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Role of the sigma factor AlgU in regulating growth, virulence, motility, exopolysaccharide production, and environmental stress adaptation of *Pseudomonas syringae* pv. *actinidiae* QSY6

Yiju Zhang[1†], Min Fu[1†], Qian Wang[1,2], Lei Zhang[1], Xiaoxi Chang[1] and Lixin Zhang[1*]

Abstract

The extracytoplasmic function (ECF) sigma factor AlgU is involved in the regulation of various virulence-related pathways in *Pseudomonas syringae*, especially alginate biosynthesis and motility, and the role of AlgU differs among *P. syringae* pathovars. However, to date, the mechanism of its regulation in virulence of *P. syringae* pv. *actinidiae* (*Psa*) is still unclear. ECF sigma factors are a class of alternative sigma factors that typically function with anti-sigma factors as part of cell-surface signaling systems. Under non-inducing conditions, AlgU remains inhibited by anti-sigma factors such as MucA and MucB. To investigate the function of AlgU in *Psa*, mutant strains lacking *algU* or lacking *algU* with *mucA* and *mucB* genes, as well as complementary and overexpression strains of *algU* were generated, respectively. The results showed that AlgU was highly conserved among *P. syringae* pathovars and positively regulated growth rate, pathogenicity, and resistance to osmotic and oxidative stress of *Psa* QSY6. While *AlgU* did not affect the motility and exopolysaccharide production of *Psa*, its abundant expression enhanced the swimming ability of QSY6 and reduced its production of extracellular polysaccharides. Furthermore, AlgU regulates a number of virulence-related factors, including the Hrp system, the type VI secretion system, and flagellar synthesis. Specifically, AlgU induced the expression of *hrpL* and *hrpRS* in vivo, and repressed the transcription of *hrpL* and *tssC* in vitro, while promoting the expression of *hrpS*, *fliC*, and *tssJ*. This study contributes to a better understanding of the mechanisms of virulence regulation of AlgU in *Psa*.

Keywords *Pseudomonas syringae* pv. *actinidiae*, Extracytoplasmic function sigma factor, AlgU

Background

Pseudomonas syringae is a well-studied plant pathogen that serves as a model for understanding bacterial pathogenicity, molecular mechanisms of plant-microbial interactions, microbial ecology, and epidemiology (Mansfield et al. 2012). To date, more than 60 pathovars have been identified in this species (Bull et al. 2010), each infecting a specific set of endemic host plants. Overall, *P. syringae* can infect almost all economically important crops, causing significant losses in agricultural production (Xin et al.

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2018). Among them, kiwifruit bacterial canker caused by *P. syringae* pv. *actinidiae* (*Psa*) has caused devastating damage to the global kiwifruit industry. The process of *P. syringae* infecting plants involves three stages: entering the plant, overcoming host resistance, and evoking disease. To enter a plant, bacteria require flagella- and type IV pili-mediated motility. Once they enter the plant's apoplastic spaces, they secrete a variety of effector proteins (such as T3SS effectors and T6SS effectors) into the plant cytoplasm to overcome host immunity, produce phytohormones and phytotoxins to suppress plant defense responses, along with extracellular polysaccharides for successful colonization (Ichinose et al. 2013).

To ensure successful infection of the host, pathogens have the ability to regulate gene expression under different environmental conditions to quickly adapt to external environmental pressure (Markel et al. 2016). Sigma factors can regulate gene expression in response to changes in the external environment. Multiple types of sigma factors are present in bacteria, which are utilized to recognize various promoters in response to diverse dynamic environmental conditions (Mascher 2013). Extracytoplasmic function (ECF) sigma factor responds to signals from the extracellular environment by regulating gene expression, thereby enabling pathogens to quickly adapt to external environmental pressure (Staron et al. 2009). It has been reported that ECF sigma factor AlgU is involved in regulating various virulence-related pathways in *P. syringae* (Xie et al. 2020). Especially, AlgU regulates alginate synthesis, flagella biosynthesis, toxin production, etc., and its effects are not exactly the same among different pathovars of *P. syringae* (Keith et al. 2001; Schenk et al. 2008; Bao et al. 2020). At present, the action mechanism of AlgU in *Psa* is still unclear.

The ECF sigma factors are usually transcribed together with anti-sigma factors, and ECF sigma factors formed a complex with anti-sigma factors in most cases, and anti-sigma factors prevented sigma factors from binding to RNA polymerase (RNAP) (Staron et al. 2009). Under the stimulation of external environmental pressure, the signal network causes the release of ECF sigma factors and then binds to the RNAP catalytic core, thus making it possible to transcribe target genes (Potvin et al. 2008). AlgU is encoded in an operon that contains up to four regulatory genes named *mucA*, *mucB*, *mucC*, and *mucD*. The *mucA* gene was near-universally associated with *algU*, while the presence of *mucB*, *mucC*, and *mucD* varied (Wang et al. 2021a).

In the present study, we investigated the function of AlgU in regulating the virulence of *Psa*. Our findings suggested that AlgU is a regulator of pathogenicity in *Psa*. In addition, we found that AlgU contributed to growth rate, swimming motility, exopolysaccharide production, resistance to environmental stress, and the expression of virulence-related genes. The findings will be helpful in understanding the mechanism of *algU* regulation of virulence of *Psa*.

Results

Cloning and sequence analysis of *algU*

Based on the sequence of *algU* from *Psa* QSY6 genomic information, a 582-bp long coding sequence was cloned from QSY6. To investigate the similarity of *algU* among different *Pseudomonas* spp., the amino acid sequences of AlgU were analyzed. A total of 13 AlgU sequences from reference strains of *P. syringae* and *P. aeruginosa* were selected to perform the homology comparison. This gene encoded 193 amino acid residues with 100% amino acid identity among different biovars of *Psa* strains, and the amino acid sequence of AlgU of the QSY6 strain showed more than 99% identity with the pathovars of *P. syringae*, indicating that AlgU shares a high homology among *P. syringae*. Meanwhile, the sequences were selected to construct a phylogenetic tree by the maximum likelihood method, and multiple sequence alignment was also analyzed using DNAMAN. The phylogenetic analysis showed that the QSY6 was most closely related to reference strains of *Psa* biovars and *P. syringae* pv. *tomato* (*Pto*) DC3000 (Fig. 1a). Based on the results of multiple sequence alignment, it showed that the amino acid sequences were highly conserved, and there was only a difference at the ninth amino acid among pathovars of *P. syringae*. However, there were 16 different amino acid sites that showed significant differences compared to *P. aeruginosa* (Fig. 1b).

Construction of deletion mutants, complementation, and overexpression strains

Using a double-crossover markerless gene deletion system with the suicide vector, pK18mobsacB, the complete ORF of *algU* (582 bp) and *algUmucAB* (2172 bp) were deleted in QSY6 based on homologous recombination, respectively. For each gene, the mutants were confirmed by PCR and Sanger sequencing. The *algU* and *algUmucAB* deletion mutants were named Δ*algU* and Δ*algUmucAB*, respectively. The complete ORF and promoter sequence region of *algU* was amplified by PCR and ligated into the plasmid, PDSK, and the resulting complementation plasmid was named PDSK-*algU*, which was subsequently introduced into Δ*algU*, Δ*algUmucAB*, and wide-type (WT), respectively, and the complementation strains and overexpression strains were confirmed by PCR amplification and Sanger sequencing. The resulting complementation strains were named Δ*algU*-C and Δ*algUmucAB*-C, and the overexpression strains were named OX-*algU*.

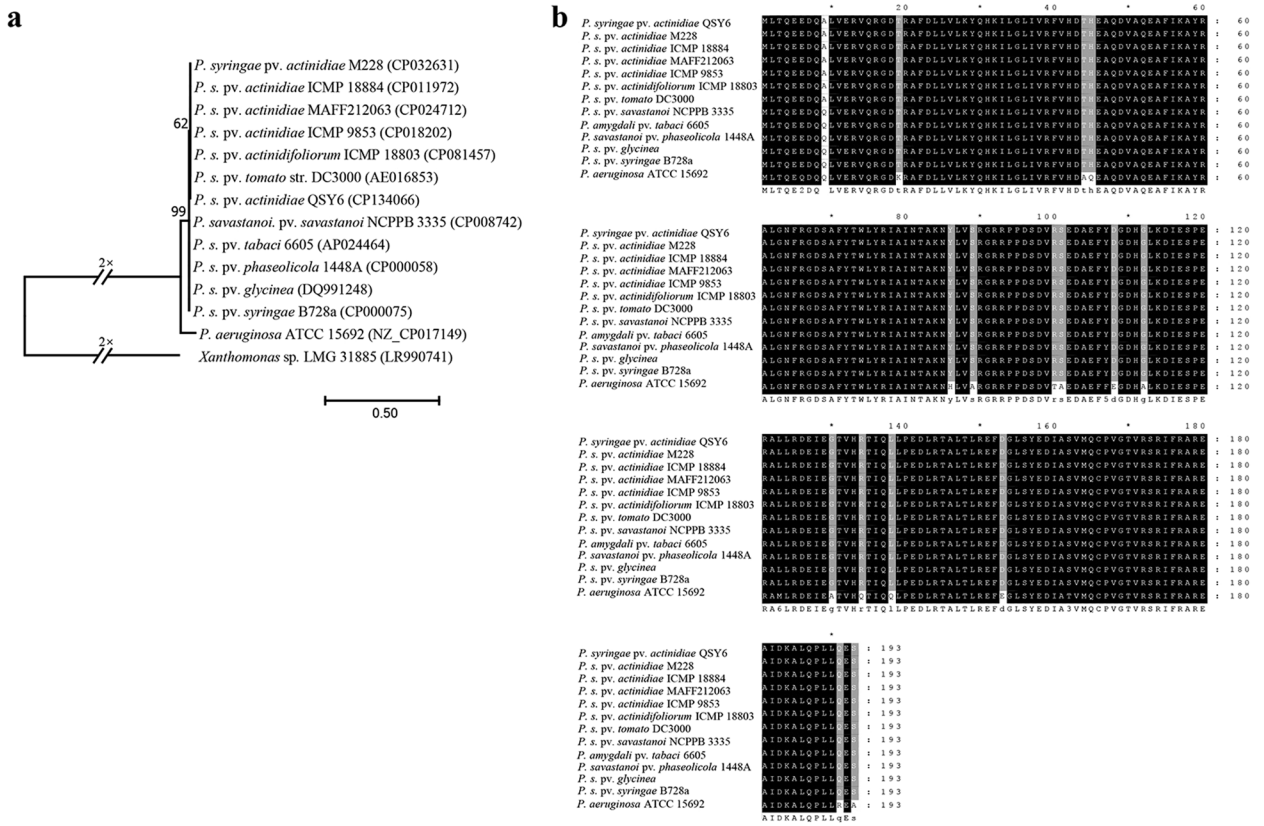

Fig. 1 Sequence similarities between AlgU proteins among *Pseudomonas* species. **a** A maximum likelihood tree of AlgU and its homolog proteins from different pathovars of *Pseudomonas syringae*, *P. aeruginosa*, and *P. savastanoi*. *Xanthomonas* sp. LMG 31885 was selected as an outgroup. The scale bar indicates 0.5 expected changes per site. **b** Multiple sequence alignment of AlgU and its homolog proteins were performed using DNAMAN version 9, and the results were visualized using GeneDoc. Black and gray backgrounds denote no less than 100% and 75% amino acid identity, respectively

algU is involved in regulating *Psa* growth in vitro

The growth rate of bacteria affects its survival and infection. To determine whether AlgU affects the growth of the strain QSY6, bacterial suspensions ($OD_{600}=0.1$) of WT and mutants were inoculated in conical flasks containing 50 mL KB liquid medium at a ratio of 1:100 at 28℃ and 200 rpm for 48 h, respectively. The knockout mutants (Δ*algU* and Δ*algUmucAB*) exhibited a lower growth rate at 24–48 h post-inoculation (hpi) compared to the WT, with a statistically significant difference observed at 32 and 36 hpi (Additional file 1: Figure S1). At 36–40 hpi, the growth of the complemented strain Δ*algU*-C was comparable to that of the WT, whereas the growth of the complemented strain Δ*algUmucAB*-C remained lower than that of the WT (Additional file 1: Figure S1). At 44 hpi, the WT strain exhibited an OD_{600} of 4, followed by a decrease in the *Psa* population. In contrast, the complementary and overexpressing strains displayed continued growth, reaching an OD_{600} of 4.3 and 4.7 at 48 hpi, respectively (Additional file 1: Figure S1).

algU is involved in regulating *Psa* motility

Bacterial motility, including swimming, swarming, and twitching motility, plays a major role in microbial surface colonization (Capdevila et al. 2004). To investigate the impact of *algU* deletion on the swimming capability of *Psa* QSY6, both WT and mutant strains were cultivated on 0.3% semi-solid agar plates. The swimming capacities of the Δ*algU* and Δ*algUmucAB* strains did not exhibit significant differences compared to those of the WT strain, as evidenced by their consistent colony diameters (Fig. 2a, b). Similarly, the swimming capacities of the complementation strains were found to be comparable to those of the WT strain. Notably, the swimming zone of the overexpression strain was significantly larger than that of the WT strain (Fig. 2a, b). The swarming motility of the strains was tested on a 0.5% semi-solid agar plate, as shown in Fig. 2c, d, and no significant differences were found in the swarming zone between mutants and WT. Together, these findings suggest that the swimming ability of *Psa* is not entirely dependent on *algU*, but the

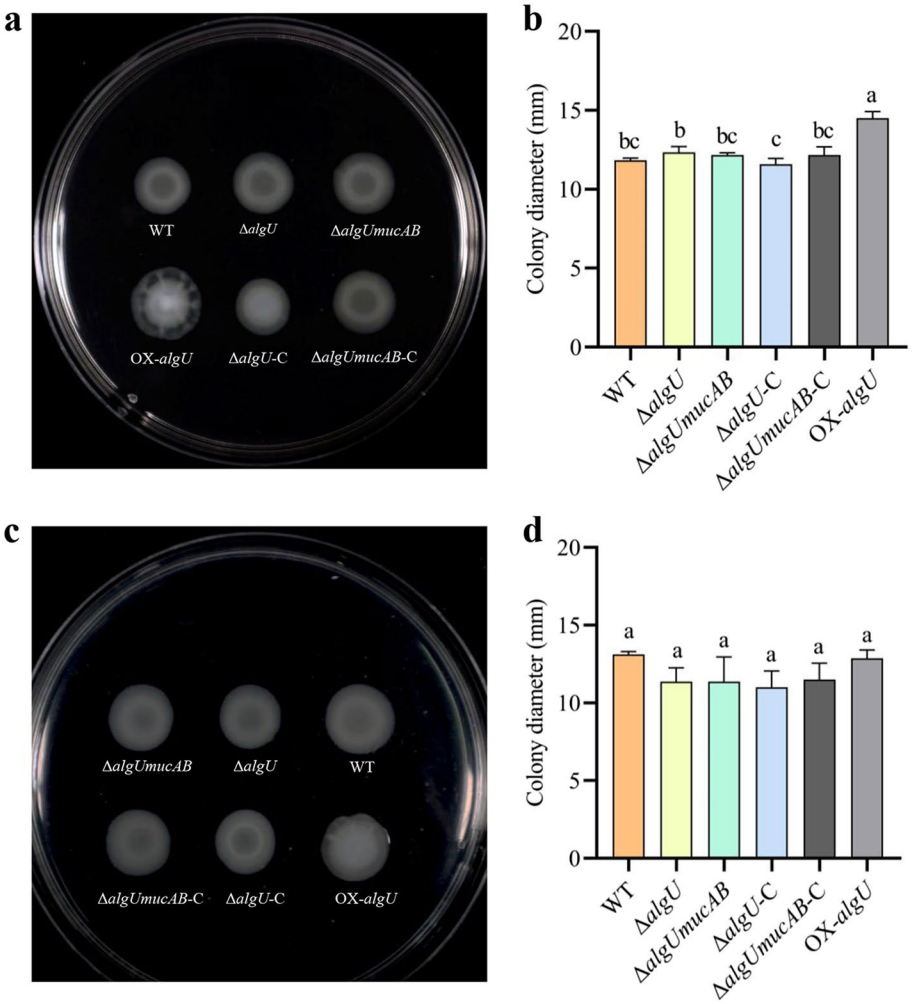

Fig. 2 Motility of QSY6 (wide-type), Δ*algU*, Δ*algUmucAB*, Δ*algU*-C, Δ*algUmucAB*-C, and OX-*algU*. **a** Swimming of different strains on 0.3% KB agar plates for 5 days at a temperature of 28℃. The experiment contains three replications. **b** The swimming abilities of different strains were determined by the diameter of the zone of motility, as shown in the bar graph. **c** Swarming of wide-type and mutants on 0.5% KB agar plates for 5 days at 28℃. The experiment contains two replications. **d** The swarming abilities of different strains were determined by the diameter of the zone of motility, as shown in the bar graph. Error bars represent the standard deviations of the mean. Different letters over the error bars indicate the significant difference at the $P = 0.05$ level

abundant expression of *algU* enhances the swimming ability of QSY6.

algU contributes to *Psa* virulence in plants

To determine whether *algU* affects the pathogenicity of QSY6 in kiwifruit, we conducted pathogenicity tests on healthy branches of kiwifruit (cv. 'Hongyang') to assess the pathogenicity of the WT and mutant strains. Visible necrotic lesions incurred by Δ*algU* (6.2 ± 2.1 mm) and Δ*algUmucAB* (6.9 ± 0.8 mm) at 21 dpi were significantly reduced compared to the WT strain QSY6 (20.0 ± 1.3 mm). The lesion length caused by the Δ*algU*-C (18.2 ± 1.2 mm) and the overexpression strains (15.3 ± 1.6 mm) were restored (Fig. 3a, b), suggesting that the deletion of *algU* significantly reduced the pathogenicity of strain QSY6. Notably, the Δ*algUmucAB*-C strain still exhibited reduced virulence, as evidenced by an average lesion length of 3.2 ± 0.3 mm (Fig. 3b). We further examined *algU* gene expression without *mucA* and *mucB*. The results showed that the expression of *algU* in Δ*algUmucAB*-C was lower than that of Δ*algU*-C and OX-*algU* both in vivo (Fig. 3c) and in vitro (Additional file 1: Figure S1c). Meanwhile, it was found that the expression levels of *hrpL*, *hrpR*, and *hrpS* in the Δ*algUmucAB*-C strain were lower than that of WT, the Δ*algU*-C strain, and overexpression strain in vivo (Fig. 3c).

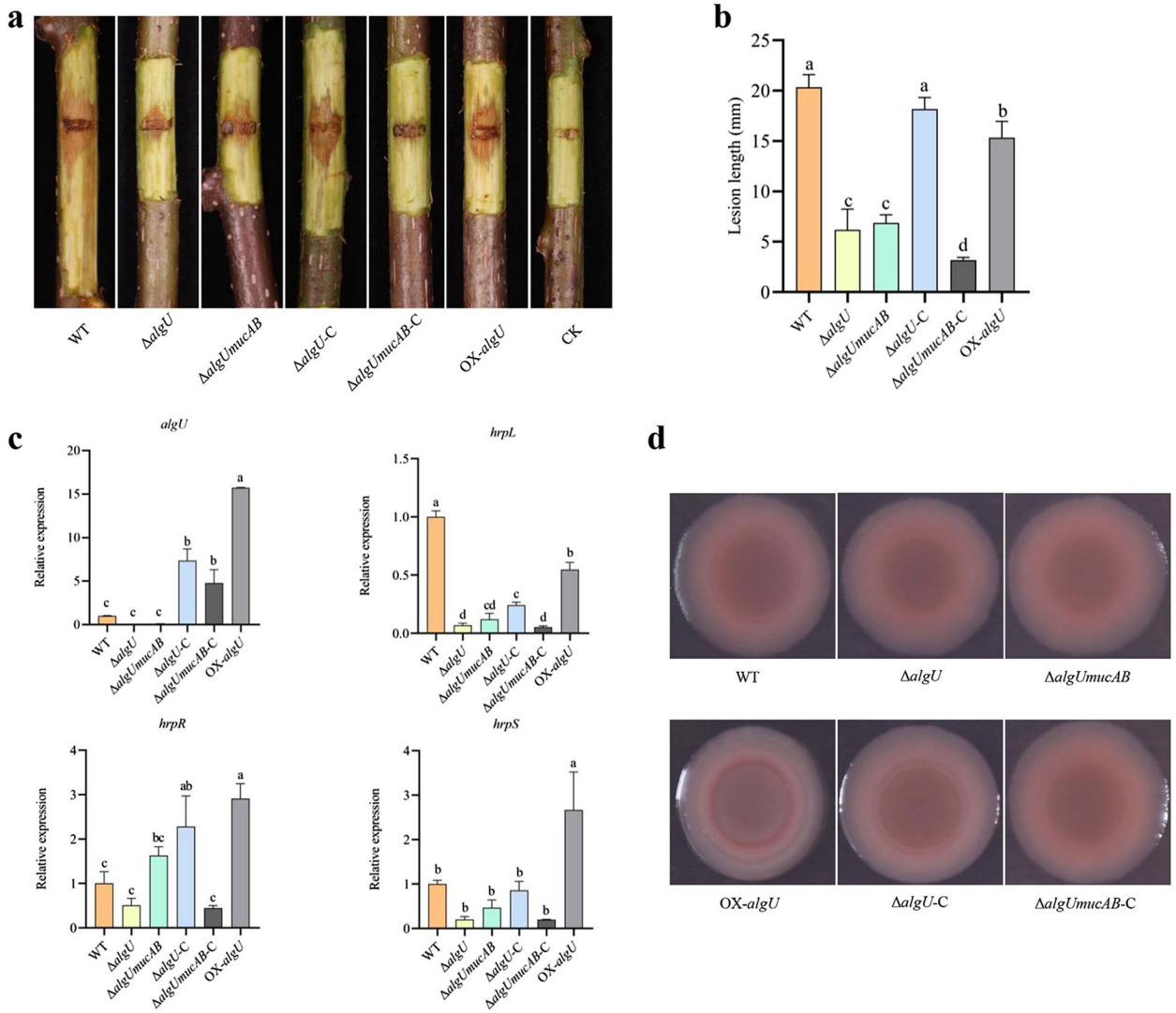

Fig. 3 Pathogenicity assays on wide-type and mutant strains of QSY6. **a** The lesion observation of the wide-type and mutant strains infecting the branches of kiwifruit. **b** The lesion lengths of the wide-type and mutants infecting the branches of kiwifruit. **c** Expression patterns of *algU* in WT and mutant strains in vivo. **d** Exopolysaccharide determination of the QSY6, Δ*algU*, Δ*algUmucAB*, Δ*algU*-C, Δ*algUmucAB*-C, and OX-*algU*. Congo red phenotypic experiment of wide-type and mutants. Photos were taken after 4 days of incubation at 28℃. The experiment contains three replications

algU affects the exopolysaccharide production of *Psa*

Bacteria generally live in communities and may adhere to solid surfaces by forming biofilms (Flemming and Wuertz 2019). Extracellular polysaccharide is one of the important components of biofilm. In this assay, the exopolysaccharide production of WT, Δ*algU*, Δ*algUmucAB*, Δ*algU*-C, Δ*algUmucAB*-C, and OX-*algU* were tested on KB agar plates containing 200 μg/mL Congo red dye. As depicted in Fig. 3d, the *algU* and *algUmucAB* mutants stained comparably on the plates compared to WT, indicating that the exopolysaccharide production of the deletion mutants was not significantly different from that of the WT strain. However, the colony morphology of the WT strain displayed a deeper red color than that of the overexpression strain, indicating that high *algU* activity suppresses the production of exopolysaccharides.

algU positively modulates *Psa* tolerance to environmental stress

Accumulating evidence suggests that the ECF sigma factor AlgU plays an important role in the adaptation of pathogenic bacteria to the external environment (Korbsrisate et al. 2005; Brooks and Buchanan 2008). We, therefore, evaluated the tolerance of the mutants to several environmental stressors, such as NaCl and hydrogen peroxide (H_2O_2). The experiments

were conducted on KB agar plates containing NaCl and H_2O_2, respectively, and the KB agar plates were set as the control. The colony diameters of the deletion mutant (8.3 ± 0.4 mm) were smaller compared to the WT (12.0 ± 0.3 mm), whereas the colony diameter of complemented (11.2 ± 0.3 mm) and overexpression strains (11.2 ± 0.3 mm) was similar to that of the WT when grown on KB agar plates containing 200 mmol NaCl (Fig. 4). Moreover, the deletion mutants did not grow in 1.0 mmol H_2O_2, while they grew well in this cultural condition for the WT, complemented strains, and overexpression strains (Fig. 4). These results suggest that AlgU plays an important role in the response of *Psa* to high external osmotic pressure and oxidative stress.

algU regulates genes with known virulence functions in vitro

To investigate the potential regulatory roles of AlgU, we conducted RT-qPCR analysis on six genes associated with the flagellar synthesis (*fliC*), type VI secretion system (*tssC* and *tssJ*), and Hrp system (*hrpR*, *hrpS*, and *hrpL*), respectively. The results showed that the levels of *fliC* and *tssJ* expression were reduced in two deletion mutants (Δ*algU* and Δ*algUmucAB*) compared to those of the WT strains (Fig. 5a, f). However, the deletion of *algU* or *algUmucAB* led to a notable increase in the expression of the *tssC* gene (Fig. 5e). In addition, the suppression of *hrpS* expression was also observed upon the knockout of the *algU* gene alone (Fig. 5d). The gene expression levels of *hrpR* and *hrpL* in Δ*algU* and Δ*algUmucAB* did not show significant differences compared to those in the WT strain (Fig. 5b, c). However, it was observed that the

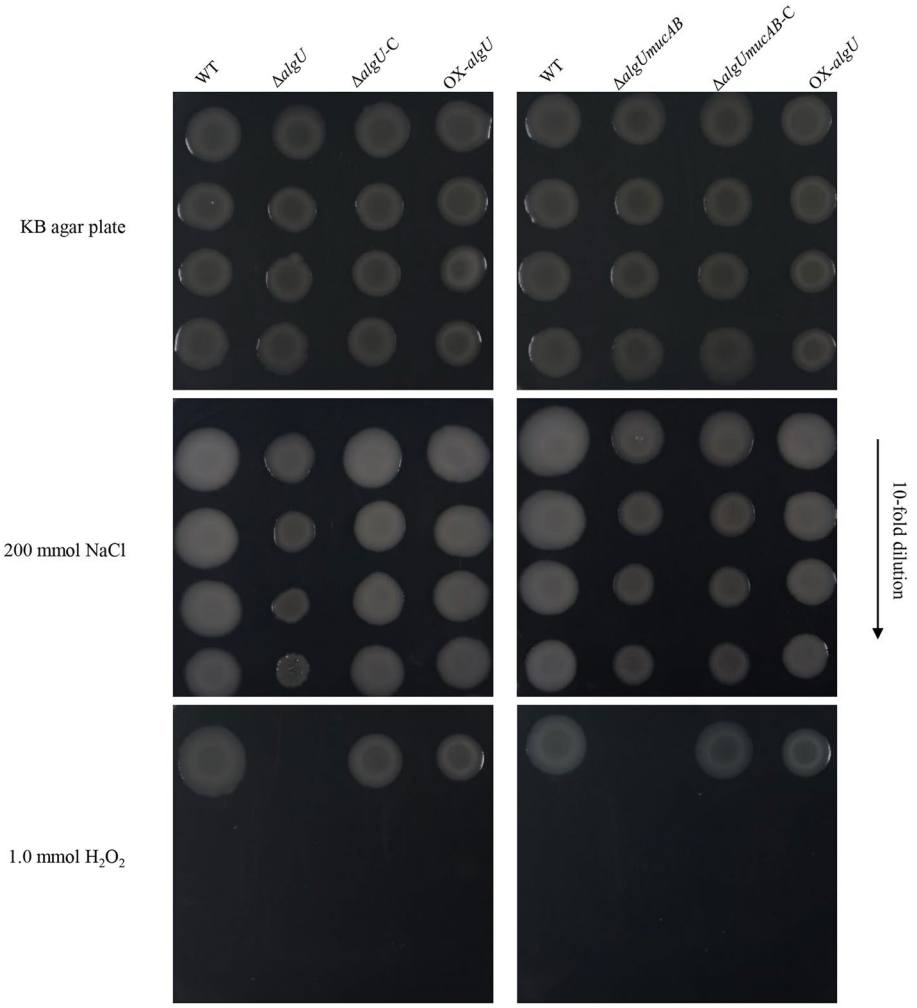

Fig. 4 The resistance to environmental stress. **a** The wide-type and mutants were cultured on KB medium. **b** The mutants were cultured on KB agar plate supplemented with 200 mM NaCl. **c** The mutants were cultured on KB agar plate with 1.0 mM H_2O_2. Photos were taken after 4 days of incubation at 28℃. The experiment contains three replications

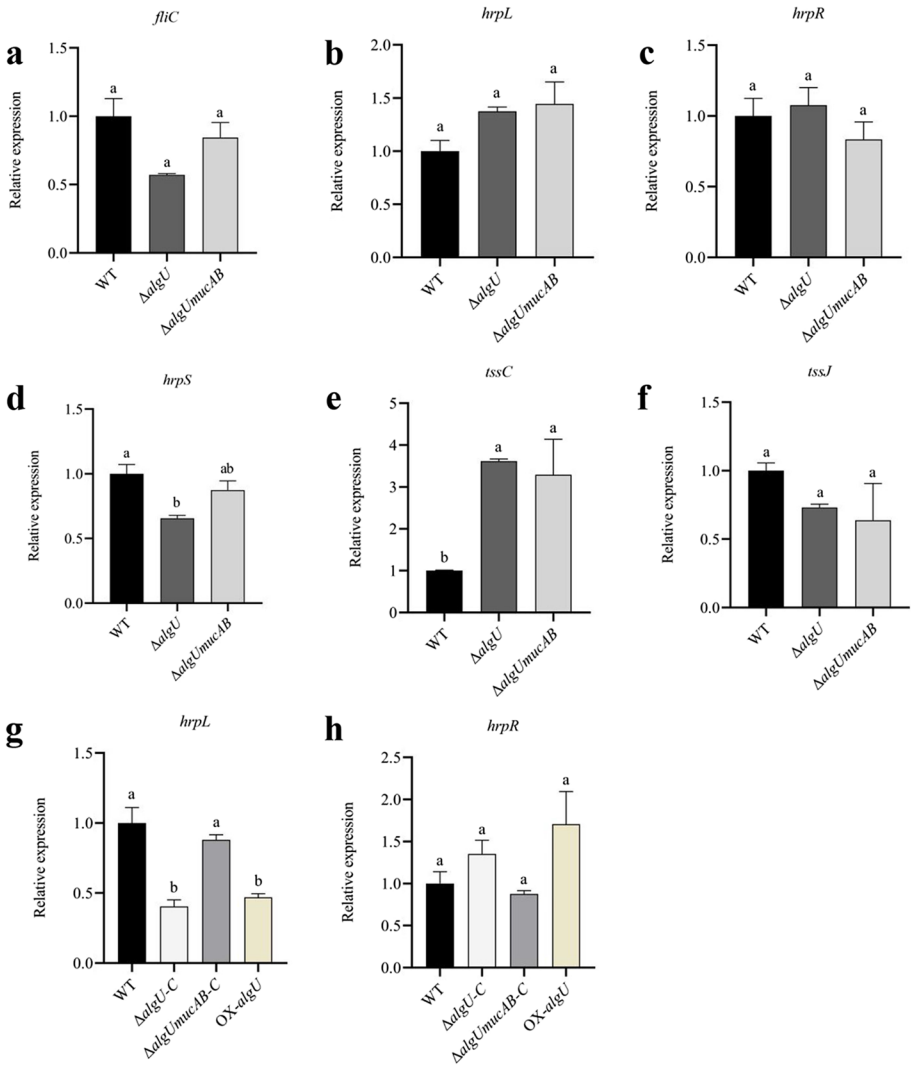

Fig. 5 Expression patterns of six genes in WT and mutant strains in vitro. **a–f** RT-qPCR of six genes (*fliC*, *hrpL*, *hrpR*, *hrpS*, *tssC*, *tssJ*) in WT, Δ*algU*, and Δ*algUmucAB*. **g–h** RT-qPCR of *hrpL* and *hrpR* in WT, Δ*algU*-C, Δ*algUmucAB*-C, and OX-*algU*. The *gyrB* was set as the reference gene. Error bars represent the standard errors of the mean. Different letters over the error bars indicate the significant difference at the $P = 0.05$ level

expression levels of the *hrpL* gene were notably reduced in the complemented strain Δ*algU*-C and overexpression strain OX-*algU* compared to the WT (Fig. 5g), suggesting that high *algU* activity negatively regulated the expression of *hrpL*.

Discussion

Plant pathogenic bacteria face various challenges throughout their life cycle, such as drought, nutrient scarcity, and host immune responses. Nevertheless, to ensure their survival, bacteria must possess the ability to withstand these adverse environmental conditions or counteract the plant's immune system (Baker et al. 2010). In general, pathogenic bacteria need to continuously adapt their gene expression profiles to the host environment during infection, and ECF sigma factors, such as AlgU and HrpL, usually mediate this adaptive regulation. Currently, there have been relevant reports on the role of AlgU in several *Pseudomonas* spp., including *P. aeruginosa*, *P. syringae* pv. *syringae* (*Pss*), *P. syringae* pv. *glycinea* (*Psg*), and *Pto* (Wood et al. 2006; Schenk et al. 2008; Freeman et al. 2013; Yu et al. 2014). Although the amino acid sequence of AlgU remains highly conserved (Fig. 1), its function may vary among different *Pseudomonas* species or pathogenic bacteria. For instance, AlgU in *Pss* B728a could induce the expression of genes involved in the T6SS (Yu et al. 2014). In contrast, the activation of AlgU in *Pto* DC3000 controls the expression of components related

to the Hrp T3SS, effectors, as well as the transcriptional regulators *hrpL* and *hrpRS* (Markel et al. 2016). In this study, we systematically investigated the function of AlgU in *Psa* and found that AlgU is an important regulator of *Psa* QSY6, which is involved in growth rate, pathogenicity, mobility, exopolysaccharides production, and tolerance to environment stresses of QSY6.

Accumulating evidence suggested that AlgU positively regulates the pathogenicity of *Pto* in tomatoes and *Psg* in soybean, respectively (Schenk et al. 2008; Markel et al. 2016; Nguyen et al. 2021). In the present study, lack of *algU* resulted in a significant decrease in the pathogenicity of *Psa* QSY6, whereas the pathogenicity of the overexpression strain increased (Fig. 3a, b), suggesting a similar function in virulence to that of *Pto* DC3000 and *Psg*. Besides, the virulence of the complementary strain Δ*algU*-C returned to levels comparable to the WT, but the introduction of AlgU in Δ*algUmucAB* did not restore pathogenicity (Fig. 3b), indicating the additional functions of *mucA* and *mucB* in *Psa* QSY6. Meanwhile, we hypothesized that the difference in MucA and MucB amino acid sequences among different pathovars in *P. syringae* may also be a reason for the different results. We also investigated the *algU* expression levels in various mutants in vivo and in vitro, respectively, and observed a decrease in the expression of *algU*, *hrpL*, *hrpR*, and *hrpS* in the Δ*algUmucAB*-C strain compared to the Δ*algU*-C strain and overexpressing strains (Fig. 3c). This finding corresponded to the pathogenic phenotype, indicating a correlation between the expression of *algU* and pathogenicity. AlgU may affect pathogenicity by regulating virulence-related genes, such as the type III secretion system.

Motility is necessary for bacterial pathogens to spread on the plant surface and colonize host plants (Josenhans and Suerbaum 2002). In *P. syringae*, mutations in flagellum-related genes may lead to a loss of motility in *P. syringae*, thereby significantly reducing its virulence (Huang et al. 2022). AlgU is a global virulence regulator that plays an essential role in regulating the motility of *P. syringae*. Previous studies have shown that AlgU is necessary for downregulating *Pto* DC3000 *fliC* expression during infection (Bao et al. 2020) and suppressing flagellar motility in *P. protegens* (Wang et al. 2023). However, Markel et al. (2018) found that AlgU induces antisense transcript expression of the *fleQ* gene, enhancing its swimming motility and positively controlling its flagellar activity in *Pto*. In this study, the swimming and swarming ability of WT and mutant strains of *Psa* QSY6 were analyzed. The results showed that the high *algU* activity of the overexpression strains promoted the swimming ability of strain. In contrast, the introduction of *algU* in the absence of an anti-sigma factor had no significant effect on mobility (Fig. 2). Extracellular polysaccharide (EPS) is an important component of bacterial biofilm. AlgU is crucial in regulating alginate synthesis, a significant constituent of EPSs (Bazire et al. 2010; Bouffartigues et al. 2015). Here, the overexpression of *algU* in QSY6 led to a decrease in the production of EPS (Fig. 3d). In contrast, *algU* positively regulated the exopolysaccharide production in *Pseudomonas stuzeri* A1501 (Shao et al. 2022) and alginate production in *Pss* (Yu et al. 2014). Similarly, in *P. aeruginosa*, the absence of *algU* led to decreased production of Psl exopolysaccharide synthesized by the *polysaccharide synthesis locus* (*psl*) (Bazire et al. 2010).

Plant pathogenic bacteria are exposed to various environmental stresses that can interfere with their growth and survival. To adapt to the environment, bacteria need specific systems to detect changes in the external environment and regulate transcriptional changes in response to environmental stimuli (Markel et al. 2016). ECF sigma factor AlgU plays an important role in this process. In *P. syringae*, *algU* mainly responds to heat, oxidative, and osmotic stress, while the role of AlgU may differ among different pathovars. It has been reported that *algU* regulated the expression of osmotic and oxidative stress-related genes in *Pto* DC3000 (Markel et al. 2016). In *P. syringae* pv. *syringae*, strains lacking *algU* were more sensitive to heat, paraquat, and H_2O_2 (Keith et al. 1999). Here, the absence of *algU* resulted in a decrease in the tolerance of the strain to environmental stresses (Fig. 4), indicating that *algU* plays a significant role in the resistance of *Psa* to osmotic and oxidative stresses.

The protein secretion system is important in virulence and interaction between pathogens and hosts in many Gram-negative bacteria (Gerlach and Hensel 2007). Among them, the T3SS is an essential virulence determinant for many Gram-negative bacterial pathogens (Peng et al. 2023), and the *hrpR*, *hrpS*, and *hrpL* genes are the indispensable virulence genes of T3SS. Studies have shown that *algU* inhibited the expression of *hrpL* in *Pss* (Yu et al. 2014); however, in *Pto* DC3000, *algU* induced the expression of *hrpL* and *hrpR* (Markel et al. 2016). In this study, the up-regulated expression of *algU* inhibited the expression of *hrpL* in vitro (Fig. 5g). However, in vivo, *algU* of QSY6 induced the expression of *hrpL* and *hrpRS* (Fig. 3c). The T3SS is regulated by the environment (Shao et al. 2021), so there are differences in the regulation of its related gene expression in vivo and in vitro culture. Moreover, T6SS was closely related to the pathogenicity of many pathogenic bacteria (Mattinen et al. 2007; Gao et al. 2021) and was also implicated in resistance to environmental stress (Ben-Yaakov and Salomon 2019). It has been reported that *algU* could activate the T6SS gene (*tssJ* and *tssC*) in *Pss* (Yu et al. 2014). However, our study showed that *algU* repressed the expression of *tssC*

and activated the expression of *tssJ* in *Psa*. It has been reported that the deletion of *tssJ* decreased the H_2O_2 tolerance in *Psa* (Wang et al. 2021b). AlgU positively regulated swimming motility in *Pto* and *P. syringae* pv. *phaseolicola* (Markel et al. 2018; Shao et al. 2021), while it did not influence the expression of *fliC* in *Pss* (Yu et al. 2014). Likewise, in *Psa*, AlgU positively regulated both swimming motility and the expression of *fliC* in this work.

Conclusions

Taken together, the ECF sigma factor AlgU globally regulates many virulence-related factors in *Psa* QSY6. Specifically, AlgU enhances pathogenicity, motility, and environmental stress tolerance and the expression of *fliC* and *tssJ* of QSY6 in vitro, and inhibits the production of exopolysaccharides and the expression of *hrpL* and *tssC*. However, in vivo, AlgU enhances the expression of *hrpL*, *hrpR* and *hrpS* in QSY6 strains. Our finding contributes to a better understanding of the mechanisms governing the virulence regulation by AlgU in *Psa*.

Methods

Bacterial strains, plasmids, and culture conditions

The bacterial strains and the plasmids used in this study are listed in Table 1. *P. syringae* pv. *actinidiae* QSY6 (GenBank accession CP134066) was cultured in King's B (KB) medium (20 g/L protease peptone, 1.5 g/L anhydrous K_2HPO_4, 15 mL/L glycerol, 1.5 g/L $MgSO_4$, with or without agar) at 28℃. *Escherichia coli* was cultured in Luria–Bertani (LB) medium (5 g/L yeast extract, 10 g/L NaCl, 10 g/L tryptone, with or without agar) at 37℃. Antibiotics were used at the following concentration: ampicillin at 50 μg/mL and kanamycin at 50 μg/mL.

Cloning and sequence analysis of *algU*

Using genomic DNA from *Psa* QSY6 as a template, the gene encoding for *algU* was amplified with the primer pair algU-F/R (Additional file 2: Table S1). The PCR was performed in a final volume of 50 μL, containing 25 μL of 2 × Taq Master Mix (CWBIO, Beijing, China), 2 μL of each primer (10 μM), 2 μL of DNA, and 19 μL of ddH_2O. The PCR conditions were as follows: pre-denaturation at 95℃ for 3 min, followed by 35 amplification cycles (95℃ for 10 s, 58℃ for 30 s and 72℃ for 30 s), before a final extension at 72℃ for 5 min. The PCR product was cloned into the pMD19-T Vector (Takara, Japan). The *algU* sequence was then determined by Sanger sequencing. Sequence similarity searches in the National Center for Biotechnology Information databases were performed using BLASTp (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Multiple alignments of amino acid sequences were performed by the DNAMAN (v. 9.0; Lynnon Biosoft), and the results were visualized using GeneDoc. The phylogenetic tree was constructed using the maximum likelihood method in MEGA v. 7.0.1 (Kumar et al. 2016).

Construction of deletion mutants, complementation, and overexpression strains

Primers used in this study were designed based on the genome sequence of *Psa* QSY6. Deletion mutant strains were generated through the process of homologous recombination. The upstream and the downstream fragments of *algU* and *algUmucAB* were amplified using the corresponding primers, respectively (Additional file 2: Table S1). The suicide plasmid pK18mobsacB was linearized with *BamHI* and *PstI*, and then the upstream and downstream fragments were cloned into the linearized pK18mobsacB using ClonExpress Ultra One Step Cloning Kit V2 (Vazyme, Nanjing, China) to construct the vectors pK18mobsacB-*algU* and pK18mobsacB-*algUmucAB*. After verification by PCR and Sanger sequencing, the vectors were transformed into the wild-type by electroporation method, and the first recombinant mutants were screened on KB agar plates with 50 μg/mL kanamycin using primers sacB-F/R (Additional file 2: Table S1),

Table 1 Strains and plasmids used in this study

Strains or plasmids	Relevant characteristics	Reference or source
QSY6	Wide-type	Laboratory collection
QSY6Δ*algU*	QSY6 *algU* gene knockout strain	This study
QSY6Δ*algUmucAB*	QSY6 *algUmucAB* gene knockout strain	This study
QSY6Δ*algU*-C	Δ*algU* complemented with PDSK-*algU*	This study
QSY6Δ*algUmucAB*-C	Δ*algUmucAB* complemented with PDSK-*algU*	This study
Escherichia coli DH5α	Gene cloning	This study
pMD19-T Vector	Cloning vector. Amp^r	Takara, Japan
pK18mobsacB	Suicide plasmid used for homologous recombination. Km^r	American-type culture collection
PDSK-GFPuv	Complement plasmid. Km^r	This study

then transferred to KB agar plates containing 15% sucrose to screen the mutants Δ*algU* and Δ*algUmucAB*, respectively. The mutants were confirmed by PCR amplification and Sanger sequencing. To obtain complementation and overexpression strains, the full-length coding sequence and the upstream predicted promoter sequence of the target gene *algU* were amplificated using corresponding primers (Additional file 2: Table S1). The fragment was cloned into plasmid PDSK (linearized by *NdeI* and *BamHI*) using ClonExpress II One Step Cloning Kit (Vazyme, Nanjing, China) to construct the complementation plasmid PDSK-*algU*. Then, the complementation plasmid was transfected into the deletion mutant using the electroporation method to generate the complementation strains. Meanwhile, the complementation plasmid was also electroporated into wild-type QSY6 to obtain the overexpression strain. The complementation and overexpression strains were screened on KB agar plates with 50 μg/mL kanamycin. The strains were confirmed by PCR amplification and sanger sequencing.

Growth rate assays

The overnight cultures were adjusted to $OD_{600}=0.1$. The diluted bacterial suspensions were inoculated into conical flasks containing 50 mL KB liquid medium at a ratio of 1:100 at 28℃ and 200 rpm for shaking culture. The OD_{600} value of the bacterial density was measured every 4 h until 48 h. The experiment was repeated three times for statistical analysis.

Motility assays

Motilities were assayed using various media. Swimming and swarming abilities were tested on a KB plate with 0.3% and 0.5% agar, respectively. A single colony was cultured in KB liquid medium overnight at 28℃ and then diluted to the same bacterial density ($OD_{600}=0.6$). For swimming motility, 2.5 μL of the cultures were spotted onto soft KB plates (0.3% agar) (Wang et al. 2019); the experiment was repeated three times for statistical analysis, and for swarming ability, the 2.5 μL were dotted on KB plate (0.5% agar), The experiment was repeated two times for statistical analysis. The plates were incubated at 28℃ for 5 days. To quantify bacterial motility, the diameter of each colony was determined.

Pathogenicity assays

The branches of healthy 1–2-year-old kiwifruit branches 'cv. Hongyang' were collected, sterilized with 0.6% NaClO solution for 20 min, washed with sterile water three times, and then cut into short branches after drying. The two ends of the branches were sealed with paraffin. A blade was used to cut a wound about 2 mm wide in the middle of the branches, and the wound was deep to the phloem. The concentration of bacterial suspensions of each strain was adjusted to 10^8 CFU/mL, then 10 μL volume was added to the wound site, and sterile water was used as a control. The inoculated branches were placed in a tray with sterile gauze, and an appropriate amount of sterile water was added. Then, the branches were moisturized with plastic wrap and placed in an artificial chamber, photoperiod L/D: 16 h/8 h; day and night temperature: 18℃/14℃, relative humidity: 90%. The lesion lengths of the branches were measured after 21 days.

Exopolysaccharide production assays

The Congo red assay was performed with minor modifications to measure the production of exopolysaccharide (Bordi et al. 2010). The overnight culture was diluted to an OD_{600} of 0.6. Germ-filtered Congo red dye (200 μg/mL) was added to KB medium with 1.5% agar, and 2.5 μL of the diluted culture was spotted onto the surface of the Congo red plates and grown at 28℃ (Xie et al. 2019). The colony morphology and staining were recorded after 4 days. The experiment was repeated three times.

Measurement of osmotic and oxidative stress resistance

In the NaCl resistance colony assay, the bacterial culture was adjusted to the same bacterial density ($OD_{600}=0.6$), and the suspensions were diluted 10 times serially. The 2.5 μL of the different concentrations of bacteria were incubated on KB agar plates with 0.2 M NaCl at 28℃ for 4 days, and the strains incubated on KB agar plates as the control group (Wang et al. 2019). In the hydrogen peroxide resistance colony assay, the bacterial culture was adjusted to an OD_{600} of 0.6. KB agar plates were supplemented with 1.0 mM H_2O_2, and 2.5 μL of serially diluted bacterial cultures were incubated on the plates at 28℃ for 4 days, and the strains incubated on KB agar plates were used as the control group. The experiment was repeated three times.

Reverse transcription-quantitative PCR assay

For reverse transcription-quantitative PCR (RT-qPCR), all strains were cultured in KB medium at 28℃ with shaking at 200 rpm overnight. RNA extraction was performed by using the RNAprep Pure Cell/Bacteria Kit (TIANGEN). To detect the expression of related genes after inoculation in vivo, kiwifruit phloem tissues were collected 21 days post-inoculation, and the RNA extraction was performed using the FastPure Universal Plant Total RNA Isolation Kit (Vazyme, Nanjing, China). RNA concentration was measured by Nanodrop 2000 spectrophotometer (Thermo Fisher, Wilmington, DE, United States). The RNA samples were reversely transcribed to cDNA using HiScript III RT SuperMix for qPCR (+ gDNA wiper) (Vazyme, Nanjing,

China). RT-qPCR was performed using ChamQ SYBR qPCR Master Mix (Vazyme, Nanjing, China) and prepared according to the manufacturer's instructions. Each reaction was performed in triplicate in 20 µL reaction volumes. The reaction program was 95℃ for 3 min, followed by 40 cycles of 95℃ for 10 s and 60℃ for 30 s. The *gyrB* was selected as the reference gene, and the expression levels of candidate genes *hrpL*, *hrpR*, *hrpS*, *fliC*, *tssC*, *tssJ*, and *algU* were detected and analyzed. The fold change represents the relative expression level of mRNA, which can be estimated by the values of $2^{-(\Delta\Delta Ct)}$. Each treatment consisted of two biological repeats and three technical replicates.

Statistical analysis

Statistical analysis was conducted with IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) by one-way analysis of variance. Means were compared using Duncan's test at a significance level of $P=0.05$. The homogeneity of variance was tested before analysis.

Abbreviations

CFU	Colony-forming units
ECF	Extracytoplasmic function
EPS	Extracellular polysaccharide
OD_{600}	Optical density at 600 nm
Psa	*Pseudomonas syringae* pv. *actinidiae*
Psg	*Pseudomonas syringae* pv. *glycinea*
Pss	*Pseudomonas syringae* pv. *syringae*
Pto	*Pseudomonas syringae* pv. *tomato*
RT-qPCR	Reverse transcription-quantitative PCR
T3SS	Type III secretion system
T6SS	Type VI secretion system

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s42483-024-00245-w.

Additional file 1: Figure S1. Growth rate and expression pattern of *algU* in WT and mutants.

Additional file 2: Table S1. Primers used in this study.

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Authors' contributions

LZ conceived and designed the project. YZ performed the experiments, MF, QW, LeiZ, and XC analyzed the data, and YZ and MF wrote the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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