Effects of \( \text{cpxR} \) on the growth characteristics and antibiotic production of \( \text{Xenorhabdus nematophila} \)

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Summary

\( \text{CpxR} \) is a global response regulator that negatively influences the antimicrobial activities of \( \text{Xenorhabdus nematophila} \). Herein, the wildtype and \( \Delta \text{cpxR} \) mutant of \( \text{X. nematophila} \) were cultured in a 5-l and 70-l bioreactor. The kinetic analysis showed that \( \Delta \text{cpxR} \) significantly increased the cell biomass and antibiotic activity. The maximum dry cell weight (DCW) and antibiotic activity of \( \Delta \text{cpxR} \) were 20.77 ± 1.56 g L\(^{-1}\) and 492.0 ± 31.2 U ml\(^{-1}\) and increased by 17.28 and 97.33% compared to the wildtype respectively. Xencoumacin 1 (Xcn1), a major antimicrobial compound, was increased 3.07-fold, but nematophin was decreased by 48.7%. In 70-l bioreactor, DCW was increased by 18.97%, while antibiotic activity and Xcn1 were decreased by 27.71% and 11.0% compared to that in 5-l bioreactor respectively. Notably, pH had remarkable effects on the cell biomass and antibiotic activity of \( \Delta \text{cpxR} \), where \( \Delta \text{cpxR} \) was sensitive to alkaline pH conditions. The optimal cell growth and antibiotic activity of \( \Delta \text{cpxR} \) occurred at pH 7.0, while Xcn1 was increased 5.45- and 3.87-fold relative to that at pH 5.5 and 8.5 respectively. These findings confirmed that \( \Delta \text{cpxR} \) considerably increased the biomass of \( \text{X. nematophila} \) at a late stage of fermentation. In addition, \( \Delta \text{cpxR} \) significantly promoted the biosynthesis of Xcns but decreased the production of nematophin.

Abbreviations

\( \pi \) average specific cell growth rate (h\(^{-1}\))

\( \gamma_p \) average specific antibiotic production rate (U mg\(^{-1}\) h\(^{-1}\))

\( \gamma_s \) average specific glucose consumption rate (h\(^{-1}\))

DCW dry cell weight (g L\(^{-1}\))

DO dissolved oxygen (%)

\( P_{\text{max}} \) maximal antibiotic activity (U ml\(^{-1}\))

\( P_p \) antibiotic productivity (U ml\(^{-1}\) h\(^{-1}\))

\( P_X \) cell production rate (g L\(^{-1}\) h\(^{-1}\))

\( q_p \) specific antibiotic production rate (U mg\(^{-1}\) h\(^{-1}\))

\( q_s \) specific glucose consumption rate (h\(^{-1}\))

\( X_{\text{max}} \) maximal cell density (g L\(^{-1}\))

\( Y_{P/S} \) antibiotic yield of glucose (U g\(^{-1}\))

\( Y_{P/X} \) antibiotic yield on the cell (U g\(^{-1}\))

\( Y_{X/S} \) cell yield on glucose (g g\(^{-1}\))

\( \mu_{\text{max}} \) maximal specific cell growth rate (h\(^{-1}\))

\( \mu \) specific cell growth rate (h\(^{-1}\))
Introduction

Xenorhabdus nematophila is a unique Gram-negative bacterium that develops a mutualistic association with an infective dauer juvenile (IJ) insect-pathogenic nematode in the genus Steinernema (Thomas and Poinar, 1979). Recent research has revealed X. nematophila to be a rich source for natural products with potent biological or pharmaceutical activities (Challinor and Bode, 2015; Bozhiiyuk et al., 2016; Tobias et al., 2017). In X. nematophila ATCC 19061, 7.5% of the genome encoding proteins is involved in secondary metabolism; however, the majority of these encoded molecules are cryptic (Chaston et al., 2011). Until now, X. nematophila has been known to produce several secondary metabolites, including indole derivatives (Paul et al., 1981; Sandar and Chang, 1993; Li et al., 1995), nematophin (Li et al., 1997), insecticidal proteins (Morgan et al., 2001; Sergeant et al., 2003; Yang et al., 2009; Sheets et al., 2011; Hinchliffe, 2010), benzylidenacetone (Ji et al., 2004), xenocoumacins (Xcns) (McInerney et al., 1991) and the major class of non-ribosomal produced secondary metabolites (Crawford et al., 2011). Due to structural diversity, these metabolites display a wide range of bioactivities with potential interest for pharmaceutical, agricultural and chemical applications. These naturally occurring antibiotics and their derivatives may provide beneficial leads in the future research and manufacturing of agrochemicals.

Nematophin is a 3-indoleethyl 3’-methyl-2’-oxopenta- namide consisting of an N-terminal α-keto acid and a C-terminal tryptamine (TRA), first isolated from strain X. nematophila BC1. It displays strong antifungal and antibacterial activities (McInerney et al., 1991; Kennedy et al., 2000). A monomodular non-ribosomal peptide synthetase (NRPS), RdpD, is responsible for the biosynthesis of nematophin (Cai et al., 2017). Xenocoumacins, including Xcn1 and Xcn2, are the principal antimicrobial compounds secreted by X. nematophila. Xcn1 exhibits marked anti-microbial activity against Gram-positive bacteria and fungal strains, while Xcn2 shows substantially reduced bioactivity (McInerney et al., 1991; Huang et al., 2005, 2006; Yang et al., 2011; Zhou et al., 2017). Meanwhile, it has been demonstrated that fourteen genes (xcnA-xcnN) are involved in the synthesis of Xcns in X. nematophila. The genes xcnA-L are responsible for Xcn1 synthesis, whereas the genes xcnM and xcnN control the conversion of Xcn1 into Xcn2 (Park et al., 2009). A prominent increase in Xcn1 along with a 20-fold reduced cell viability following attenuated Xcn2 production suggests that the conversion of Xcn1 to Xcn2 is a resistance mechanism to circumvent self-toxicity (Park et al., 2009).

The rapid bacterial adaptation ability to changing environmental conditions is crucial for their growth and pathogenicity. Reports have shown that the growth of X. nematophila occurs in the nematode vesicle, a stressful and nutrient-deficient environment. Nevertheless, X. nematophila is speculated to encounter a relatively nutrient-rich environment, when released from nematode vesicles to insect haemolymph (Goodrich-Blair and Clarke, 2007; Herbert and Goodrich-Blair, 2007). In response to nutrient upshift, the bacteria grow rapidly and produce several metabolites to overcoming the insect immune system (Forst and Nealson, 1996) and preventing the growth of various bacterial and fungal competitors (Akhurst, 1982; Chen et al., 1994). Several reports confirmed that the antimicrobial activity of X. nematophila varies according to the environment conditions (Chen et al., 1996; Wang et al., 2008a,b, 2010, 2011). However, it remains unclear how X. nematophila recognizes these changes or how these signals are associated with the antimicrobial activity. Increasing evidence indicates that two two-component systems (CpxRA and EnvZ/OmpR) are involved in responding to both of the nematode and insect environments (Park and Forst, 2006; Herbert et al., 2007; Tran and Goodrich-Blair, 2009; Tran and Goodrich-Blair, 2009).

As a global regulator, OmpR also represses antibiotic production, in ΔompR mutant strain, Xcn1 level and expression of xcnA–L were increased, while Xcn2 level and xcnMN expression were decreased (Park and Forst, 2006; Park et al., 2009). Interestingly, CpxR also exhibits a similar regulatory mechanism for Xcns biosynthesis as OmpR. In ΔcpxR mutant strain, the levels of xcnA–L expression were amplified, while xcnMN expression was diminished (Tang, 2015). However, to date, there is no report concerning the effects of CpxR on the growth characteristics and antibiotics biosynthesis potential of X. nematophila. Therefore, the present study investigated the effects of CpxR on the antibiotics production and growth of X. nematophila. The kinetics analysis of batch fermentation of the wildtype and ΔcpxR mutant of X. nematophila YL001 was carried out in a 5-l laboratory-scale bioreactor. Specifically, we tested whether CpxR influenced the production of nematophin and Xcns.

Results

Effects of cpxR disruption on cell growth and antibiotics production

The batch fermentation kinetics of the wildtype and ΔcpxR of X. nematophila YL001 were studied in a 5-l bioreactor. Evidently, a distinct exponential and stationary
phase was observed from the bacterial growth curve (Fig. 1A). During 0–36 h of the bioprocess, the DCW of the wildtype strain was higher than that of ΔcpxR; however, after 36 h, the DCW of ΔcpxR was found to be higher in comparison with the wildtype strain. Due to the rapid growth of wildtype compared with ΔcpxR at earlier fermentation stage, DO concentration and pH decreased rapidly (Fig. 2). The maximum DCW of the wildtype and ΔcpxR showed an optimal value of 17.71 ± 1.35 and 20.77 ± 1.56 g L⁻¹, respectively, when fermentation proceeded to the stationary phase, where the DO concentration was 17.8 and 3.5% respectively (Figs 1A and 2). However, the maximum specific cell growth rate ($\mu_{\text{max}}$) of ΔcpxR was 0.099 ± 0.007 h⁻¹ and found to be lower compared with the wildtype strain (Fig. 1B). The cell yields on glucose ($Y_{X/S}$) and cell production rate ($P_X$) of ΔcpxR were higher than that of the wildtype strain (Table 1). It is obvious that ΔcpxR mutation favoured the cell production. In consonance with diminishing cell biomass of ΔcpxR during 0–36 h, the consumption of glucose was declined.

During the bioprocess, ΔcpxR showed a significantly higher ($p < 0.05$, $t = 43.33$, $d_f = 10$) antibiotic activity (Fig. 1C). The maximum antibiotic activity (492.0 ± 31.2 U ml⁻¹) of ΔcpxR, which was increased by 97.33% compared with the wildtype (249.33 ± 15.23 U ml⁻¹), was achieved at 60 h. Profiles of the specific antibiotic production rate ($q_p$) of the wildtype strain and ΔcpxR showed identical trends. The time duration to reaching the maximum $q_p$ was similar, whereas the declining rate was found to be different after $q_p$ reached the maximum (Fig. 1D). The maximum $q_p$ of the wildtype and ΔcpxR was 16.35 ± 1.23 and 42.87 ± 3.67 U mg⁻¹ h⁻¹ respectively. During the bioprocess, the average specific antibiotic biosynthesis rate of

![Figure 1](image-url)
Fig. 2. The variations in dissolved oxygen concentration and pH of wildtype and ΔcpxR mutant of Xenorhabdus nematophila YY001 during cultivation in the 5-l bioreactor.

Table 1. Batch fermentation parameters of wildtype and ΔcpxR of Xenorhabdus nematophila YY001 at pH 7.0 in 5- and 70-l bioreactor.

| Parameters      | 5 L         | ΔcpxR       | 70 L         |
|-----------------|-------------|-------------|--------------|
|                 | YY001       | ΔcpxR       | ΔcpxR        |
| X_max (g L⁻¹)   | 17.71 ± 1.35| 20.77 ± 1.56| 24.71 ± 2.31 |
| P_max (U ml⁻¹)  | 249.33 ± 15.23| 492.0 ± 31.20| 381.07 ± 15.74|
| P_x (g L⁻¹ h⁻¹) | 0.30 ± 0.05 | 0.35 ± 0.03 | 0.41 ± 0.06  |
| P_y (U ml⁻¹ h⁻¹) | 3.78 ± 0.28 | 8.20 ± 1.20a | 9.07 ± 2.53a |
| µ (h⁻¹)         | 0.107 ± 0.02 | 0.099 ± 0.007 | 0.035 ± 0.003b |
| π (h⁻¹)         | 0.037 ± 0.003 | 0.036 ± 0.004 | 0.021 ± 0.002b |
| Y_P/S (U mg⁻¹ h⁻¹) | 16.35 ± 12.3 | 42.87 ± 3.67a | 6.410 ± 0.89b |
| Y_P/X (U mg⁻¹ h⁻¹) | 2.29 ± 0.26 | 6.04 ± 1.25a | 1.348 ± 0.26a |
| Y_X/S (g g⁻¹) | 2.82 ± 0.08 | 3.05 ± 0.55a | 5.626 ± 1.06b |
| Y_P/S (U g⁻¹) | 55.05 ± 6.21 | 116.52 ± 9.35a | 87.32 ± 12.3 |
| Y_P/X (U g⁻¹) | 14.99 ± 1.36 | 23.69 ± 0.89a | 16.44 ± 1.42b |
| q_P (h⁻¹) | 0.187 ± 0.06 | 0.252 ± 0.03a | 0.132 ± 0.002b |
| q_S | 0.028 ± 0.004 | 0.036 ± 0.002a | 0.024 ± 0.003b |

Data are presented as a mean ± standard deviation from three replicated experiments.
a Followed by the data in the column indicates significant differences of ΔcpxR and YY001 in 5-l bioreactor at P = 0.05.
b Followed by the data in the column indicates significant differences of ΔcpxR between fermentation in 5- and 70-l bioreactor at P = 0.05.

the wildtype and ΔcpxR was 6.04 ± 1.25 and 2.29 ± 0.26 U mg⁻¹ h⁻¹ respectively. Similarly, the optimum antibiotic yield on glucose (YP/S), the antibiotic productivity (P_y) and the optimum antibiotic yield on cell (YP/X) of ΔcpxR, which were 116.52 ± 9.35 U g⁻¹, 8.20 ± 1.20 U ml⁻¹ h⁻¹ and 23.69 ± 0.89 U g⁻¹, respectively, were higher than that of the wildtype strain (Table 1). It can be concluded that ΔcpxR could significantly increase the antibiotic activity of X. nematophila.

Based on our findings that ΔcpxR mutant exhibited higher antibiotic activity against B. subtilis, we also investigated the variations of nematophin and Xcn1. At 24 and 48 h of the bioprocesses, ΔcpxR had no obvious (p > 0.05, t = 1.22, d_2 = 4, 24 h; t = −2.20, d_2 = 4, 48 h) effect on nematophin production, but at 72 h, the levels of nematophin in ΔcpxR were significantly decreased by 48.7% compared with the wildtype strain (Figs 3A and S1). In addition, Y_P/S, P_y, Y_P/X and q_P of nematophin in ΔcpxR were decreased by 41.12, 32.04, 45.68 and 45.67%, respectively, compared with the wildtype strain (Table 2). However, Xcn1 in ΔcpxR was 6.41-fold greater in contrast with the native strain (Fig. S2). These results indicate that CpxR positively regulates the production of nematophin while negatively regulates Xcn1 synthesis.

The rapid growth and increase in biomass of the wildtype at an early phase of fermentation resulted in the rapid decrease in DO concentration compared with ΔcpxR (Fig. 2). Correspondingly, glucose uptake of the wildtype was faster than ΔcpxR (Fig. 1E). At the late stage of fermentation, an increase in biomass of ΔcpxR (Fig. 1E). At the end of the fermentation, the glucose concentration in the broth of the strains was 1.51 and 1.76 g L⁻¹, and the total glucose consumption was 74.8 and 70.67%. Profiles of the specific glucose consumption rate (q_S) of the wildtype and ΔcpxR displayed comparable trends. The time duration of attaining the maximum q_S was identical, but a different decaying trend...
were observed after \( q_p \) reached the maximum (Fig. 1F). Notably, the maximum \( q_p \) and the average specific glucose consumption rate of the wildtype and \( \Delta cpxR \) showed a significant difference (Table 1).

**Effects of pH on the cell growth and antibiotics production of \( \Delta cpxR \)**

At different initial pH, cell growth was observed to be considerably changed with the pH operation applied (Fig. 4A). At pH 8.5, the presence of an obvious lag phase was characterized by a higher DO concentration at an early phase of fermentation (Fig. 5). During 0–42 h of the fermentation, the DCW was reduced at pH 8.5 in comparison with other pH conditions. After 48 h, the DCW was observed to be higher than that at pH 5.5 and lower than that at pH 7.0. The highest DCW of each original pH displayed an optimum value of 20.44 ± 2.65 g L\(^{-1}\) at pH 7.0, which was reduced by 31.5% and 21.14% at pH 5.5 and 8.5 respectively. The maximum specific cell growth rate (\( \mu_{\text{max}} \)) at pH 5.5, 7.0 and 8.5 was 0.065 ± 0.013, 0.065 ± 0.009 and 0.054 ± 0.011 h\(^{-1}\), and the average specific cell growth rate during the bioprocess was 0.030 ± 0.01, 0.030 ± 0.005 and 0.024 ± 0.007 h\(^{-1}\) respectively (Fig. 4B, Table 3). Likewise, the optimal cell yield on glucose (\( Y_{X/S} \)) and the maximum cell productivity (\( P_X \)), that is, which were 4.03 ± 1.25 g g\(^{-1}\) and 0.357 ± 0.005 g L\(^{-1}\) h\(^{-1}\) at pH 7.0, were higher than that at other pH conditions (Table 3). The optimum original pH for maximal cell proliferation of \( \Delta cpxR \) was found to be pH 7.0.

The influence of different pH on the antibiotic production potential of \( \Delta cpxR \) is portrayed in Fig. 4C. A higher antimicrobial activity was observed at pH 8.5 than that at pH 5.5 and 7.0 during the first 0–18 h. However, after 24 h of bioprocess, the antibiotic activity was significantly increased at neutral pH (pH 7.0) in contrast with pH 5.5 and 8.5. The maximum antibiotic activity (470.71 ± 15.67 U ml\(^{-1}\)), which was 28.68% and 27.67% higher compared with pH 5.5 and 8.5, was obtained at pH 7.0, where the maximum DCW was obtained. Profiles of the specific antibiotic production rate (\( q_p \)) exhibited similar trends at varying pH, and \( q_p \) reached the maximum value at 6 h of the bioprocesses. The highest \( q_p \) was achieved at pH 8.5 and reduced by 61.97% and 57.71% at pH 5.5 and 7.0 respectively (Fig. 4D, Table 3). Consistent with the \( q_p \) of \( \Delta cpxR \) at different pH, during the bioprocess, the \( P/S \) of \( \Delta cpxR \) at pH 8.5 was also significantly higher than that at pH 5.5 and 8.5 (Table 3). Similarly, the highest antibiotic yield on glucose (\( Y_{P/S} \)) and the antibiotic productivity (\( P_S \)) achieved at pH 7.0 were 92.79 ± 2.58 U g\(^{-1}\) and 6.54 ± 0.58 U ml\(^{-1}\) h\(^{-1}\) respectively. Nevertheless, the maximum antibiotic yield on the cell (\( Y_{P/X} \)) was 24.00 ± 1.09 U g\(^{-1}\) at pH 5.5 (Table 3). The findings inferred that among the different pH investigated, pH 7.0 was found to be promising for antibiotic biosynthesis due to the elevated cell growth at this pH.

The influence of varying pH on the biosynthesis of nematophin and Xcn1 was also determined. A noticeable effect (\( p < 0.05 \)) of different pH was observed in Xcn1 biosynthesis. At pH 7.0, Xcn1 production was 5.45- and 3.87-fold higher than that at pH 5.5 and 8.5 respectively (Table 4, Figs 3A and 3C). In addition, pH also had a significant effect on nematophin production (Fig. S5). In comparison with pH 5.5 and 7.0, the nematophin concentration was recorded to be significantly higher at pH
8.5. At 72 h, the concentration of nematophin under pH 8.5 was $156.25 \pm 3.67 \mu g \text{ ml}^{-1}$, 1.59- and 3.69-fold greater than that at pH 7.0 and 5.5. Likewise, the optimal $Y_{P/S}$, $P_{p}$, $Y_{P/X}$ and $q_p$ of nematophin at pH 8.5 were 86.71, 58.39, 101.04 and 101.49% higher compared with that at pH 7.0 (Fig. 3B, Table 2).

### Table 2. Parameters of the batch fermentation of nematophin produced by *Xenorhabdus nematophila* YL001 wildtype and ΔcpxR at different pH in 5- and 70-l bioreactor.

| Parameters | 5 L | 70 L |
|------------|-----|------|
|            | YL001 | ΔcpxR | pH 7.0 | pH 5.5 | pH 7.0 | pH 8.5 | pH 7.0 |
| Nematophin (μg ml$^{-1}$) | 148.06 ± 5.61 a | 42.30 ± 3.67 c | 100.67 ± 3.38 b | 156.2 ± 3.67 a | 72.85 ± 2.58* |
| $P_{p}$ (μg ml$^{-1}$ h$^{-1}$) | 2.06 ± 0.35 a | 0.59 ± 0.12 c | 1.40 ± 0.26 b | 2.17 ± 0.18 a | 1.01 ± 0.03* |
| $q_p$ (μg mg$^{-1}$ h$^{-1}$) | 0.127 ± 0.015 a | 0.59 ± 0.011 c | 1.40 ± 0.012 b | 2.17 ± 0.005 a | 0.042 ± 0.012* |
| $Y_{P/S}$ (μg g$^{-1}$) | 32.98 ± 2.58 b | 9.86 ± 1.67 d | 19.42 ± 2.31c | 36.26 ± 0.58 a | 16.70 ± 0.37* |
| $Y_{P/X}$ (μg g$^{-1}$) | 9.15 ± 1.32 a | 3.02 ± 0.85 c | 4.97 ± 0.78 b | 9.69 ± 1.35 a | 3.04 ± 0.79* |

Data are presented as a mean ± standard deviation from three replicated experiments. Different lowercase letters followed by the data in each row indicate significant differences at $P = 0.05$. ** followed by the data in the column indicates significant differences of ΔcpxR fermentation in pH 7.0 between in 5- and 70-l bioreactor at $P = 0.05$. 

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**Fig. 4.** Time profiles of ΔcpxR mutant of *Xenorhabdus nematophila* YL001 at different pH during cultivation in the 5-l bioreactor.  
A. Cell growth.  
B. Specific cell growth rate ($\mu$).  
C. Antibiotic activity.  
D. Specific antibiotic production rate ($q_p$).  
E. Glucose.  
F. Specific glucose consumption rate ($q_s$).  

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The relative amount of xenocoumacins (Xcn1 and Xcn2) at different pH in the 5-l bioreactor.

Table 3. Parameters of the batch fermentation of ΔcpxR mutant of Xenorhabdus nematophila YL001 at different pH in the 5-l bioreactor.

| Parameters     | ΔcpxR | pH 5.5 | pH 7.0 | pH 8.5 |
|----------------|-------|--------|--------|--------|
| $X_{max}$ (g L$^{-1}$) |      | 13.99 ± 1.38 c | 20.44 ± 2.65 a | 16.12 ± 0.57 b |
| $P_{max}$ (U ml$^{-1}$) |      | 335.71 ± 25.73 b | 470.71 ± 15.67 a | 340.48 ± 20.36 b |
| $P_{P}$ (g L$^{-1}$ h$^{-1}$) |      | 0.231 ± 0.03 b | 0.357 ± 0.005 a | 0.166 ± 0.02 b |
| $P_{C}$ (U ml$^{-1}$ h$^{-1}$) |      | 4.66 ± 1.13 b | 6.54 ± 0.58 a | 5.67 ± 1.67 ab |
| $\mu$ (h$^{-1}$) |      | 0.065 ± 0.013 a | 0.065 ± 0.009 a | 0.054 ± 0.011 ab |
| $\pi$ (h$^{-1}$) |      | 0.030 ± 0.01 a | 0.030 ± 0.005 a | 0.024 ± 0.007 b |
| $q_{c}$ (U mg$^{-1}$ h$^{-1}$) |      | 3.098 ± 0.556 b | 3.192 ± 0.796 b | 8.392 ± 1.875 a |
| $Y_{C/S}$ (g g$^{-1}$) |      | 3.26 ± 0.34 b | 4.03 ± 1.25 a | 3.74 ± 1.13 ab |
| $Y_{R/S}$ (U g$^{-1}$) |      | 78.28 ± 5.30 b | 92.79 ± 2.58 a | 76.18 ± 6.13 b |
| $Y_{P/S}$ (U g$^{-1}$) |      | 24.00 ± 1.09 a | 23.03 ± 3.24 a | 22.95 ± 0.67 a |
| $q_{s}$ (h$^{-1}$) |      | 0.241 ± 0.052 b | 0.247 ± 0.007 b | 0.314 ± 0.021 a |
| $q_{E}$ (h$^{-1}$) |      | 0.042 ± 0.006 a | 0.036 ± 0.013 a | 0.057 ± 0.015 a |

Data are presented as a mean ± standard deviation from three replicated experiments. Different lowercase letters followed by the data in each row indicate significant differences at $P = 0.05$.

Table 4. The relative amount of xenocoumacins (Xcn1 and Xcn2) in ΔcpxR of Xenorhabdus nematophila YL001 at different pH.

| pH | Relative amount of Xcn$^a$ | Xcn1 | Xcn2 |
|----|---------------------------|------|------|
| 5.5| 1 c                       | 1 b  |      |
| 7.0| 5.46 a                    | 1.06 b|      |
| 8.5| 1.41 b                    | 2.4 a |      |

$^a$ The peak area of extracted ion chromatogram (EIC) of Xcn at different pH/the peak area of extracted ion chromatogram (EIC) of Xcn at pH 5.5, the peak area was calibrated by its OD$_{600}$ value, and the relative amount of Xcn at pH 5.5 was referred to 1. Different lowercase letters followed by the data of Xcn1 and Xcn2 at different pH indicate significant differences at $P = 0.05$.

The rapid growth and rise in biomass at an early phase of fermentation at pH 7.0 resulted in the rapid decrease of DO concentration compared with that at pH 5.5 and 8.5 (Fig. 5). Consistently, glucose uptake at pH 7.0 was faster than that at pH 5.5 and 8.5 (Fig. 4E). Similarly, at the late stage of fermentation, a higher biomass at pH 7.0 resulted in the increased uptake of glucose. During 0–42 h of the fermentation, the total glucose consumption at pH 5.5, 7.0 and 8.5 was 76.0, 87.67 and 69.67% respectively. At the end of the fermentation, the glucose concentration was 1.71, 0.93 and 1.69 g L$^{-1}$ at pH 5.5, 7.0 and 8.5 respectively. However, the highest $q_{c}$ (0.314 ± 0.021 h$^{-1}$) was obtained at pH 8.5 and decreased by 30.29% and 27.13% at pH 5.5 and 7.0 respectively (Fig. 4D). The average specific glucose consumption rate during the bioprocess at pH 5.5, 7.0 and 8.5 was 0.042 ± 0.006, 0.036 ± 0.013 and 0.057 ± 0.015 h$^{-1}$ respectively (Table 3).

Batch fermentation characteristics of ΔcpxR mutation in the 70-l bioreactor

Based on the 5-l bioreactor-based fermentation results, the batch fermentation kinetics of ΔcpxR mutation was carried out in the 70-l bioreactor. The growth profile displayed a distinctive exponential and stationary phase, and antibiotic production occurred throughout the
bioprocess (Fig. 6). The maximum DCW and antibiotic activity ($P_{\text{max}}$) in 70-l bioreactor were $24.71 \pm 2.31 \text{ g L}^{-1}$ and $381.07 \pm 15.74 \text{ U ml}^{-1}$, which was increased by 18.97%, and decreased by 27.71% compared to that in 5-l bioreactor respectively. The concentration of nematophin ($72.85 \pm 2.58 \text{ mg ml}^{-1}$) produced by $\Delta cpxR$ at 72 h was decreased by 26.06% compared to the 5-l bioreactor. $Y_{P/S}$, $P_{D}$, $Y_{P/X}$ and $q_{p}$ of nematophin in 70-l bioreactor were decreased by 14.01, 26.28, 36.93 and 37.31% compared with that in the 5-l bioreactor (Fig. 3C, Table 2). Xcn1 of $\Delta cpxR$ was decreased by 11.0% relative to that in the 5-l bioreactor (data not shown).

**Discussion**

In contrast to the wildtype strain, $\Delta cpxR$ mutant exhibited a prolonged lag phase, and lower DCW at early- and mid-stage. These findings were consistent with the previous report, in which $cpxR$ inactivation results in a prolonged lag phase when cultivated in either LB culture or haemolymph (Herbert et al., 2007). At the late stage of fermentation, the DCW of $\Delta cpxR$ was enhanced relative to the wildtype, whereas, in the previous study, the cell biomass between a $cpxR$ deletion mutant and wildtype of *X. nematophila* had no significant difference (Herbert et al., 2007). The difference in growth profiles between the parent strain and $\Delta cpxR$ derivative may be associated with the rapid adaptability to fluctuating environmental perturbations. CpxRA could sense the changing environment and regulate the growth and metabolisms to adapt the environment of insect haemolymph (Herbert et al., 2007; Tran and Goodrich-Blair, 2009; Herbert et al., 2009). However, $\Delta cpxR$ mutant was defective in adapting to this nutrient source and displayed a protracted lag phase. This may be due to the lack of metabolism adaptability of $\Delta cpxR1$ mutant to the nutrients available in haemolymph but is more prone to insect antimicrobial activities (Herbert et al., 2007). Previously, it has been found that the $\Delta cpxR1$ exhibited a prolonged lag phase during *in-vitro* growth in haemolymph (Herbert et al., 2007), a finding consistent with the idea that it is defective in acclimatizing to this nutrient source.

Generally, antibiotic biosynthesis is induced at high cell density and is regulated, in most of the cases by quorum sensing (Romero et al., 2011). In this study, in all cases, the nematophin biosynthesis could be improved by raising the concentration of antibiotic-producing cells (Fig. 3). Li et al. (1997) and Park et al. (2009) reported comparable outcomes that the concentration of nematophin and Xcns increased by increasing the bacterial cell density and attained the maximal level at the stationary phase. However, in $\Delta cpxR$ mutant, the level of Xcn1 was increased significantly while nematophin was decreased during the stationary phase, where the DCW of $\Delta cpxR$ was higher. Our findings confirmed that quorum sensing is not an essential process to regulate the antimicrobial biosynthesis (Singh and Forst, 2016), and global regulators particularly govern the biosynthesis of small molecules in *X. nematophila* (Park et al., 2009; Engel et al., 2017). Therefore, identifying and engineering of these regulators can provide a noteworthy approach for the discovery of new secondary metabolites and increased production of useful molecules.

In this study, the $\Delta cpxR$ strain exhibited an increase in antibiotic activity. This suggested that the biosynthetic pathway of secondary metabolites was induced in $\Delta cpxR$. However, although the production of Xcn1 in...
ΔcpxR was 3.07-fold higher, nematophin production was significantly decreased. Meanwhile, we did not rule out the possibilities that the cpxR gene disruption could induce the production of previously cryptic metabolites, and the biological activities of these induced cryptic metabolites need to be further validated by purification and characterization. Taken together, the improved antibiotic activity of ΔcpxR might be attributed to the mutual effect of higher Xcn1 or the cryptic antibiotics produced in ΔcpxR strain.

In CpxRA signal transduction system, CpxA senses a variety of stresses and changes in external conditions, including pH, phosphorylates CpxR, and activates this response regulator to regulate the expression of its regulator (Herbert et al., 2007). Previously, we have demonstrated the significant influence of different pH on the growth profile and antibiotic biosynthesis titre of X. nematophila YL001 (Wang et al., 2011). However, it is uncertain whether CpxA can sense the pH changing and affect the growth and antibiotic production of cpxR-inactivated mutant. Our study indicated that pH had a marked consequence of the growth and production of antibiotic by ΔcpxR strain. The cell biomass, the maximum DCW and antibiotic production of ΔcpxR strain all were varied at different pH conditions. However, there was no significant difference in cell biomass of the wildtype at different pH conditions (Wang et al., 2011). The growth difference between the wildtype and ΔcpxR strain might be related to the hypersensitivity of ΔcpxR strain to alkaline conditions. Danese and Silhavy (1998) found that the cpxR null mutant of E. coli was the most hypersensitive to alkaline condition, and showed marked growth defects at an extreme alkaline pH. Also, the antibiotic production of ΔcpxR strain had a significant difference between pH 5.5, 7.0 and 8.5 (Table 1, Fig. S3). Our previous study revealed that antibiotic activity and the relative amount of Xcn1 of wildtype at pH 8.5 were higher relative to that at pH 5.5 and pH 7.0 (Guo, 2015). Moreover, in X. nematophila, CpxR and OmpR negatively regulate the production of Xcn1 (Park et al., 2009; Tang, 2015). Although CpxR can be activated by alkaline pH, it works as a CpxA-dependent response (Siryaporn and Goulian, 2008; Wolfe et al., 2008). At pH 8.5, the expression level of cpxR and ompR of wildtype of X. nematophila YL001 was significantly lower than that at pH 5.5 and 7.0 (Guo, 2015). Therefore, the Xcn1 levels of the wildtype were increased at pH 8.5. However, the ompR expression level of ΔcpxR strain at pH 8.5 was significantly greater than that at pH 5.5 and 7.0 (Tang, 2015). Thus, at pH 7.0 the ΔcpxR strain exhibited the higher levels of Xcn1 and antibiotic activity. The ability of ΔcpxR strain to sense the changes in pH, and regulating the growth and antibiotic production might be related to the cross-talk between a histidine kinase and a non-cognate response regulator of different two-component signalling systems. Siryaporn and Goulian (2008) detected the cross-talk between the histidine kinase CpxA and non-cognate response regulator OmpR in E. coli. Hence, we speculated that OmpR of ΔcpxR strain could be triggered by alkaline pH conditions.

Based on the scale-up method of a constant mass transfer coefficient, X. nematophila YL001 was successfully scaled-up from shaking flask to a 5-l bioreactor, and further to a 70-l bioreactor (Wang, 2004). In this study, according to the fermentation condition of X. nematophila YL001 in a 70-l bioreactor, ΔcpxR strain was cultured in a 70-l bioreactor. ΔcpxR strain exhibited the similar kinetics profiles, compared with fermentation in the 5-l bioreactor. However, the profiles of DO concentration of ΔcpxR strain in 5- and 70-l bioreactor had a significant difference (p < 0.05. t = 5.491, d = 16). In the 70-l bioreactor, the DO concentration was above 20% during the whole bioprocess but was maintained to around zero level in a growth phase in the 5-l bioreactor. The fermentation condition in 70-l bioreactor could ensure the sufficient supply of oxygen in the fermentation process that is favourable to both the cell growth and metabolic activity. So, the maximum DCW of ΔcpxR strain in the 70-l bioreactor was increased by 18.97%, but the maximum antibiotic activity was decreased by 27.71%, compared to that in the 5-l bioreactor. These findings were consistent with the results of scale-up of X. nematophila YL001 from a 5-l to the 70-l bioreactor. These showed that the fermentation conditions used in the 70-l bioreactor were beneficial for cell growth, but not favourable to the biosynthesis of antibiotics. Thus, the optimum fermentation condition for antibiotic production by ΔcpxR strain will be further investigated.

These findings confirmed that ΔcpxR mutation significantly increased the biomass of X. nematophila at a late stage of fermentation, and promoted the production of Xcns, but decreased the production of nematophin. In addition, pH had remarkable effects on the bacterial cell growth and antimicrobial activity of ΔcpxR mutant. Thus, this work provides an efficient way to obtain massive antimicrobial production of Xcns or nematophin in X. nematophila.

Experimental procedures

Microorganism

The X. nematophila wildtype strain was isolated from the soil in Yangling, China. The cpxR-null mutant strain (ΔcpxR) derived from YL001 by the allelic exchange that contains a kanamycin (Km) cassette instead of the cpxR gene was used (Tang, 2015). Phase I variant of X.
nematophila preserved in glycerol at −70°C was used as seed inoculum for cultures. NBTA NA medium supplemented with triphenyl tetrazolium chloride (0.040 g L⁻¹) and brothomyl blue (0.025 g L⁻¹) was employed to test the phase variant of X. nematophila.

Preparation of inoculum
A loopful of phase I of X. nematophila YL001 wildtype and ΔcpxR was inoculated into 250-ml Erlenmeyer flasks comprising 100 ml sterilized NB (NA without agar) medium with the final pH of 7.2. The flasks were then incubated in dark at 28°C in a temperature-controlled rotary shaker at an agitation speed of 150 rpm for 16 to 24 h.

Batch fermentation characteristics in the laboratory-scale bioreactor
Fermentation of ΔcpxR of X. nematophila YL001 was investigated in batch fermentation using a 5-l bioreactor (Eastbio, China). The pH, temperature, DO and agitation speed were monitored online by a computer-coupled system. The medium containing (g L⁻¹) glucose 6.13, peptone 21.29, KH₂PO₄ 0.86, MgSO₄·7H₂O 1.50, Na₂SO₄ 1.72, K₂HPO₄ 1.11 and (NH₄)₂SO₄ 2.46 was adjusted to pH 7.2 by the addition 1 M HCl or 1 M NaOH. The influence of varying pH on the growth pattern and antibiotic production of ΔcpxR was also evaluated in batch fermentation using the same bioreactor. Details have been shown in the Supporting Information.

Batch fermentation process in 70-l bioreactor
Fermentation of ΔcpxR was also carried out in a 70-l bioreactor (Eastbio, China) with a working volume of 50 L. The bioreactor was equipped with two six-blade disc turbine impeller. The pH, temperature, DO and agitation speed were monitored online. The fermentation medium, pH, temperature and inoculum level used were identical with the 5-l bioreactor. Throughout the fermentation bioprocessing, the temperature levels were regulated automatically. The bioreactors were operated under well-controlled conditions as mentioned above for 5-l bioreactor for 72 h.

Analytical methods
The method for the determination of DCW (g L⁻¹), the specific cell growth rate (μ), the glucose concentration and the antibiotic activity assay was shown in Supporting Information. All of the results were averaged and presented as the mean ± standard deviation from triplicate experiments. Student’s t-test using SPSS 18.0 (SPSS, Chicago, IL, USA) statistically analysed the data.

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Conflict of interest
The authors have no conflict of interest.

Author Contributions
Y.H.W. proposed and supervised the project. X.Z. designed the experiments. S.Q.G., Z.Y.W., B.L.L., J.T.G., X.L.F. and Q.T. conducted the experiments. S.Q.G., M.B., X.L.F. and Y.H.W. analysed the data and drafted the manuscript. All authors read and approved the final manuscript.

References
Akhurst, R.J. (1982) Antibiotic activity of Xenorhabdus spp., bacteria symbiotically associated with insect pathogenic nematodes of the families Heterorhabditidae and Steinernematidae. J Gen Microbiol 128: 3061–3065.
Bozhüyük, K. A. J., Zhou, Q., Engel, Y., Heinrich, A., Pérez, A., and Bode, H. B. (2016) Biosynthesis of the antibiotic nematophin and its elongated derivatives in entomopathogenic bacteria. In The Molecular Biology of Photorhabdus Bacteria. Cham: Springer, pp. 59–79.
Cai, X., Challinor, V.L., Zhao, L., Reimer, D., Adihou, H., Grün, P., et al. (2017) Biosynthesis of the antibiotic nematophin and its elongated derivatives in entomopathogenic bacteria. Org Lett 19: 806–809.
Challinor, V.L., and Bode, H.B. (2015) Bioactive natural products from novel microbial sources. Ann N Y Acad Sci 1354: 82–97.
Chaston, J.M., Suen, G., Tucker, S.L., Andersen, A.W., Bhasin, A., Bode, E., et al. (2011) The entomopathogenic bacterial endosymbionts Xenorhabdus and Photorhabdus: Convergent Lifestyles from Divergent Genomes. PLoS ONE 6: e27909.
Chen, G., Dunphy, G.B., and Webster, J.M. (1994) Antifungal activity of two Xenorhabdus species and Photorhabdus luminescens, bacteria associated with the nematodes Steinernema species and Heterorhabditis megidis. Biol Control 4: 157–162.
Chen, G., Maxwell, P., Dunphy, G.B., and Webster, J.M. (1996) Culture conditions for Xenorhabdus and Photorhabdus symbionts of entomopathogenic nematodes. Nematologica 42: 124–130.
Crawford, J.M., Portmann, C., Kontrik, R., Walsh, C.T., and Clardy, J. (2011) NRPS substrate promiscuity diversifies the Xenematidae. Org Lett 13: 5144–5147.
Danese, P.N., and Silhavy, T.J. (1998) CpxR, a stress-combative member of the Cpx regulon. J Bacteriol 180: 831–839.

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Engel, Y., Windhorst, C., Lu, X., Goodrich-Blair, H. and Bode, H.B. (2017) The global regulators Lrp, LeuO, and HexA control secondary metabolism in entomopathogenic bacteria. *Front Microbiol* **8**: 209. https://doi.org/10.3389/fmicb.2017.00209.

Forst, S. and Nealon, K. (1996) Molecular biology of the symbiotic-pathogenic bacteria *Xenorhabdus* spp. and *Photorhabdus* spp. *Microbiol Rev* **60**: 21.

Goodrich-Blair, H., and Clarke, D.J. (2007) Mutualism and pathogenesis in *Xenorhabdus* and *Photorhabdus*: two roads to the same destination. *Mol Microbiol* **64**: 260–268.

Guo, S.Q. (2015) *Study on Antibacterial Mechanism of Xenorhabdus Nematophila Regulated by pH*. China: Northwest A&F University.

Herbert, E.E., and Goodrich-Blair, H. (2007) Friend and foe: the two faces of *Xenorhabdus nematophila*. *Nat Rev Microbiol* **5**: 634–646.

Herbert, E.E., Cowles, K.N., and Goodrich-Blair, H. (2007) CpxRA regulates mutuality and pathogenesis in *Xenorhabdus nematophila*. *Appl Environ Microbiol* **73**: 7826–7836.

Herbert, E.E., Andersen, A.W., and Goodrich-Blair, H. (2009) CpxRA influences *Xenorhabdus nematophila* colonization initiation and outgrowth in *Steinernema carpocapsae* nematodes through regulation of the *nil* locus. *Appl Environ Microbiol* **75**: 4007–4014.

Hinchliffe, S.J. (2010) Insecticidal toxins from the *Photorhabdus* and *Xenorhabdus* bacteria. *Open Toxins J* **3**: 101–118.

Huang, W.R., Zhu, C.X., Yang, X.F., Yang, H.W., Xu, H.Z., Xie, Y.Y., and Jian, H. (2005) Isolation and structural identification of main component CB6-1 produced by *Xenorhabdus nematophilus* var. pekingensis. *Chin J Antibiot* **30**: 513–515.

Huang, W.R., Yang, X.F., Yang, H.W., Liu, Z., and Yuan, J.J. (2006) Identification and activity of a antibacterial substance from *Xenorhabdus nematophilus* var. Pekingense. *Nat Prod Res Dev* **18**: 25–28.

Ji, D., Yi, Y., Kang, G.H., Choi, Y.H., Kim, P., Baek, N.I., and Kim, Y. (2004) Identification of an antibacterial compound, benzylideneacetone, from *Xenorhabdus nematophilus* against major plant-pathogenic bacteria. *FEMS Microbiol Lett* **239**: 241–248.

Kennedy, G., Viziano, M., Winders, J.A., Cavallini, P., Gevi, M., Micheli, F., et al. (2000) Studies on the novel anti-staphylococcal compound nematin. *Bioorg Med Chem Lett* **10**: 1751–1754.

Li, J., Chen, G., Webster, J.M., and Czyzewska, E. (1995) Antimicrobial metabolites from a bacterial symbiont. *J Nat Prod* **58**: 1081–1086.

Li, J., Chen, G., and Webster, J.M. (1997) Nematophila, a novel antimicrobial substance produced by *Xenorhabdus nematophilus* (Enterobacteriaceae). *Can J Microbiol* **43**: 770–773.

McInerney, B.V., Taylor, W.C., Lacey, M.J., Akhurst, R.J., and Gregson, R.P. (1991) Biologically active metabolites from *Xenorhabdus* Spp., Part 2. Benzopyran-1-one derivatives with gastroprotective activity. *J Nat Prod* **54**: 785–795.

Morgan, J.A., Sergeant, M., Ellis, D., Ousley, M., and Jarrett, P. (2001) Sequence analysis of insecticidal genes from *Xenorhabdus nematophilus* PMFI296. *Appl Environ Microbiol* **67**: 2062–2069.

Park, D., and Forst, S. (2006) Co-regulation of motility, exoenzyme and antibiotic production by the EnvZ-OmpR-FliDC-FiiA pathway in *Xenorhabdus nematophila*. *Mol Microbiol* **61**: 1397–1412.

Paul, V.J., Frautschy, S., Fenical, W., and Nealon, K.H. (1981) Antibiotics in microbial ecology. *J Chem Ecol* **7**: 589–597.

Romero, D., Traxler, M.F., López, D., and Kolter, R. (2011) Antibiotics as signal molecules. *Chem Rev* **111**: 5492–5505.

Sergeant, M., Jarrett, P., Ousley, M., and Morgan, J.A. (2003) Interactions of insecticidal toxin gene products from *Xenorhabdus nematophilus* PMFI296. *Appl Environ Microbiol* **69**: 3344–3349.

Sheets, J.J., Hey, T.D., Fencil, K.J., Burton, S.L., Ni, W., Lang, A.E., et al. (2011) Insecticidal toxin complex proteins from *Xenorhabdus nematophilus*: structure and pore formation. *J Biol Chem* **286**: 22742–22749.

Singh, S. and Forst, S. (2016) Antimicrobials and the natural biology of a bacterial-nematode symbiosis. In *The Mechanistic Benefits of Microbial Symbionts*. Cham: Springer, pp. 101–119.

Siryporn, A., and Goulian, M. (2008) Cross-talk suppression between the CpxA-CpxR and EnvZ-OmpR two-component systems in *E.coli*. *Mol Microbiol* **70**: 494–506.

Sundar, L., and Chang, F.N. (1993) Antimicrobial activity and biosynthesis of indole antibiotics produced by *Xenorhabdus nematophilus*. *J Gen Microbiol* **139**: 3139–3148.

Tang, Q. (2015) Effect of CpxR Gene Knockout on the Antibacterial Activity and Metabolism of *Xenorhabdus Nematophila*. China: Northwest A&F University.

Thomas, G.M., and Poinar, G.O. (1979) *Xenorhabdus* gen. nov., a genus of entomopathogenic, nematophilic bacteria of the family enterobacteriaceae. *Int J Syst Bacteriol* **29**: 352–360.

Tobias, N.J., Wolff, H., Djahanschiri, B., Grundmann, F., Kronenwerth, M., Shi, Y.M., et al. (2017) Natural product diversity associated with the nematode symbionts *Photorhabdus* and *Xenorhabdus*. *Nat Microbiol* **2**: 1676–1685.

Tran, E.E.H., and Goodrich-Blair, H. (2009) CpxRA contributes to *Xenorhabdus nematophilus* virulence through regulation of irhA and modulation of insect immunity. *Appl Environ Microbiol* **75**: 3998–4006.

Wang, Y.H. (2004) *Studies on the Bioactivity and Culture of Bacterial Symbiont of Entomopathogenic Nematodes*. China: Northwest A&F University.

Wang, Y.H., Feng, J.T., Zhang, Q., and Zhang, X. (2008a) Optimization of fermentation condition for antibiotic production by *Xenorhabdus nematophilus* with response surface methodology. *J Appl Microbiol* **104**: 735–744.

Wang, Y.H., Li, Y.P., Zhang, Q., and Zhang, X. (2008b) Enhanced antibotic activity of *Xenorhabdus nematophilus* by medium optimization. *Bioresource Technol* **99**: 1708.
Wang, Y.H., Fang, X.L., Li, Y.P., and Zhang, X. (2010) Effects of constant and shifting dissolved oxygen concentration on the growth and antibiotic activity of *Xenorhabdus nematophila*. *Bioresource Technol* **101**: 7529–7536.

Wang, Y., Fang, X., Cheng, Y., and Xing, Z. (2011) Manipulation of pH shift to enhance the growth and antibiotic activity of *Xenorhabdus nematophila*. *J Biomed Biotechnol* **2011**: 672369.

Wolfe, A.J., Bruno, N.P., Lima, P., and Zemaitaitis, B. (2008) Signal Integration by the two-component signal transduction response regulator CpxR. *J Bacteriol* **190**: 2314–2322.

Yang, X.F., Qiu, D.W., Zhang, Y.L., Zeng, H.M., Liu, Z., Yang, H.W., *et al.* (2009) A toxin protein from *Xenorhabdus nematophila* var. pekingensis and insecticidal activity against larvae of *Helicoverpa armigera*. *Biocontrol Sci Techn* **19**: 943–955.

Yang, X., Qiu, D., Yang, H., Liu, Z., Zeng, H., and Yuan, J. (2011) Antifungal activity of xenocoumacin 1 from *Xenorhabdus nematophilus* var. pekingensis against *Phytophthora infestans*. *World J Microb Biot* **27**: 523–528.

Zhou, T., Yang, X., Qiu, D., and Zeng, H. (2017) Inhibitory effects of xenocoumacin 1 on the different stages of *Phytophthora capsici* and its control effect on *Phytophthora blight* of pepper. *Biocontrol* **62**: 1–10.