Glucose 6-phosphate transport regulatory protein UhpA regulates the virulent genes in *Edwardsiella piscicida*

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Research

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Abstract

Edwardsiella piscicida (*E. piscicida*) is an important zoonotic pathogen, which infects animals by colonizing the intestine. Glucose 6-phosphate (Glu6P) was an important carbohydrate in intestine and could be used as a regulate signal. Here we identify a virulence-regulating pathway named Glu6P transport regulatory protein UhpA, which affects the virulent genes of hemolysins, flagellar, T3SS, T6SS and metabolism related genes how to promote *E. piscicida* infect the host. The results showed that the metabolism related gene expression of cysteine synthase (orf 1134) and sulfate transporter (*ych*M) in the *uhp*A mutant strain Δ*uhpA* was 0.76-fold and 0.68-fold lower than the ones in the wild strains ($P < 0.05$), the gene expression of ethA and *ethB* in the Δ*uhpA* strain was 0.80-fold and 0.72-fold lower than the ones in the wild strains ($P < 0.05$). However, the gene expression of *fliC* and *flgN* in the Δ*uhpA* was 1.51-fold and 1.21-fold higher than the ones in the wild strains ($P < 0.05$), the gene expression of T3SS (*esrB* and *esrC*) and T6SS (*evpB* and *evpC*) in the Δ*uhpA* was 1.27-fold, 1.13-fold 1.28-fold and 1.23-fold higher than the ones in the wild strains ($P < 0.05$). Besides, the survival rate of fish challenged with *E. piscicida* EIB202 and Δ*uhpA* was 50% and 30% respectively. These suggested that although the *uhp*A gene deletion decreased the metabolic level and the hemolysins related gene expression in *E. piscicida*, the *uhpA* gene could down regulate the key virulent gene expression to decrease the pathogenicity of *E. piscicida* in fish.

Importance

Edwardsiella piscicida (*E. piscicida*) is an important zoonotic pathogen, which is widely distributed in aquatic environments and infects hosts mostly via the intestine. The intestine provides a nutritional and competitive environment for the pathogens. Successful colonization by pathogens requires scavenging nutrients, sensing chemical signals, competing with the resident bacteria and precisely regulating the expression of virulence genes. The Glucose-6-phosphate (Glu6P) in the intestine could trigger a novel signaling system named UhpA, which enables the cell to regulate the virulent genes. Here we found that the UhpA could down regulate the key virulent gene expression to decrease the pathogenicity of *E. piscicida* in fish.

1. Introduction

Edwardsiella piscicida (*E. piscicida*) is a rod-shaped Gram-negative, facultatively anaerobic, non-capsulated, motile, intracellular bacteria, which belong to an *Enterobacteriaceae, Edwardsiella* species, the old name was called as *E. tarda* (1, 2). This bacterium could infect fish and move through the food chain to infect amphibians, reptiles, and birds, and humans, which particularly infects fish and causes large economic losses (3, 4). *E. piscicida* infected host meanly through the intestine because it provides nutrition for bacteria (5, 6). The microbiotas rely on intestine nutrient availability and invading pathogens compete for these resources and regulate its virulent gene expression to enhance its pathogenicity (6–8).

The pathogenesis of *E. piscicida* include many virulence factors such as the production of hemolysis (9, 10), filament structural protein of flagellar (11), possession of type Ш secretion system (T3SS) (2, 9), and type VI secretion system (T6SS) (12), production of growth metabolism (13, 14), ability to adhere, invade, survive and replicate in host cells (2). Additionally, most of them were regulated by the two-component signal transduction system (TCS), such as UhpA/UhpB-UhpC, which enables the cell to acquire phosphorylated sugars from its environment that can be used as carbon or energy sources (15). Although *uhp*A gene deletion affects Glucose 6-phosphate (Glu6P) usage in *E. piscicida* and UhpA could decrease its pathogenicity in zebrafish (15), UhpA how to regulate the main virulent genes of hemolysis, flagellar, T3SS, T6SS and metabolism are still not available.

The study aims to find out the *uhpA* gene in *E. piscicida* on how to affect the main virulent gene expression and pathogenicity of *E. piscicida*. Elucidate the key virulent gene expression of hemolysis, flagellar, T3SS, T6SS and metabolism in the *uhpA* gene mutant strain and the wild type strain of *E. piscicida*. We believe that understanding this mechanism of *E. piscicida* would be helpful to explain the key role of UhpA in the pathogenicity of *E. piscicida*.

2. Results

2.1 The uhpA gene deletion down-regulates the gene expression of metabolism

To evaluate the function of the *uhp*A gene in *E. piscicida* how to affect the metabolism related genes. The gene expression was carried out in the *uhp*A mutant strain and the wildtype strain, and the results were shown in (Fig. 1). Results demonstrated that the metabolism related gene expression of cysteine synthase (orf 1134) and sulfate transporter (*ych*M) in the mutant strains was 0.76-fold and 0.68-fold lower than the ones in the wild strains ($P < 0.05$). This suggested that the *uhp*A gene deletion decreased the metabolic level *E. piscicida*.

2.2 The uhpA gene deletion down-regulates the gene expression of hemolysis

Hemolysins are lipids and proteins that cause lysis of red blood cells by destroying their cell membrane. The primary function of hemolysins is that of hemolysis, the result of two key hemolysis related gene expressions was shown in (Fig. 2). Results showed that the gene expression of *eth*A and *eth*B in the mutant strains was 0.80-fold and 0.72-fold lower than the ones in the wild strains ($P < 0.05$). This suggested that the *uhp*A gene deletion decreased the hemolysis related gene expression in *E. piscicida*.

2.3 The uhpA gene deletion up-regulates the gene expression of flagellar

The primary function of a flagellum is that of locomotion, the result of two flagellar related gene expressions was shown in (Fig. 3). Results showed that the gene expression of *fliC* and *flgN* in the mutant strains was 1.51-fold and 1.21-fold higher than the ones in the wild strains ($P < 0.05$). This suggested that the *uhp*A gene deletion increased the flagellar related gene expression in *E. piscicida*.

2.4 The uhpA gene deletion up-regulates the gene expression of T3SS and T6SS

The result of T3SS and T6SS related gene expressions was shown in (Figs. 4 and 5). Results showed that the gene expression of T3SS (*esrB* and *esrC*) and T6SS (*evpB* and *evpC*) in the mutant strains was 1.27-fold, 1.13-fold 1.28-fold and 1.23-fold higher than the ones in the wild strains ($P < 0.05$). This suggested that the *uhp*A gene deletion increased the T3SS and T6SS related gene expression in *E. piscicida*.

2.5 The uhpA gene deletion of E. piscicida attenuates virulence in fish

E. piscicida EIB202 caused mortalities in tilapia at first day after being orally injected infection, whereas the death occurred at 1–3 days after infection with the mutant strain *E. piscicida* Δ*uhpA* (Fig. 6). The survival rate of *E. piscicida* EIB202 and Δ*uhpA* groups was 50% and 30% respectively. None of the fish in the control group died and pure cultures of *E. piscicida* strains were recovered from the kidney and liver of dead fish.

3. Discussion

Host-bacterial interactions are very complex, the fundamental relationship between them are competition between host immunity and bacterial pathogenicity (6, 16–18). For example, host immunity after vaccination was greater than the invading ability of bacteria; fish could not be dead appearing (4, 19, 20). As we know *E. piscicida* is an important zoonotic pathogen, although *Edwardsialla* has been known as a serious pathogen of aquatic animals for a long time, its pathogenicity mechanisms are yet to be fully elucidated. The pathogenesis of *E. piscicida* appears to be multifactorial virulence factors. One of them, the UhpA was regulated by the transport protein UhpT, to transport a broad range of phosphorylated sugars (15). In this system, the Glu6P has been used as a broad specificity of sugar phosphates (21), which decreased the pathogenicity of *E. piscicida* in zebrafish (15). Although UhpA decreased its pathogenicity has been reported, UhpA how to regulate the main virulent gene are still not available.

Cysteine synthase coded by open reading frame 1134 in *E. piscicida* genome, have been reported to participate in the regulation of ethanol utilization and production of antioxidant respectively (13). With the growing knowledge on the physiology of dissimilatory sulfate, one of them coded by *ych*M gene has been identified in bacteria including *E. piscicida* (14, 22). The virulence element hemolysis (Eth) system, which comprised EthA and EthB, widely distributed in this bacterium, which is essential for the fish invasion in vivo and in vitro (9, 10). In this study, our results showed that the metabolism related gene and the hemolysis related gene expression were lower than the ones in the wild strains. This suggested that the *uhp*A gene deletion decreased the metabolic level *and* hemolysis ability in *E. piscicida*.

On the other hand, a flagellar protein encoded by *flgN* and *fliC* plays an important role in the adhesion and the proptosis in *E. piscicida* respectively (11, 23, 24). Meanwhile, EsrA and EsrB are responsible for T3SS regulation in *E. piscicida* (2, 9). Also, EvpB and EvpC proteins regulated iron-restricted conditions in vitro had a crucial role in the T6SS mediated pathogenesis of *E. piscicida* (12). The result in this study showed that the flagellar related gene, T3SS and T6SS related gene expressions were higher than the ones in the wild strains. Importantly, the survival rate of fish infected with *E. piscicida* EIB202 was higher than fish infected with Δ*uhpA*. In general, the *uhp*A gene deletion more effect on the flagellar protein, T3SS and

T6SS, enhanced the pathogenicity of *E. piscicida*. In summary, the pathogenicity of the *E. piscicida* mutant strain Δ*uhpA* increased because the key virulent gene upregulated after the *uhp*A gene deletion.

4. Materials And Methods

4.1 Bacterial strains and culture conditions

Bacterial strains include the wild type strain *E. piscicida* EIB202, the *uhpA* gene mutant strain Δ*uhpA* (15, 25). *E. piscicida* strains were grown in tryptic soy broth (TSB) at 28℃.

4.2 The Expression Of Main Virulent Genes

The *E. piscicida* EIB202 and Δ*uhpA* were grown in TSB cultures at 28℃. Total RNA was extracted at 0.5 OD_{600} using a total RNA isolation system (TaKaRa company, Dalian, China). To verify that the RNA was free of DNA contamination, PCR amplification was performed using the RNA as a template. The primers for virulent genes are shown in (Table 1). The RT-PCR and qPCR was carried out performing our previous research (8, 26, 27).

Table 1
Primers used in this study

Primer names	Primer sequence (5’-3’)
EsrB-F	CGCCTACGTCCTGAAGCAA
EsrB-R	CCTGAGCCATATCGGACGAG
EsrC-F	AGGCTGGTCTTGGCCTACAG
EsrC-R	CGGTAGAGCGTGTCGAACAG
FliC-F	ACCGCTTCACCGCCAATATCAAC
FliC-R	GTTAGAGCCGTTCTGTGCCTGTAC
FlgN-F	GAGAATCTGCTGGCCGATCTGC
FlgN-R	CCGTTGTGCTGATTCATGTCATGC
EthA-F	CGGCTGCGGCTTTATCAATA
EthA-R	ACCAGATCCAGCACGCTGTT
EthB-F	CAGCCGCGACGTCAA
EthB-R	CCCTCGATACGCTCGATAAA
EvpB-F	AGCCGAGCCGATCTGATCCG
EvpB-R	CCGCCGCAACGTGTGAGATC
EvpC-F	GTCGCCGTCTCCAGCTATCAATG
EvpC-R	CGCCTTGCCGTTCTGATCCTG
1134-F	CCGAGATCCACCGCCAGACC
1134-R	CTCGACGGCCACAATGCTGAC
YchM-F	CGTCACCGATATGCCGTTCCAG
YchM-R	TAGCGTTCCTCCTCCAGACTGC
16 s rRNA-F	ACCCTGGTAGTCCACGCTGTAAACG
16 s rRNA-R	CATCGAATTAAACCACATGCTCCACC

4.3 Test Of Bacterial Virulence In Fish Model

The tilapia was prepared as our previous paper and has been approved by the Animal Ethics Committee of Shandong Agricultural University (8), the mean weight of the tilapia was 73.09 ± 3.07 g, and the cultured water’s temperature was 28.0 ± 1.0 °C. These fish were recognized as disease-free animals to assess the virulence of *E. piscicida* strains (EIB202 and Δ*uhpA*). Fish from each group were orally

infected with 100 μL PBS containing bacterial cells at 10^{10} CFU/mL according to the previous research. The number of dead fish was calculated observed over for 14 days.

4.4 Statistical Analysis

Statistical significance was determined by ANOVA analysis. Differences were considered significant at $P < 0.05$.

Declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Availability of data and material

All data generated or analyzed during this study are included in this published article.

Acknowledgments

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Figures

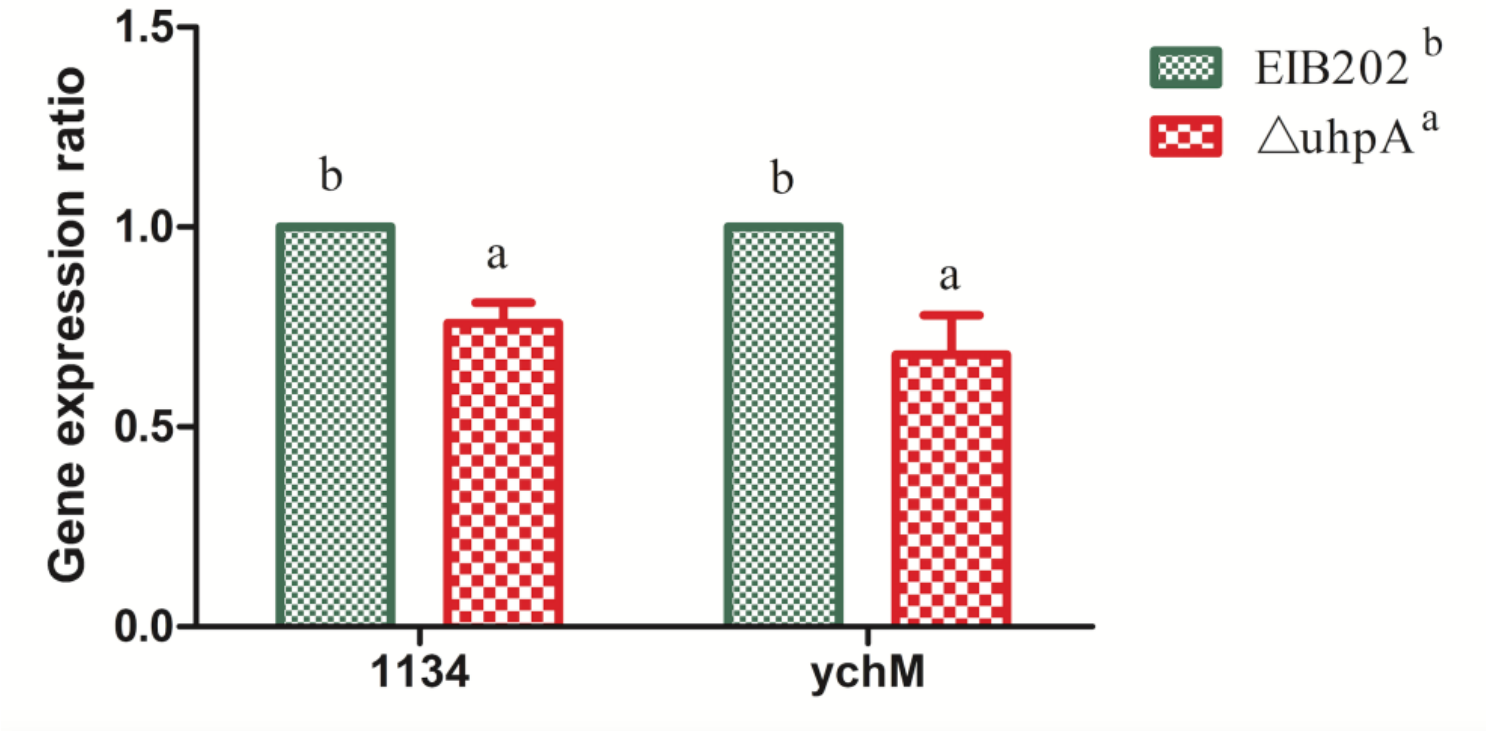

Figure 1

The difference of transcript levels of the cysteine synthase and sulfur metabolism related genes in E. piscicida EIB202 and mutant strain ΔuhpA. Alphabet a and b: Values indicate significant difference at $P<0.05$.

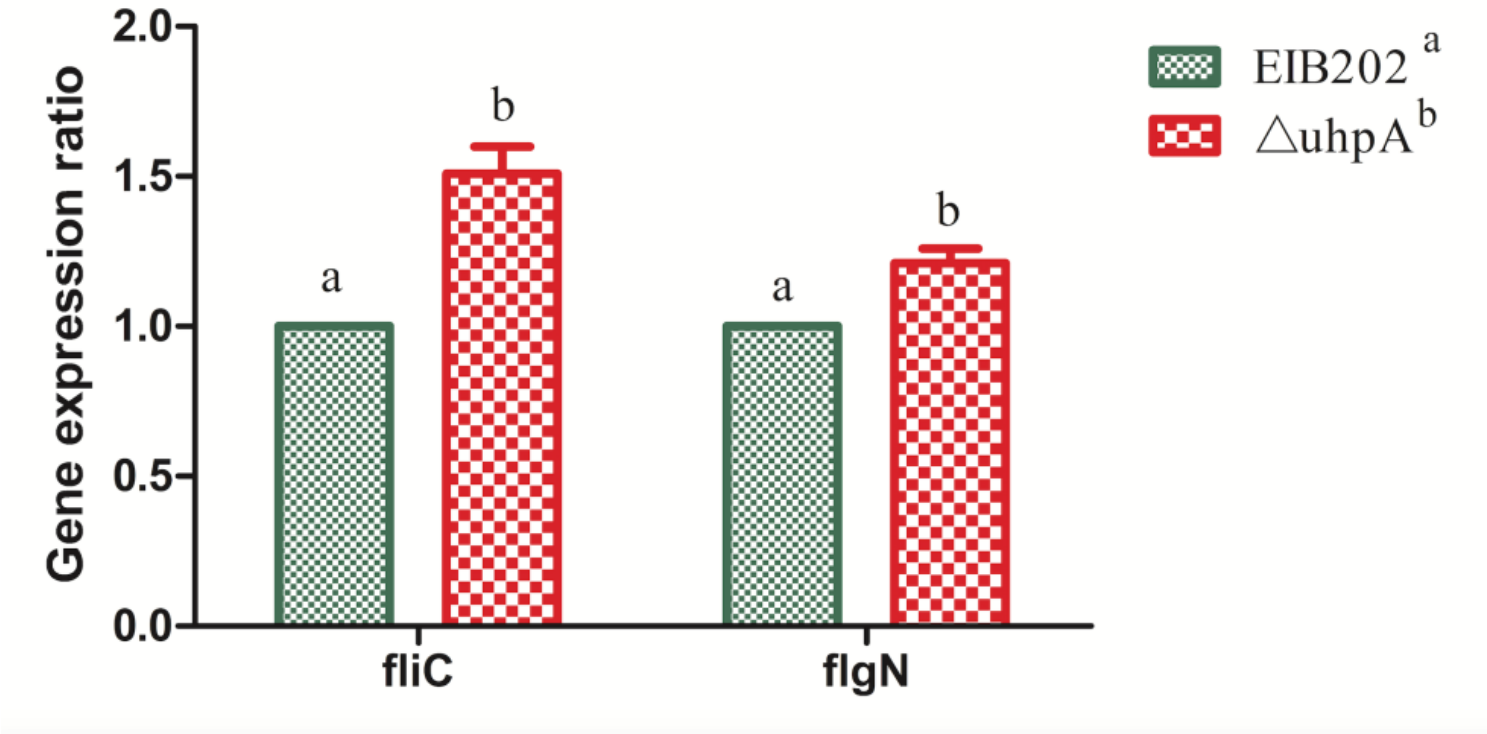

Figure 2

The difference of transcript levels of the flagellar related genes in E. piscicida EIB202 and mutant strain ΔuhpA. Alphabet a and b: Values indicate significant difference at P<0.05.

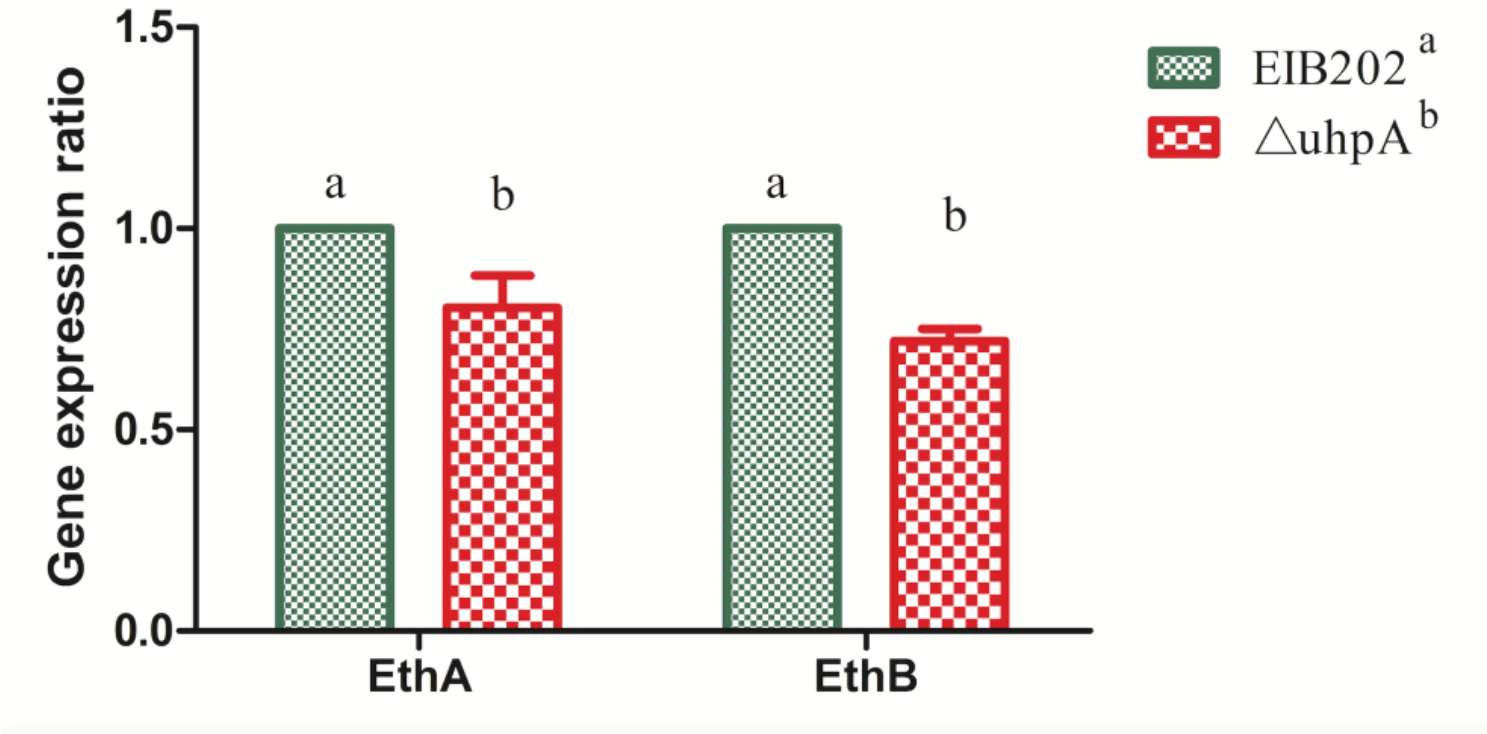

Figure 3

The difference of transcript levels of the hemolysis related genes in E. piscicida EIB202 and mutant strain ΔuhpA. Alphabet a and b: Values indicate significant difference at P<0.05.

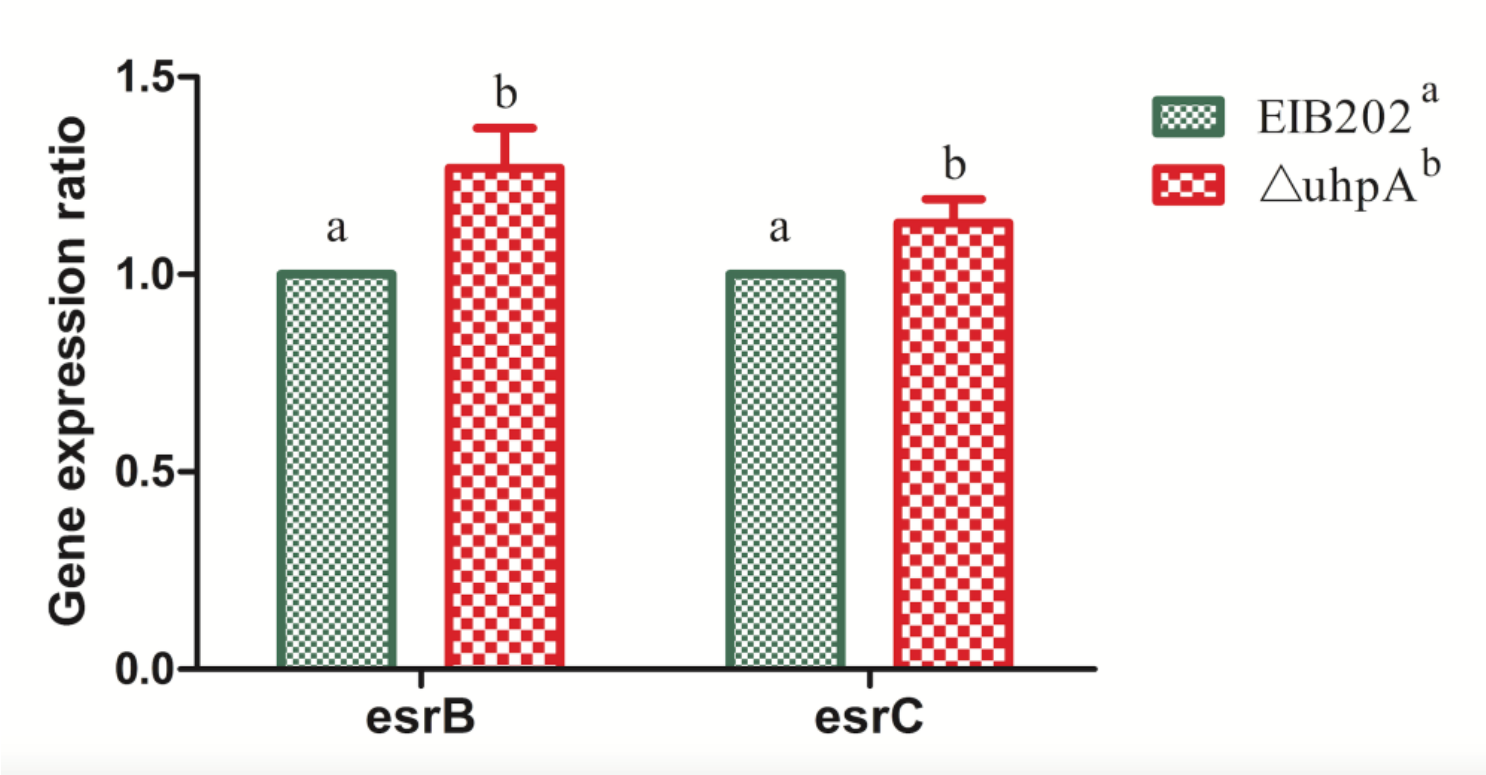

Figure 4

The difference of transcript levels of the key T3SS related genes in E. piscicida EIB202 and mutant strain ΔuhpA. Alphabet a and b: Values indicate significant difference at P<0.05.

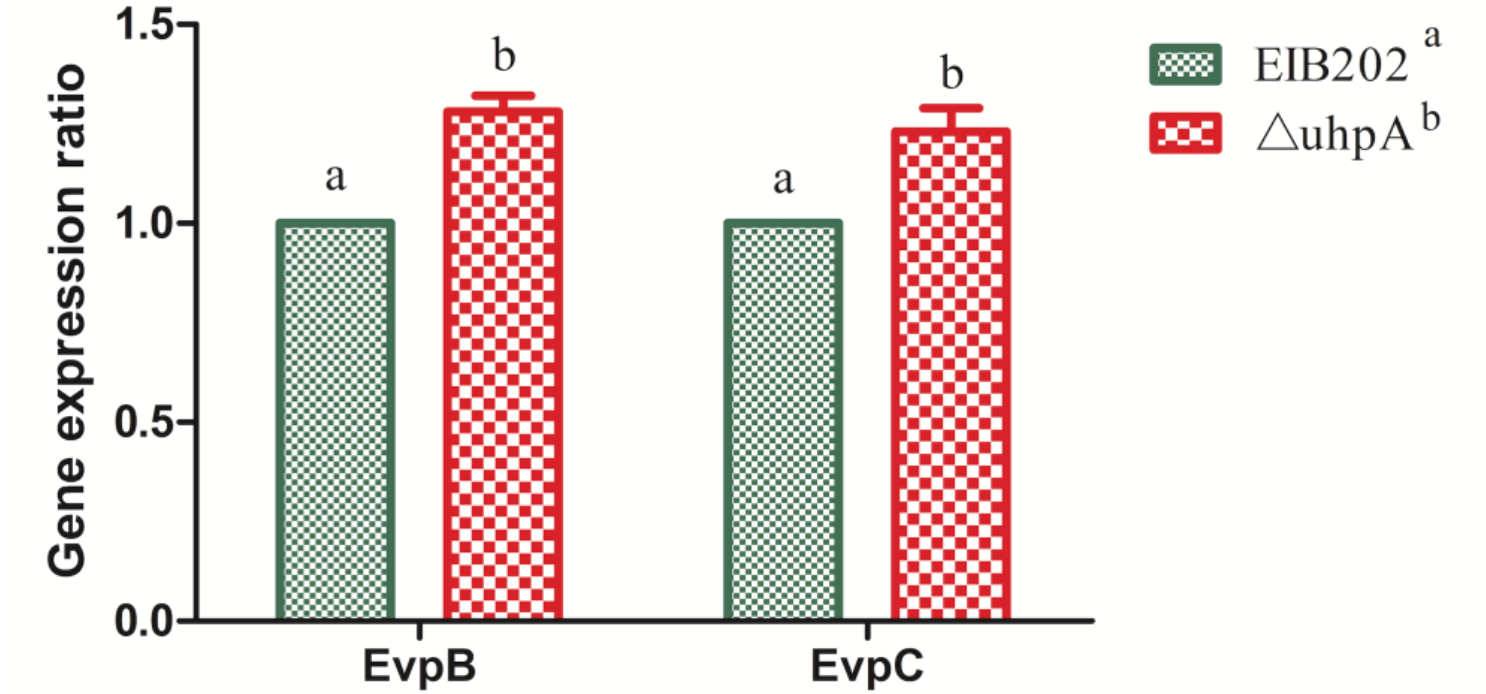

Figure 5

The difference of transcript levels of the key T6SS related genes in E. piscicida EIB202 and mutant strain ΔuhpA. Alphabet a and b: Values indicate significant difference at P<0.05.

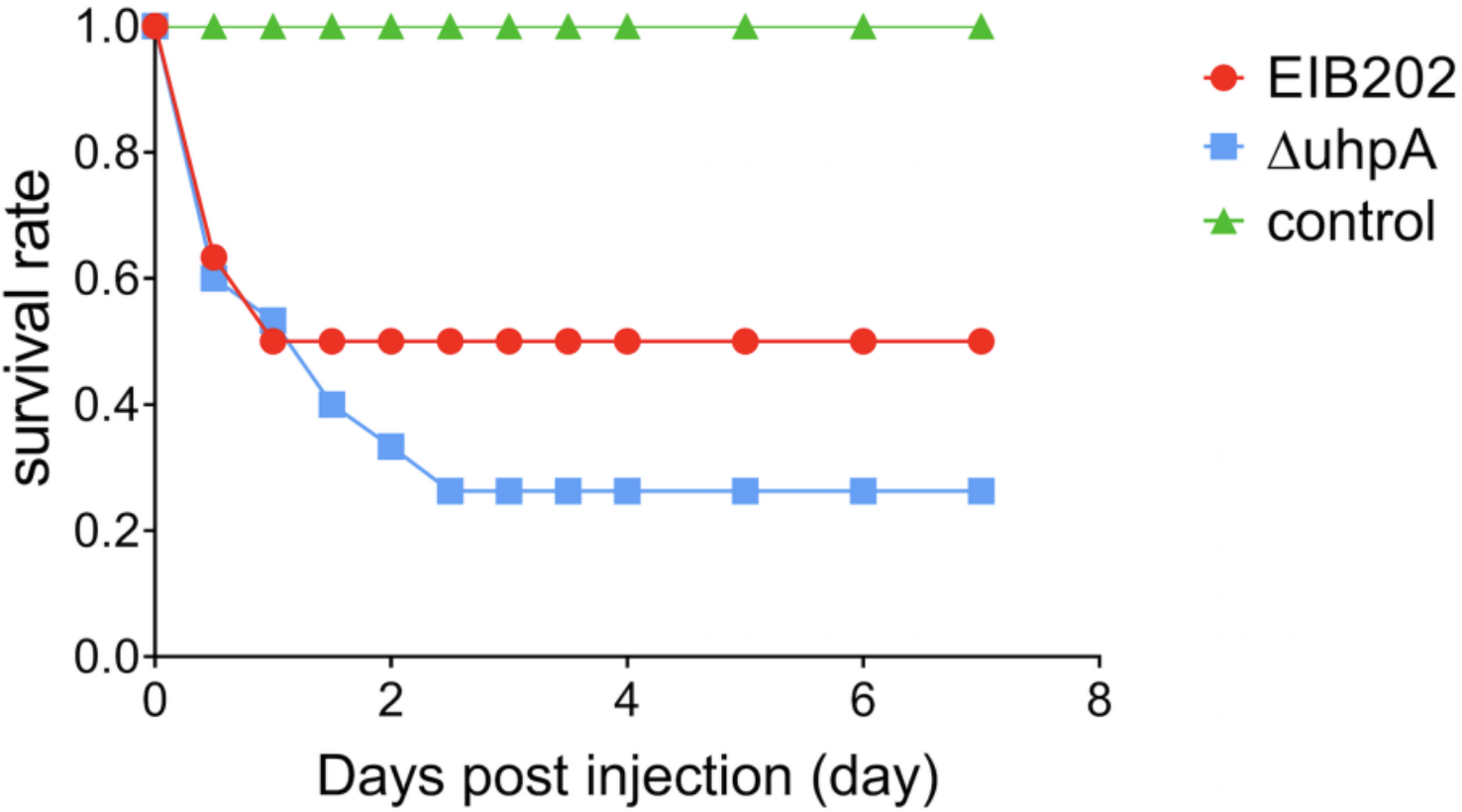

Figure 6

The difference of transcript levels of the key T6SS related genes in E. piscicida EIB202 and mutant strain ΔuhpA. Alphabet a and b: Values indicate significant difference at P<0.05.