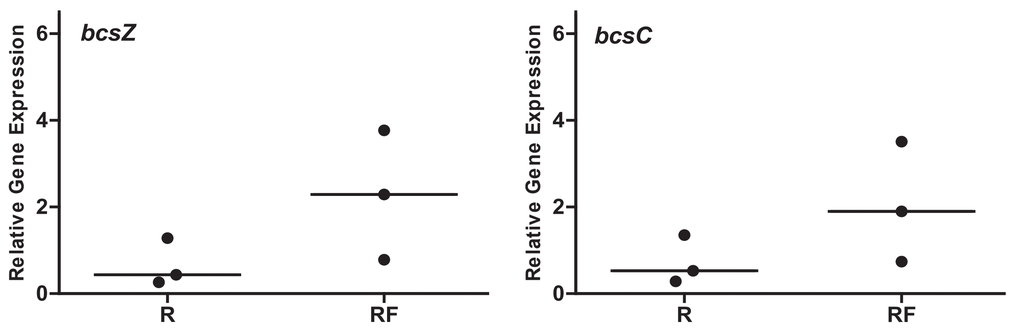
Figure 3. The effects of the intracellular c-di-GMP level on the UPEC aggregation and dispersal cycle. Diguanylate cyclases increase c-di-GMP levels which promotes cellulose biosynthesis, the expression of adhesive molecules and hence promote a biofilm formation and sessility[5,8]. Phosphodiesterases hydrolyse c-di-GMP, decreasing its intracellular concentration thus supporting a planktonic form of life[7].

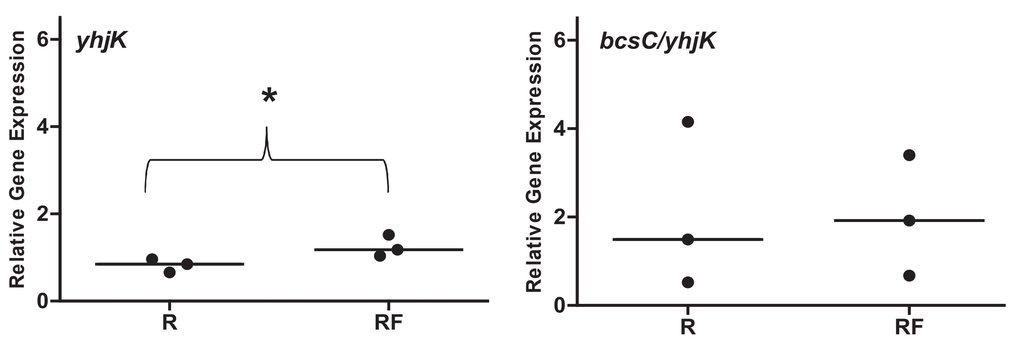
Is *yhjK* Expressed as Part of the Cellulose Biosynthesis Operon?

Using RT-qPCR the mRNA products of *bcsC*, *yhjK* and the junction between *bcsC* and *yhjK* were amplified to determine relative gene expression (Figure 4). Expression of *yhjK* as part of the operon was confirmed by amplification of the junction region in an equivocal level to those yielded from *bcsC* and *yhjK* (Figure 5).

Figure 4. Diagram of the primers used for RT-qPCR to investigate the hypothesis that *yhjK* is expressed as part of the cellulose biosynthesis operon using primers targeted at amplifying the region between *bcsC* and *yhjK*.







Iron Starvation of UPEC 536 Favours the Formation of Biofilm Aggregates with a Cellulosic Matrix

In an iron-restricted environment, UPEC 536 forms biofilm-like aggregates with a cellulosic matrix (Figure 1A); the provision of iron stimulates aggregate dispersal[3].

Staining of the aggregates with Calcofluor White and visualisation using fluorescence microscopy demonstrates that cellulose is an important component of the UPEC 536 biofilm matrix (Figure 2B)[3].

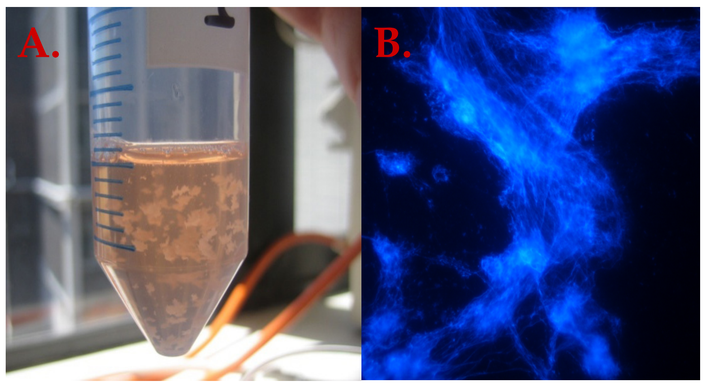


Figure 1. (A) UPEC 536 cells aggregate to Rmax at 4 hours when grown in RPMI 1640 to simulate iron restricted conditions (R)[3]. (B) Visualisation of the cellulose component of UPEC 536 aggregates using Calcofluor White[3].

The Cellulose Biosynthesis Operon

The genes required for cellulose biosynthesis in UPEC 536 are encoded by the constitutively expressed *bcsABZC* (bacterial cellulose synthesis) operon (Figure 2) which encodes a trans-membrane cellulose-synthase complex that is responsible for the production of cellulose[4]. A *bcsA* deletion mutant fails to form biofilm-like aggregates.

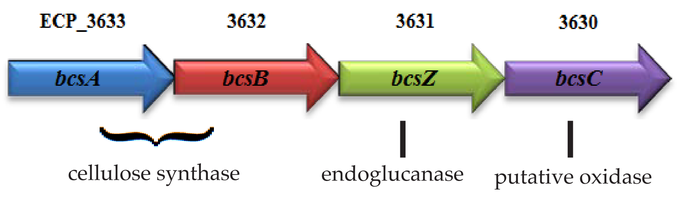


Figure 2. Representative arrangement and basic functions of the genes comprising the cellulose biosynthesis operon in UPEC 536.

Regulation of Cellulose Synthesis

In *E. coli* and *Salmonella typhimurium* the activation of cellulose biosynthesis is allosterically controlled by the reversible binding of c-di-GMP and the products of both *bcsA* and *bcsB* are implicated as containing possible binding sites for this molecule[5]. The synthesis of c-di-GMP is catalysed by diguanylate cyclases (DGCs); phosphodiesterases (PDEs) catalyse the hydrolysis of the molecule[6] (Figure 3). Increased levels of c-di-GMP promote biofilm formation, while lower levels are associated with planktonic cells[7]. The first gene downstream of the known UPEC 536 cellulose biosynthesis operon, *yhjK*, encodes a phosphodiesterase and *yhjK* mutants form aggregates which do not disperse upon the provision of iron.

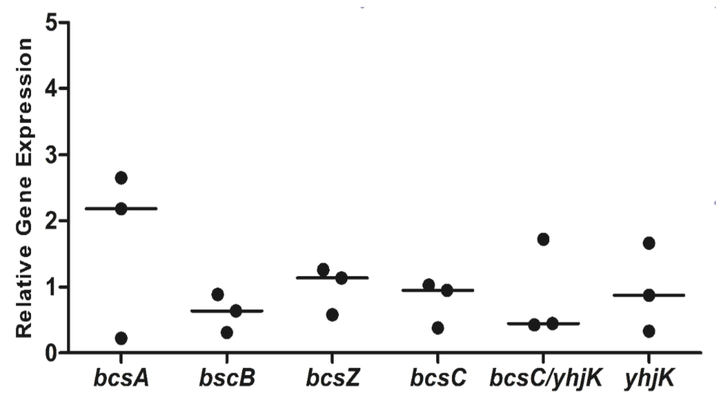


Figure 5. Expression of the junction between *bcsC* and *yhjK* is equivocal to the expression of both *bcsC* and *yhjK* and the remainder of the operon in UPEC 536 cells grown overnight in Luria Bertani broth. Medians are not significantly different; $p > 0.05$, Kruskal-Wallis test.

Transcription of *bcsABZC* is not Activated in Iron Restricted Conditions That Favour Aggregation

In many bacteria, iron uptake is depended on Fur, the Ferric Uptake Regulator. The iron dependent aggregation and dispersal cycle of UPEC 536 is not regulated by Fur, as *fur* mutants behave in the same manner as the parental strain. To investigate whether new gene expression in the *bcsABZC* operon is involved in the control of iron-induced aggregation and dispersal, the transcription of *bcsA*, *B*, *Z*, *C*, the *bcsC/yhjK* junction and *yhjK* was analysed in response to iron restricted (R) and iron replete (RF) environments using RT-qPCR.

Comparison of *bcs* gene expression in aggregating and dispersing populations does not reflect an upregulation of genes during the formation of the cellulose-based aggregates. An up-regulation of *bcs* genes during aggregate dispersal was proposed to reflect a growth surge due to the addition of iron to cells starved of this vital nutrient. The data supports the theory that the activation of cellulose production occurs at a post-transcriptional level, where the relative gene expression of *bcsABZC* is not expected to significantly change between aggregate formation and dispersal.

UPEC 536 cells dispersing under the influence of 40 µM $FeCl_3$ (RF) have slightly higher relative levels of *bcs* gene expression than those aggregating in response to an iron restricted environment (R). * $p < 0.05$ Mann-Whitney one-tailed t-test.

Conclusions

Using RT-qPCR this study has produced the novel finding that *yhjK* is expressed as part of the cellulose biosynthesis operon. Comparison of *bcsABZC* expression during the formation and dispersal of cellulosic biofilm-like aggregates supports the hypothesis that cellulose production is regulated post-transcriptionally.

References

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