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Larval settlement and metamorphosis of *Mytilus coruscus* in response to varying bacterial capsular polysaccharide

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Marine invertebrates are the backbone of marine biodiversity and play a pivotal role in the marine ecosystem. The life cycle of most marine invertebrates includes the settlement and metamorphosis stage, which is induced by marine biofilms, but the mechanism is still enigmatic. In the present study, we constructed the capsular polysaccharide (CPS) synthesis gene *capC*-deleted mutant of *Pseudoalteromonas marina* by gene knockout and then compared the phenotype, the biofilm-forming ability, the effect on settlement and metamorphosis of *Mytilus coruscus*, and the exopolysaccharide and CPS levels between the mutant and the wild-type strains to explicate the relationship between bacteria and mussels. The study presented that the phenotype and biofilm-forming ability between the wild-type and Δ*capC* strains had no significant difference, but the inducing activity of Δ*capC* biofilms on larval settlement and metamorphosis decreased significantly ($p < 0.05$). Compared with the wild-type, the CPS content of Δ*capC* strain significantly decreased by 38.07%, accompanied by the increase of c-di-GMP. Meanwhile, the biomass of α-polysaccharides and β-polysaccharides on Δ*capC* biofilms decreased significantly ($p < 0.05$). Thus, the CPS synthesis gene could modulate c-di-GMP, which regulates bacterial polysaccharide secretion, and then impact larval settlement and metamorphosis of mussels. This work brings an entry point to deeply understand the interaction between bacterial polysaccharide and larval recruitment.

KEYWORDS

***Mytilus coruscus*, settlement and metamorphosis, biofilm, capsular polysaccharide, capC gene**

1 Introduction

Marine invertebrates are of great ecological value in the marine biodiversity system (Lotze, 2021). Marine bivalve shellfish, a major representative class of marine invertebrates, are distributed in coastal and estuarine areas of the whole world and often become the dominant group of local biological communities. *Mytilus coruscus* is a major economic shellfish in East Sea, China (Chang, 2007; Wang et al., 2012). The life history of *M. coruscus* is similar to that of most marine invertebrates, settlement and metamorphosis are the essential steps for motile larvae to become adults (Cavalcanti et al., 2020), which can contribute to the persistence of *M. coruscus*. This process is often induced by chemical signals within marine biofilms (Zardus et al., 2008; Hadfield, 2011; Liu et al., 2022).

Biofilms exist in almost all of the matrix surfaces in the marine environment, the chemical cues within biofilms, such as extracellular polymeric substances and c-di-GMP, can induce larvae settlement and metamorphosis (Hadfield, 2011; Cavalcanti et al., 2020; Dobretsov and Rittschof, 2020; Rischer et al., 2022). For instance, the exopolysaccharide secreted by a marine *Pseudomonas* sp. could promote *Ciona intestinalis* larvae to metamorphose (Szewzyk et al., 1991). Bao et al. (2007) found that exopolysaccharides or glycoproteins on the biofilm surface may participate in the metamorphosis of *Mytilus galloprovincialis* by binding with lectins (Bao et al., 2007). Freckelton et al. (2022) found that lipopolysaccharides in the OMVs produced by *Cellulophaga lytica* could induce the metamorphosis of *Hydroides elegans* (Freckelton et al., 2022). Moreover, our previous work demonstrated that some polysaccharide biosynthesis genes of marine bacteria promote or inhibit larval settlement and metamorphosis through regulating the content of biofilm extracellular polymeric substances (Zeng et al., 2015; Liang et al., 2020; Peng et al., 2020; Liang et al., 2021). It is worth noting that a point mutation in *AT00-17125* of *Pseudoalteromonas* resulted in a translucent morphology and showed less capsular polysaccharide (CPS) by phenotypic observation, and the biofilm formed by the mutant inhibited larvae metamorphosing to post-larvae (Zeng et al., 2015), but the mechanism of CPS regulating larval settlement and metamorphosis is still unclear.

CPS is an essential component of bacterial capsule in the outer membrane polysaccharides, and it makes a difference in the formation of bacterial biofilm (Badel et al., 2011). CPS can inhibit biofilm formation by preventing adhesion molecules on cell surface (Nagar and Schwarz, 2015). Previous studies have found that the mutants of *Vibrio vulnificus* (Lee et al., 2013), *Pasteurella multocida* (Petruzzi et al., 2017) and *Bacteroides thetaiotaomicro* (Bechon et al., 2020) with defected capsular CPS can enhance the adhesion and cell aggregation capacity of the non-biological surface, and the mutant strain produce more biofilms. However, the interaction between CPS and recruitment of marine benthic animals remains little known.

In this study, we investigated the involvement of CPS synthesis gene *capC* of *Pseudoalteromonas marina* (Yang et al., 2013; Peng et al., 2018) in biofilm development and *M. coruscus* larval settlement and metamorphosis. It provides a supplement for the mechanism of the interaction between bacteria and marine invertebrates and provides a new sight for the study of marine biodiversity conservation from a microscopic perspective.

2 Materials and methods

2.1 Larval culture

Mussel larvae used in larval induction bioassays were all produced in Shengsi Islands (122°76 E; 30°72 N), Zhenjiang, China. The method of larval culture was the same as in a previous study (Liang et al., 2021). Basically, *M. coruscus* developed into swimming straight-hinge veliger larvae within 2 days after fertilization, and then usually need to be cultivated at 16-20°C for about 20 days to develop into pediveliger stage. During the temporary culture in the laboratory, the larvae was cultured at 18°C in darkness, and fed with *Isochrysis zhanjiangensis* every day. The larval induction test was organized when the shell length of pediveligers was longer than 300 μm.

2.2 Strains and plasmids

The tested bacterium *P. marina* was isolated from the natural biofilms and stored at −80°C. The wild-type and Δ*capC* were both cultured in Zobell 2216E medium (2216E, Sigma, St Louis, MO, USA) at 25°C (Peng et al., 2018). The *Escherichia coli* WM3064 was grown in Luria-Bertani (Sigma, St Louis, MO, USA) at 37°C (Peng et al., 2020). When culturing strains containing the pK18mobsacB-ery plasmid, kanamycin and erythromycin should be added to maintain the resistance of the strain (Wang et al., 2015). Other relevant information is shown in Table 1.

2.3 Construction of Δ*capC* mutant strains

A mutant strain of *P. marina* with the deletion of *capC* gene was built by homologous recombination technology (Wang et al., 2015). The upstream and downstream primers used to amplify the target gene *capC* were shown in Table 2. Recombinant plasmids are built by restriction enzyme ligation and transferred into *E. coli* WM3064. The suicide plasmid in *E. coli* WM3064 was transferred into *P. marina*, and colonies that could grow on Zobell 2216E medium which contained erythromycin were chosen and verified by single-

TABLE 1 Strains and plasmids.

Strains or plasmids	Correlation properties	References or source
Pseudoalteromonas marina ECSMB14103	Wild-type	(Peng et al., 2018)
Escherichia coli WM3064	RP4(tra) in chromosome, DAP	(Peng et al., 2020)
Δ*capC*	The mutant strain with the deletion of *capC* gene	This lab
pK18mobsacB-ery	(Peng et al., 2020)	(Wang et al., 2015)
pK18mobsacB-ery-*capC*	Recombinant plasmid	This study

TABLE 2 Primers used to construct Δ*capC* strain.

Primer name	Sequence (5′→3′)	Product length (bp)
capC-up-S	AGGGAGTTGTTGCCAGTTCA	514
capC-up-A	TACTAGCAAGGTGTTTAAAT	514
capC-down-S	AATAACGTTATTTACGACTG	914
capC-down-A	TACGCACCAAAGGACCCAAG	914
capC-SF	CATCTGGCTGGTTTGTAC	284
capC-SR	GTGACGCACTCGTTATTG	148
capC-LF	TTTGGAGGGAGTTGTTGC	519
capC-LR	AGCTCGCCAGGTGAAGGT	587

crossover primer pairs *capC*-SF/LR and *capC*-LF/SR. The correct Δ*capC* strains were screened on 20% sucrose plates and validated by utilizing four primer pairs: *capC*-SF/*capC*-LR, *capC*-LF/*capC*-SR, *capC*-SF/*capC*-SR, *capC*-LF/*capC*-LR.

2.4 Effects of *capC* gene deletion on phenotype of *P. marina*

The experimental methods referred to Zeng et al. (Zeng et al., 2017) and Hu et al. (2021). *P. marina* and Δ*capC* strains were cultivated in Zobell 2216E for 16 - 18 h (200 r/min, 25°C), diluted and coated the bacterial fluid on Zobell 2216E solid plate according to concentration gradient, and then cultured at 25°C for 2 - 5 days for observation.

2.5 Biofilm formation of experimental strains

The wild-type and Δ*capC* biofilms were produced in accordance with previous studies (Wang et al., 2012). The culture conditions of *P. marina* and Δ*capC* strains used to form biofilms were identical to those described previously (Peng et al., 2020). Bacterial cells were obtained by centrifuging at 1600 × g for 15 min, washed with autoclaved filtered seawater (AFSW) and diluted to 50 mL. According to the obtained bacterial density, the corresponding amount of bacterial solution was added to a sterile petri dish (64.0 mm (Φ) × 19.0 mm) which contained sterile glass slide (12.7 mm × 38.1 mm), and the AFSW was added to obtain 1×10^8, 3×10^8, 5×10^8, and 1×10^9 colony-forming unit (CFU) mL^{-1} as initial concentrations. The culture dishes were placed at 18°C for 48 h to develop biofilms.

2.6 Cell density of biofilms

The pre-treatment steps of the glasses used to calculate the bacterial density of the biofilm were identical to those described previously (Peng et al., 2020). Briefly, after the biofilms were soaked in 5% formaldehyde solution for 24 h, then rinsed with aseptic saline (0.9% NaCl) once, and stained with acridine orange solution (0.1%) for 5 min. There were 10 fields of view (random observation) were selected under a microscope at 1000 × magnification to calculate the final density of biofilms, and each biofilm pattern contained three duplicates.

2.7 Settlement and metamorphosis bioassays

With regard to detecting whether the larvae have the ability of settlement and metamorphosis, 10^{-4} mol L^{-1} of epinephrine (EPI) was used as inducers (Yang et al., 2013). The biofilm slide and 20 larvae were added into a sterile glass Petri dish equipped with 20 mL of AFSW, and the slides without biofilms were built as blank control. Nine repetitions were operated in each test group. After being placed at 18°C without light, the number of post-larvae was recorded at 24, 48, 72, and 96 h.

2.8 Effects of *capC* gene deletion on growth ability of *P. marina*

The growth of *P. marina* and Δ*capC* strains was measured by turbidimetry. The two strains were grown overnight for 16 - 18 h. When the OD_{600} absorbance reached 1.0, 5 μL of bacterial solution was sucked into 5 mL of culture medium respectively (200 r/min, 25°C). Then the OD_{600} absorbance of 2, 4, 6, 9, 12, 15, 18, and 24 h was measured by a spectrophotometer, respectively.

2.9 Swimming motility assay

P. marina and Δ*capC* strains were grown for 16 -18 h in a table concentrator (200 r/min, 25°C). To observe the swimming trajectory, the bacterial solution (1 μL) from *P. marina* and Δ*capC* strains were pointed on the swimming medium (Marine Broth 2216E medium, 0.3% agar), respectively, and kept at 25°C for 18 h. The test consisted of 9 iterations per strain.

2.10 The thickness of biofilms

The morphology of biofilm cells was maintained by 5% formalin solution (30 min). After being dyed with iodide solution (5 g/L) for 20 min in complete darkness, the stained biofilms were washed three times with 0.9% NaCl. To determine the thickness of biofilms, the confocal laser scanning microscopy (CLSM) images were conducted using Leica TCS SP8 with LAS X software. The confocal microscope and software were set to objective magnification of 63 ×, z-step of 0.2 μm and pixels of 1024×1024. Imaging analysis was performed on 9 randomly chosen fields of view.

2.11 Extraction of bacterial CPS

The method of bacterial CPS extraction was identical to that of a previous study (Zhang et al., 2013; Zhang et al., 2017; Lin et al.,

2018). Typically, 4 mL of sterile saline was added to each Zobell 2216E agar medium to dissolve the bacterial cells. Bacteria from 24 plates were collected for each strain. The bacterial suspension was centrifugated for 30 min at 12000 × g, then the bacterial sediments were collected for resuspended in 30 mL of sterile buffered saline (10 mM EDTA, 0.9% NaCl). After stored at 4°C for 2 h, the bacterial suspension was centrifugated for 30 min at 12000 × g at 4°C. To eliminate bacteria from the supernatant, the 0.22 μm vacuum filters were utilized to filter the liquid. Next, the filtration liquid was concentrated through 10 kD ultrafiltration and 3 times the volume of anhydrous ethanol was added to the mixture. After cooled at 4°C for 12 h, the mixture was centrifugated (4°C, 1800 × g) for 30 min to remove nucleic acid impurities. Then added 4-times the volume of anhydrous ethanol into the supernatant, and stored at 4°C for 24 h. Next, the suspension was centrifugated for 1h (4°C, 1800 × g), and the polysaccharide precipitation was collected and washed with anhydrous ethanol and acetone twice, respectively. After lyophilized, the polysaccharide was dissolved into a 5% aqueous solution and 1/3 volume of Sevage (Chloroform: N-butanol = 4: 1, v/v) was added. After shaking for 25 min, the mixture was centrifuged for 10 min (4°C, 1800 × g), then the protein precipitation was discarded. The supernatant was dialyzed for 48 h and freeze-dried to obtain CPS.

2.12 Quantification of bacterial CPS and c-di-GMP

The bacterial CPS quantification method was identical to that of a previous study (Zhang et al., 2017), and the extracted CPS powder was dissolved in 5 mL sterilized distilled water to form a stock solution. The stock solution was diluted 10 folds with distilled water as a test solution. Then, 1 mL distilled water, 1 mL 3% phenol and 5 mL sulfuric were successively added into a sterile tube containing 1 mL test solution. And 2 mL of distilled water served as a blank control. The samples were allowed to react for 20 min at 80°C. Then, the samples were kept at 25°C, and the OD_{485} values of samples were detected. Three replicates were performed for each sample. The value of the A485 was calculated using a glucose concentration standard curve to calculate the content of the CPS in the sample. The c-di-GMP content was quantitatively analyzed by LC-MA/MS with reference to Peng et al. (2020).

2.13 Analysis of biofilm exopolysaccharide content scanning by CLSM

Biofilms of experimental strains were dyed as in a previous study (Peng et al., 2020; Hu et al., 2021). Confocal images of the processed samples were acquired using CLSM and LAS X software with the same conditions as described in 2.10. Three replicate samples were prepared for wild-type and mutant biofilms, respectively. Each experimental sample consisted of three randomly chosen fields of vision. The Image J software (NIH, Madison, WI, USA) was utilized to analyze confocal images and calculate the content of polysaccharides in extracellular products of biofilms.

2.14 Data statistical analysis

Data analysis was carried out using JMP™ software (Liang et al., 2021).

3 Results

3.1 Effects of *capC* deletion on phenotype and growth ability of *P. marina*

To understand the effect of *P. marina* gene *capC* on settlement and metamorphosis of *M. coruscus* larvae, a mutant with deletion of CPS synthesis gene was created (Figure 1A). The phenotypic observation revealed that the formats of the wild-type and Δ*capC* strains were smooth and circular (Figures 1B, C). Compared with *P. marina*, the growth of Δ*capC* strain increased significantly during 3 – 12 h ($p < 0.05$, Figure 1D). When it entered the stationary phase, the growth situation of the two strains was similar.

3.2 Δ*capC* strain reduced larval settlement

The results demonstrated that the inductive ability of Δ*capC* biofilms to *M. coruscus* larvae was significantly reduced at each initial bacterial concentration ($p < 0.05$). Compared with wild-type biofilms, the largest reduction was 23.1% ($p < 0.05$, Figure 2A), whereas the bacterial cell density of Δ*capC* biofilms did not differ significantly ($p > 0.05$, Figure 2B). There was no significant correlation between biofilm-inducing activity and bacterial density of biofilms ($p > 0.05$, Table S1).

3.3 Effects of *capC* deletion on swimming motility and biofilm thickness

The swimming motility of Δ*capC* strain was not significantly different from *P. marina* by observing (Figure 3A) and calculating the formation of the migration zone ($p > 0.05$, Figure 3B).

The CLSM scanned data indicated that the bacterial aggregation of *P. marina* and Δ*capC* biofilms was similar (Figures 4A, B). Biofilms were formed separately by the two strains with the initial density of 5×10^8 cells mL^{-1} ($p > 0.05$, Figure 4C). The rate of post-larvae showed no significant correlation with the thickness of biofilms ($p > 0.05$, Table S1).

3.4 Comparison of content of bacterial CPS extraction and c-di-GMP

According to the results of the bacterial CPS analysis, the Δ*capC* strain generated significantly less CPS (38.07% decrease) than *P.*

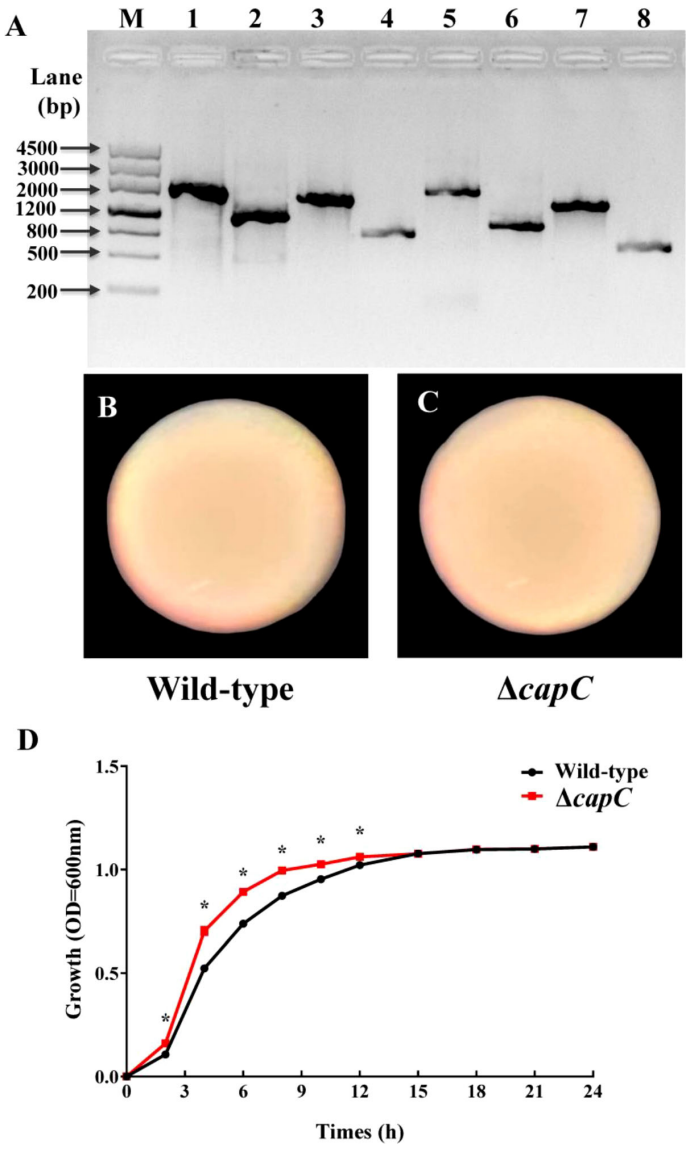

FIGURE 1
Construction of Δ*capC* strain and the colony morphology and growth of wild-type and Δ*capC* strains. **(A)** PCR verified the deletion of the *capC* gene. M: DNA Maker III; 1: *capC*-LF/LR, 1 835 bp; 2: Δ*capC*-LF/LR, 1 106 bp; 3: *capC*-LF/SR, 1 396 bp; 4: Δ*capC*-LF/SR, 667 bp; 5: *capC*-SF/LR, 1 600bp; 6: Δ*capC*-SF/LR, 871 bp; 7: *capC*-SF/SR, 1 161 bp; 8: *capC*-SF/SR, 435bp. The orf (open reading frame) length of *capC* is 729 bp. **(B, C)** Bacterial colony characteristics of experimental strains. **(D)** Growth curve of experimental strains at 25°C. The * indicates a significance level of $p < 0.05$. The same as below.

marina ($p < 0.05$, Figure 5A). The CPS content significantly correlated with the inducing capacity of biofilms on the settlement and metamorphosis rate (r = 0.592, $p < 0.05$, Table S1). The results of bacterial c-di-GMP analysis showed that the c-di-GMP produced by Δ*capC* strain was significantly higher than *P. marina*, and the increase was 1.94 times higher ($p < 0.05$, Figure 5B).

3.5 CLSM images of ΔcapC biofilms exopolysaccharide

CLSM scanned images exhibited that the production of exopolysaccharide was different between Δ*capC* biofilms and wild-type biofilms (Figure 6A). There was also a significant difference in biomass between the two strains ($p < 0.05$, Figure 6B). The content of α-polysaccharides and β-polysaccharides produced by Δ*capC* biofilms was 35.93% and 35.85% less than that in biofilms of *P. marina*, respectively ($p < 0.05$, Figure 6B).

4 Discussion

Marine invertebrates and marine microorganisms are crucial components of marine ecosystems (Falkowski, 1998; Paredes et al., 2021), and participate in the maintenance of marine biodiversity. Many studies have shown that the larval settlement and metamorphosis of various marine invertebrates are influenced by the chemical cue on marine biofilms (Dworjanyn and Pirozzi, 2008; Tamburri et al., 2008; Webster et al., 2011; Whalan and Webster, 2014). CPS, as a bacterial polysaccharide, has been demonstrated to

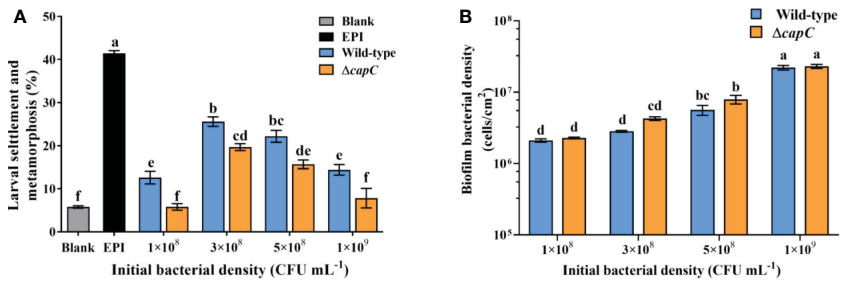

FIGURE 2
The biofilms of Δ*capC* reduced the inducing activity. **(A)** The effects of biofilms generated by experimental strains on larval settlement and metamorphosis (96h). **(B)** Biofilm density produced by experimental strains with diverse initial CFU.

affect the formation of bacterial biofilms (Petruzzi et al., 2017; Eberle et al., 2020), whether CPS synthesis gene regulates biofilm formation of marine bacterial biofilm and larval settlement and metamorphosis is still undefined. Here, a *capC* deletion strain of *P. marina* was created, and it was found that the Δ*capC* strain with lower CPS production and lower exopolysaccharide on Δ*capC* biofilms significantly prevented mussel settlement and metamorphosis. This revealed that *capC* gene regulates the production of bacterial CPS, which participate in larval settlement and metamorphosis (Figure 7).

CPS is a major structural component outside the bacterial cell wall, appearing in many Gram-positive bacteria and Gram-negative bacteria (Whitfield, 2006; Cuthbertson et al., 2009). The general biosynthesis pathway of CPS is wzy dependent pathway, monosaccharide passes through the flipper enzyme to the outer periplasmic, and the high-level polymerization of CPS requires the alternation of phosphorylation of tyrosine kinase and homologous phosphatase (Wugeditsch et al., 2001; Hagelueken et al., 2009). It is reported that in *E. coli*, tyrosine kinase wzc and homologous phosphatase wzb have been proved to be pivotal genes during CPS synthesis and assembly (Wugeditsch et al., 2001; Cuthbertson et al., 2009). In *Streptococcus Pneumoniae*, the *cpsCD* complex is considered to be similar to *wzc*, and phosphatase *cpsB* is its corresponding homologous phosphatase (Grangeasse et al., 2007). In this study, homologous sequence alignment found that the *capC* gene of *P. marina* was similar to *cpsB*, and the deletion of *capC* gene led to a significant reduction in CPS content. Thus, it can be speculated that the *capC* gene plays a role in homologous phosphorylation in the *P. marina* CPS synthesis.

Previous studies have shown that the deletion of CPS synthesis gene can promote the formation of biofilms (Petruzzi et al., 2017; Eberle et al., 2020). The deletion of *wza* gene of *V. vulnificus* CPS transfer protein caused that the CPS in bacterial capsule cannot be secreted to extracellular surface, and the formation of biofilms was significantly increased. (Wright et al., 1999; Wright et al., 2001). During the formation of *S. Pneumoniae* bacterial biofilm, the decrease of *cps3A* gene expression led to the reduction of CPS content and promoted the formation of biofilms (Hall-Stoodley et al., 2008). Nevertheless, this study showed that the deletion of *capC* gene of *P. marina* did not alter the final growth ability, motility, colony morphology and bacterial density of biofilms, as well as biofilm thickness. It was showed that the *capC* gene of *P. marina* did not influence the ability of biofilm formation. Considering previous studies, it was speculated that the function of CPS synthesis gene is different in various bacteria. This situation may be caused by various bacterial characteristics and living environments. Furthermore, the analysis of CLSM images confirmed that the deletion of *capC* gene of *P. marina* resulted in a significant decline in α-polysaccharides and β-polysaccharides of biofilms. These findings showed that the effects of capsular polysaccharide biosynthesis genes on biofilm formation ability were different from those found in previous studies, the deletion of *capC* gene in this study only altered the exopolysaccharide secretion of *P. marina* biofilms, without affecting the ability of bacteria to aggregate.

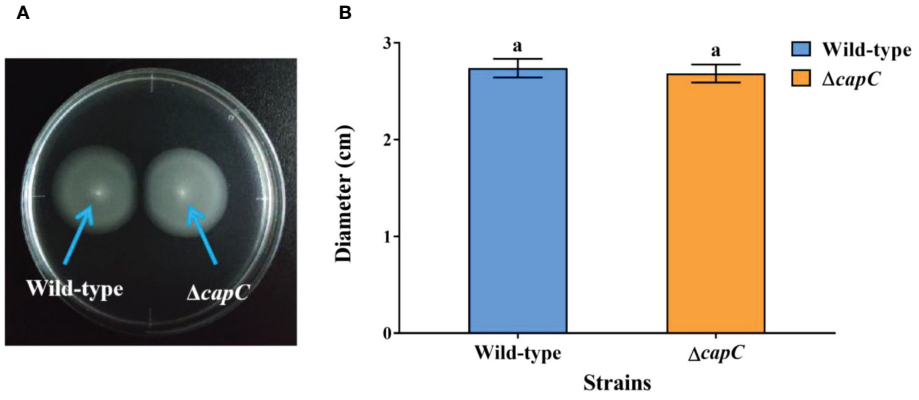

FIGURE 3
The swimming motility of experimental strains. **(A)** The swimming motility phenotypes of *P. marina* and Δ*capC* strains. **(B)** The migration zone diameter of *P. marina* and Δ*capC* strains.

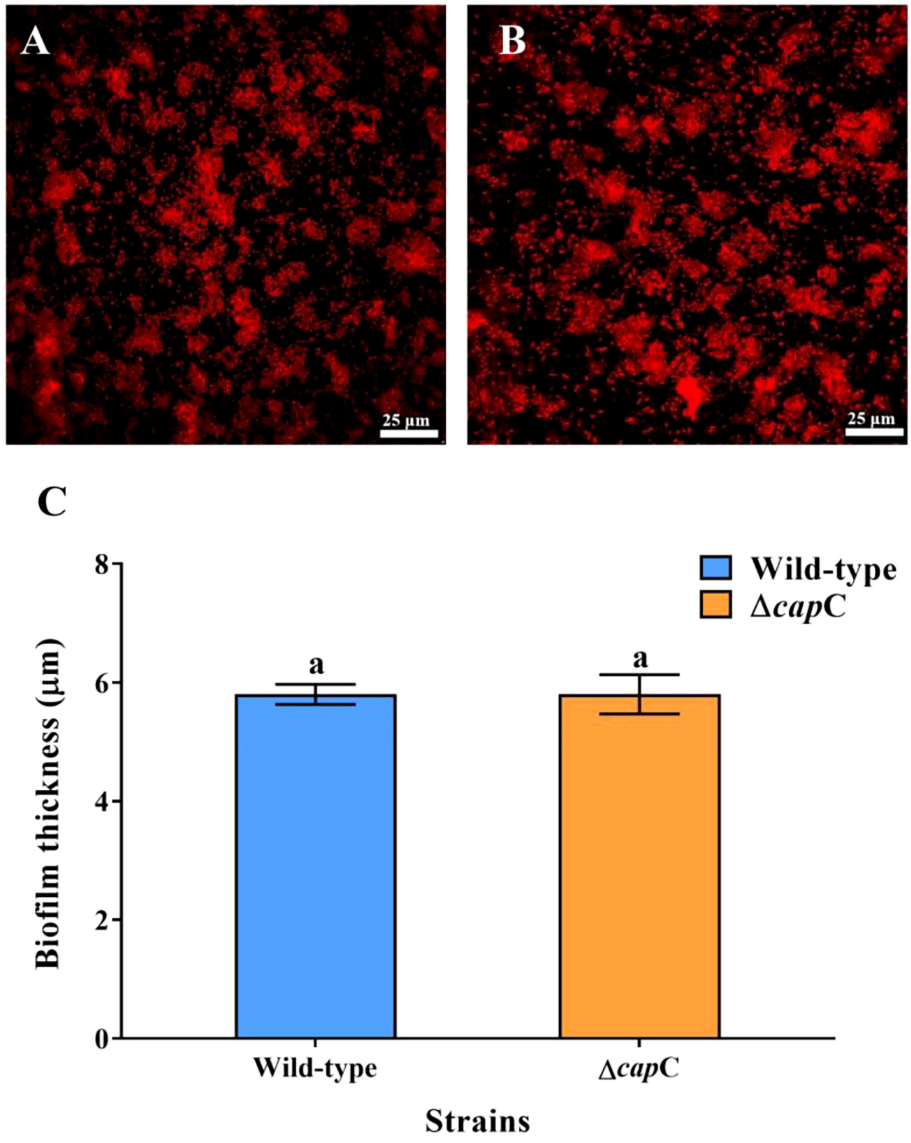

FIGURE 4
Biofilm-forming ability of experimental strains. **(A, B)** The CLSM images of wild-type and Δ*capC* biofilms. **(C)** Quantitative results of biofilm thickness of wild-type and Δ*capC* biofilms.

Exopolysaccharide is the main component of bacterial biofilms, and it has great biological activity (Sutherland, 2001; Flemming et al., 2016; Moradali and Rehm, 2020). Many studies have found that a variety of exopolysaccharides in biofilms can regulate the larval settlement and metamorphosis in marine invertebrates (see Table S2) (Kirchman et al., 1982; Liang et al., 2021; Freckelton et al., 2022). The settlement and metamorphosis of *Janua brasiliensis* larvae is thought to be caused by the

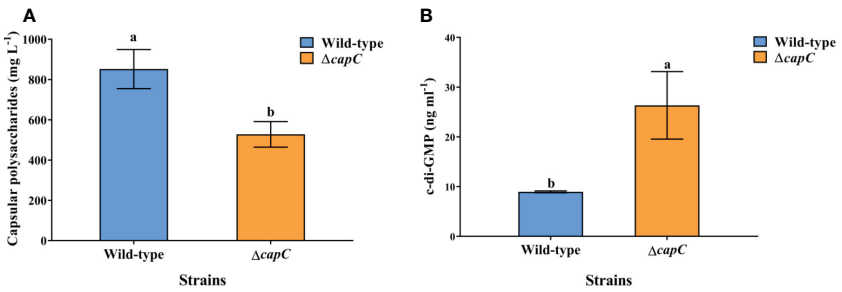

FIGURE 5
Difference in CPS secretion **(A)** and c-di-GMP **(B)** of experimental strains.

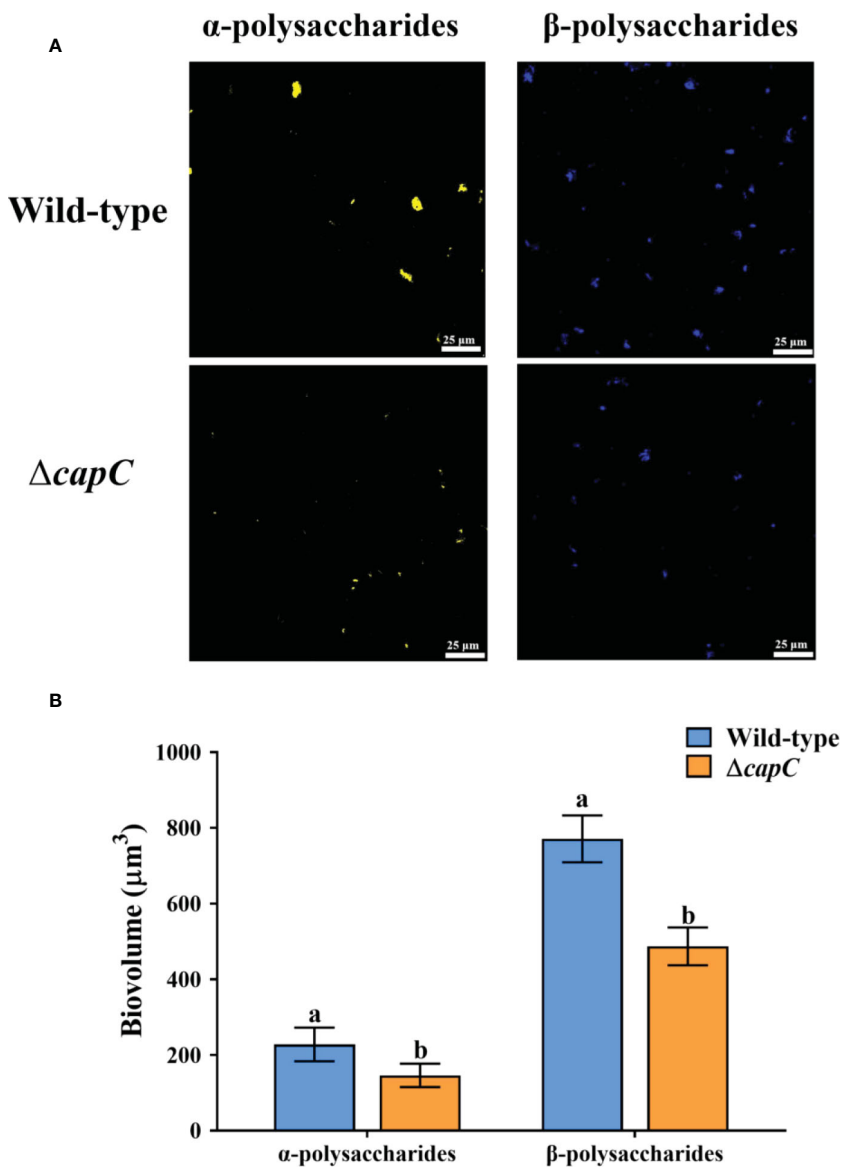

FIGURE 6
The CLSM analysis of exopolysaccharide in wild-type and Δ*capC* biofilms. **(A)** Distribution of extracellular polysaccharides in two experimental biofilms. **(B)** Analysis of exopolysaccharide.

associated exopolysaccharides on the bacterial surface, rather than the water-soluble metabolites of the bacteria (Kirchman, 1982). Szewzyk et al. (1991) found that the decrease in the content of exopolysaccharide in *Pseudomonas* sp. (strain S9) reduced the settlement response of the larvae of *C. intestinalis*. Holmström et al. (1992) predicted the involvement of exopolysaccharide in larval settlement by analyzing the effects of 40 strains of marine bacteria on *Balanus amphitrite* and *Ciona integrinalis* (Holmström et al., 1992). And in the mutant strain of *Pseudoalteromonas lipolytica AT00-17125* gene, the bacterial capsule became thinner, the content of colanic acid decreased, and significantly reduced the inducing activity on *M. coruscus* (Zeng et al., 2015). These above studies indicated that different polysaccharides have different inducing effects on the settlement and metamorphosis of marine invertebrate larvae.

This current investigation demonstrated that the knockout of CPS synthesis gene *capC* caused the increasing the bacterial c-di-GMP and reduction of the secretion of CPS and exopolysaccharide of biofilms, and the inducing activity of Δ*capC* biofilms to *M. coruscus* larvae decreased significantly. Our previous work also found that bacterial colanic acid concentration nearly quadrupled after deleting gene *01912* associated to polysaccharide biosynthesis, accompanied with increasing c-di-GMP caused the significant upregulation in mussel larval settlement and metamorphosis (Peng et al., 2020). In addition, knockout cellulose synthesis gene *bcsQ* caused significantly a reduction in exopolysaccharide (including cellulose) and c-di-GMP, and inhibited the settlement of *M. coruscus* larvae (Liang et al., 2021). Thus, these findings suggested that bacterial c-di-GMP mediates polysaccharide secretion and subsequent changed the inducing capability of biofilm on larval recruitment. Further molecular basis is needed.

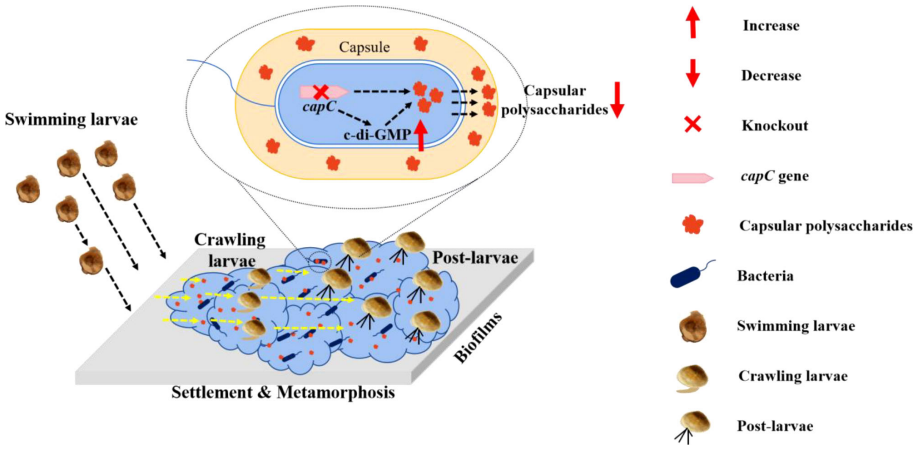

FIGURE 7
Model of *capC* regulation on larval settlement and metamorphosis: the capsular polysaccharide synthesis gene could modulate bacterial c-di-GMP which regulates the secretion of capsular polysaccharide, and then impact mussel larval settlement and metamorphosis. Once *capC* is deficient, bacterial biofilm biomass including cell density and thickness shows no change and capsular polysaccharide secretion is reduced accompanied with upregulation of c-di-GMP.

5 Conclusion

The present findings indicated that deletion of the gene *capC* in *P. marina* bring about the decrease in exopolysaccharides and CPS of biofilms, upregulation of bacterial c-di-GMP and significantly lower levels of biofilm-inducing activity. Thus, the CPS synthesis gene could modulate mussel recruitment *via* c-di-GMP and CPS. This study contributes a fresh insight to our understanding of the molecular processes that lead to the production of bacterial exopolysaccharides and recruitment of marine invertebrates.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by The Experimental Animal Ethics Committee of Shanghai Ocean University.

Author contributions

J-LY and XL: supervision, funding acquisition, conceptualization, validation, data curation, writing original draft preparation, writing review and editing, visualization. C-HH and WZ: methodology, software, formal analysis, investigation, data curation, writing original draft preparation. X-MH: methodology, formal analysis, data curation, writing original draft preparation. AY and KO: writing original draft preparation, writing review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1089024/full#supplementary-material

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