Characterization of Quroum Sensing System in *Acinetobacter baumannii* that enhances the pili assembly and biofilms proficiency

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Introduction

Acinetobacter baumannii, presenting an enhanced resistance to antibiotics, survives in hospitalenvironments and often causes nosocomial infection. The factors associated with its ability of virulence, antibiotic resistance, remarkable adaptability to hospital conditions and capacity to form biofilms are largely unknown. Quroum Sensing (QS) system influences biofilms formation which represents an important virulence factor related to the survival and antibiotic resistance. In *A. baumannii*, biofilms formation depends on production of pili, which assembled via the CsuAB-A-B-C-D-E chaperone-usher secretion system. It is hypothesized that pili proficiency was affected by QS signal molecules. However, the mechanism remains unclear. The aim of this study was to demonstrate the possible role of QS signal molecules regulated pili proficiency and mediated the ability to form biofilms on abiotic surfaces.

Materials and Methods

In this study , the analysis of the processes of pili expression and surface attachment of *A. baumannii* ATCC19606 was initiated. Real-time quantitative PCR (RT-PCR) was used to detect the gene expression of CsuAB-A-B-C-D-E chaperone-usher secretion system and their regulators, *bfmS* and *bfmR*. Subsurface twitching assay was used to detect the pili motility, which also implicated in biofilm development. By a morphologic approach using transmission electron microscopy (TEM) and laser scanning confocal microscope (LSCM), we compared the pili and biofilms formation in two groups of *A. baumannii*, the control group and treatment group (co-cultured with 100 μmol/L N-hexanoyl-homoserine lactone, C6-HSL).

Results

RT-PCR analysis showed that co-cultured with C6-HSL, the chaperone-usher secretion system, including all of csuA/B, csuA, csuB, csuC, csuD and csuE, distinctly increased expression (P<0.05, Fig 1). Interestingly, at the same experimental conditions, expression of chaperone-usher regulators (*bfmS* and *bfmR*) were significantly higher than those of the control strain (P<0.05, Fig 1). Subsurface twitching assay showed there was a switch from a small to a large and structured clone that may result from enhanced twitching motility (P<0.05, data/fig not show). TEM analysis of cells lifted from a LB broth co-cultured with C6-HSL showed the pili were more abundant than control strain (data/fig not show). We then tested the idea that the addition of QS signal, and therefore induction of chaperone-usher secretion system genes, provides a greater benefit at higher biofilms densities. The total fluoreseence intensity of biofilms assay obviously increased revealed by LSCM (data/fig not show).

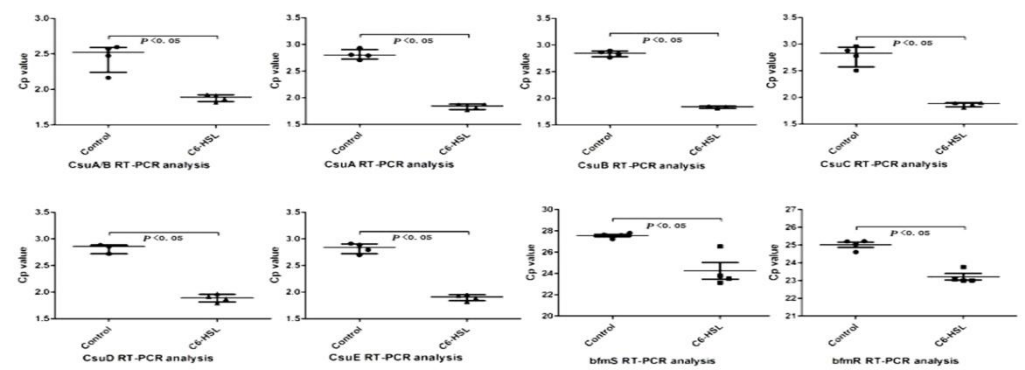

Fig. 1 RT-PCR analysis of csuA/BABCDE and bfmS/R between control and C6-HSL group

Conclusions

Our study demonstrated that, through the genes of *bfmS* and *bfmR,* QS signal molecules enhance the chaperone-usher secretion system expression and this is required for twitching motility in *baumannii.* The concomitant of pili expression and strain twitching was *A. baumannii* easily attached to abiotic surfaces and the ensuing formation of biofilms.