Transcriptional factor OmpR positively regulates prodigiosin biosynthesis in *Serratia marcescens* FZSF02 by binding with the promoter of the prodigiosin cluster

Xianbo Jia¹, Ke Zhao²†, Fangchen Liu², Junjie Lin³, Chenqiang Lin¹ and Jichen Chen¹*

¹Institute of Soil and Fertilizer, Fujian Academy of Agricultural Sciences, Fujian Key Laboratory of Plant Nutrition and Fertilizer, Fuzhou, China, ²College of Resources and Environment, Fujian Agriculture and Forestry University, Fuzhou, China, ³College of Life Sciences, Fujian Agriculture and Forestry University, Fuzhou, China

Prodigiosin is a promising secondary metabolite mainly produced by *Serratia marcescens*. The production of prodigiosin by *S. marcescens* is regulated by different kinds of regulatory systems, including the EnvZ/OmpR system. In this study, we demonstrated that the regulatory factor OmpR positively regulated prodigiosin production in *S. marcescens* FZSF02 by directly binding to the promoter region of the prodigiosin biosynthesis cluster with a lacZ reporter assay and electrophoretic mobility shift assay (EMSA). The binding sequence with the *pig* promoter was identified by a DNase I footprinting assay. We further demonstrate that OmpR regulates its own expression by directly binding to the promoter region of *envZ*/OmpR. For the first time, the regulatory mechanism of prodigiosin production by the transcriptional factor OmpR was revealed.

**KEYWORDS**

*Serratia marcescens*, prodigiosin, OmpR, two-component system, regulatory mechanism

**Introduction**

Prodigiosin, an important secondary metabolite produced by *S. marcescens* and some other bacteria, is of particular interest for its potential applications, including various pharmacological activities, food colorants, and potential sunscreens (Stankovic et al., 2014; Darshan and Manonmani, 2015). In the genus *Serratia*, biosynthesis of prodigiosin is regulated not only by external factors, including temperature, pH and medium composition (Paul et al., 2020) but also by various genes (Williamson et al., 2006). Nearly 30 genes have been reported to be involved in prodigiosin biosynthesis in *S. marcescens* (Pan et al., 2021).
and more studies should be carried out to search for new regulatory genes and uncover the complex regulatory mechanisms of this secondary metabolite.

The two-component system is a family of signal transduction proteins reported to be present in all types of life (Stock et al., 1999; Scharf, 2010; Papon and Stock, 2019). In bacteria, the classical two-component system consists of a sensor protein and a regulatory protein (Yuchuan et al., 2019). Sensor proteins respond to chemical or physical signals by phosphorylating regulatory proteins, and phosphorylated regulatory proteins can regulate the expression levels of downstream genes by binding to certain DNA sequences (Groisman, 2016). Two-component systems regulate many processes of bacteria, such as adaptation to environmental changes: osmolarity (Boyce et al., 2016), temperature (Dhiman et al., 2015; Nainin et al., 2016), oxygen (Dixon, 1998; Wright et al., 2018), regulation of developmental pathways, and behaviors, such as sporulation (Sarwar and Garza, 2015), biofilm formation (Lai et al., 2005), quorum sensing (Krumpa et al., 2004), regulation of secondary metabolite biosynthesis (Sola-Landa et al., 2003), virulence (Beier and Gross, 2006), and antibiotic resistance (Bhagirath et al., 2019; Tierney and Rather, 2019). Biosynthesis of prodigiosin was also regulated by different types of two component systems in various Serratia strains, including PigQ/W and PhoB/PhoR in Serratia 39,006 (Finefrock et al., 2005; Gristwood et al., 2009); EepR/EpsP in S. marcescens CMS376, S. marcescens K904, and S. marcescens Nima (Stella et al., 2015); RsSB/RssA in S. marcescens CH-1 (Horng et al., 2010); and CpxR/A in S. marcescens FS14 (Qiu et al., 2021).

The two component system EnvZ/OmpR is an important signal transduction system in bacteria responding to various environmental stress and growth conditions (Qin et al., 2001). We have previously demonstrated that when envZ or ompR was knocked out, S. marcescens FZSF02 lost its prodigiosin biosynthesis ability (Jia et al., 2021), and OmpR was also recently found to control prodigiosin biosynthesis in S. marcescens JNBS-1 (Pan et al., 2022). However, the regulatory mechanism of the two-component EnvZ/OmpR system on prodigiosin production is still unknown.

In this study, with LacZ-reporter studies and an electrophoretic mobility shift assay (EMSA), we demonstrated that OmpR positively regulated prodigiosin biosynthesis by directly binding to the promoter region of the prodigiosin biosynthesis gene cluster. We also found that OmpR can regulate its own expression level by binding the promoter of the EnvZ/OmpR genes.

Materials and methods

Bacterial strains, plasmids, and culture conditions

The bacterial strains and plasmids used in this study are listed in Supplementary Tables S1, S2. Serratia marcescens FZSF02 and its related mutants were incubated in lysogeny broth (LB) at 28°C and 180 rpm. E. coli DH5α and E. coli Rosetta (DE3) were cultured in LB medium at 37°C and 220 rpm. The final concentrations of antibiotics used in this study were as follows: 100 mg/L ampicillin, 100 mg/L kanamycin, and 50 mg/L chloramphenicol.

Construction of the in-frame deletion mutant and complementary strains

FZSF02ompR and FZSF02envZ were constructed in our previous study (Jia et al., 2021). FZSF02envZompR was also constructed with the homologous recombination method (Jia et al., 2021). Complementary strains were constructed with the plasmid pRK415 as we have reported in our previous study (Jia et al., 2021). pRK415-ompR, pRK415-envZ and pRK415-envZompR were transformed into FZSF02ompR, FZSF02envZ, and FZSF02envZompR, respectively, to construct the complementary strains. The primers used in these experiments are listed in Supplementary Table S2.

lacZ reporter assays

The pig promoter and ompR promoter were inserted upstream of lacZ in the plasmids pTOPO-lacZ-Cmr to construct the plasmids pTOPO-Pigpro-lacZ-Cmr and pTOPO-OmpRpro-lacZ-Cmr. The primers used in this experiment are listed in Supplementary Table S2. The constructed plasmids with the Pig promoter and ompR promoter were transformed into the wild-type strain S. marcescens FZSF02 and the ompR-knockout strain FZSF02ompR, respectively. The plasmids pTOPO-Pigpro-Cmr and pTOPO-ompRpro-Cmr were also transformed into S. marcescens FZSF02 and FZSF02ompR as controls. For liquid β-galactosidase assays, constructed strains were cultured at 28°C and 180 rpm for 16 h, and enzyme activities were measured in sonicated extracts according to the method described by Pardee et al. (1959).

Electrophoretic mobility shift assay

The coding sequence of ompR was cloned into pEASY®-Blunt E2 (TransGen Biotech, Beijing, China). The plasmid pEASY®-Blunt E2-ompR was transformed into E. coli Rosetta (DE3). The DNA fragments containing the pig promoter (406 bp) and ompR promoter (257 bp) were cloned into the pTOPO-Blunt simple vector (Aidlab, China), respectively, and then promoter probes were obtained through polymerase chain reaction (PCR) with primers M13F and M13R labeled with Cy5.5 at the 5’ end. The Pigpro probe and ompRprobe probe were 556 and 407 bp, respectively. Electrophoretic mobility shift assay (EMSA) was carried out with an EMSA/Gel-Shift kit (Beyotime, Shanghai, China). The purified probe and protein were mixed with EMSA/
Gel-Shift binding buffer (5×), and a total of 10 μl of the reaction system was supplied with distilled water and incubated for 30 min at 28°C. 6% native PAGE was prepared as the kit protocol described, and the reaction mixture was loaded onto the PAGE. Electrophoresis was performed at 60 V for 3 h in 0.5× TBE buffer, and the gels were exposed to an Odyssey CLx (LI-COR® Biosciences).

DNase I footprinting assay

The pig promoter probe was prepared by polymerase chain reaction (PCR) with primers M13F and M13R (labeled with Hex). The DNase I footprinting assay was carried out as described by Shi et al. (2017).

Results

OmpR activates the transcription level of the pig gene cluster

We have previously demonstrated that gene deletion of ompR (GenBank: QIU42212.1) or envZ (GenBank: QIU42211.1) would result in loss of prodigiosin producing ability in S. marcescens FZSF02; transcription levels of pigA also down-regulated significantly when ompR or envZ was knocked out assayed by qPCR (Jia et al., 2021). The effect of OmpR and EnvZ on prodigiosin-producing ability was further demonstrated by knockout ompR and envZ, double knockout of envZ and ompR in this study; gene deletion strains all lost prodigiosin-producing ability, prodigiosin-producing ability restored in complementary strain (Figures 1A–C). OmpR was very conserved in amino acid sequences among many Gram-negative bacteria, such as Rouxiella aceris, Ewingella americana, Versinia enterocolitica, Citrobacter youngae, Hafnia psychrotolerans, Escherichia coli and Klebsiella pneumoniae (Figure 1D). Many studies have demonstrated that OmpR influences a wide variety of cellular processes in E. coli, Salmonella sp. and Shigella sp., as well as pathogenic species of Versinia sp. (Jaworska et al., 2021), but the influence of secondary metabolite biosynthesis by OmpR has rarely been reported. We have previously demonstrated that OmpR can positively regulate the production of the secondary metabolite prodigiosin in S. marcescens FZSF02, but the regulatory mechanism was unknown.

To further test whether the regulation of prodigiosin biosynthesis by OmpR is at the transcriptional level, the β-galactosidase activity of FZSF02 and FZSF02ΔompR was assayed when the lacZ gene was first controlled by the pig cluster promoter. The results showed that the β-galactosidase activity of FZSF02ΔompR (pTOPO-Pigpro-lacZ-Cmr) decreased by 92.3% compared with that of the wild-type strain FZSF02 WT (pTOPO-Pigpro-lacZ-Cmr; Figure 2A), and almost no β-galactosidase activity was tested in the control group of WT (pTOPO-Pigpro-Cmr) and ΔompR (pTOPO-Pigpro-Cmr; Figure 2A). This result indicates that OmpR directly or indirectly activates the transcription level of the pig gene cluster, and influences prodigiosin synthesis in strain FZSF02.

OmpR activates the transcription level of the pig gene cluster by directly binding to its promoter sequence

To study whether OmpR regulates the expression of the pig gene cluster by binding with its promoter directly, EMSA was used to detect the binding ability between OmpR and the pig gene cluster promoter sequence. The results showed that OmpR can bind with the probe prepared with the prodigiosin cluster promoter sequence (Figure 2B). A DNase I footprinting assay showed that the proposed binding sequence of OmpR on the pig promoter was 5’CATTTATCATATTAC3’ (Figure 3), which located on −103 bp to −86 bp relative to the A of the ATG start codon of pigA. Pig promoter sequence is between pigA (QIU38817.1) and cueR (QIU38818.1) on the genome of FZSF02.

OMPR activates its own expression level

To test whether the autoregulation of OmpR exists in S. marcescens FZSF02, the β-galactosidase activity of FZSF02 and FZSF02ΔompR was assayed when the lacZ gene was first controlled by the ompR promoter. The results showed that the β-galactosidase activity of FZSF02ΔompR (100 U/ml) decreased by 92.3% compared with that of the wild-type strain FZSF02 (1,300 U/ml; Figure 2C). This finding demonstrates that OmpR can activate its own expression level.

OmpR can directly bind to the envZ/ompR promoter

To test whether the activation of OmpR on its own expression is performed by binding with the envZ/ompR promoter, EMAS was used to examine the binding ability between OmpR and the envZ/ompR promoter sequence. The results showed that when the envZ/ompR promoter sequence was used as a probe, OmpR could bind with the labeled probe (Figure 2D). The addition of unlabeled probe can compete with the labeled probe, which further demonstrates the binding ability.

Discussion

Prodigiosin was a kind of bacterial secondary metabolites produced mainly by many S. marcescens strains. Various regulating genes involved in prodigiosin biosynthesis have been found in the
Past two decades, but new regulators, such as RcsB (Pan et al., 2021), CpxA/R (Qiu et al., 2021) and Fnr (Sun et al., 2021), have still been reported continuously. Research of these genes may help to uncover the regulatory mechanism behind prodigiosin biosynthesis in S. marcescens.

EnvZ/OmpR is known to control motility (Prüß, 2017), intracellular survival (Du et al., 2022), antibiotic resistance (Ko and Choi, 2022), virulence (Tipton and Rather, 2017), and other characteristics of different bacterial strains, but few studies have reported the role of EnvZ/OmpR in S. marcescens. We have demonstrated previously that mutation of ompR or envZ would cause the loss of prodigiosin producing ability in S. marcescens FZSF02 and confirmed that the two-component system EnvZ/OmpR was a newly found system that can regulate prodigiosin biosynthesis (Jia et al., 2021). In this study, the regulatory function of EnvZ/OmpR on prodigiosin biosynthesis was further confirmed by gene deletion and complementation (Figure 1C). For the EnvZ/OmpR system, OmpR was reported to play the role by binding to the gene promoters and regulating the expression of other genes (Wang et al., 2021). LacZ reporter assays and EMSA assay in this study also showed OmpR regulate prodigiosin biosynthesis by directly binding to the promoter region of pig cluster (Figures 2A,B). The binding region of the OmpR was identified as 5′CATTTATTTACATTTAC3′ (Figure 3) by a DNase I footprinting assay. The binding sequence showed 45% identity to the E. coli consensus sequence (5′TTTTACTT TTGTAACATAT3′; Maeda et al., 1991) and 55% identity to that of Y. enterocolitica (5′ATTATTGATGGTAACAATT3′; Nieckarz et al., 2020).
Many two-component systems regulating proteins can autoregulate their own expression by binding to their promoters, and this kind of feedback allows the regulatory functions of the system to be more flexible (Groisman, 2016). For the EnvZ/OmpR two-component system, autoregulation differs among different strains; it exists in Salmonella enterica (Bang et al., 2002; Cameron and Dorman, 2012) but not in E. coli (Ochman and Wilson, 1987; Doolittle et al., 1996) and Acinetobacter baumannii (Tipton and Rather, 2017). The results of the LacZ reporter assay (Figure 2C) and EMSA assay (Figure 2D) indicated that OmpR can bind to the promoter region of envZ/ompR and promote the expression of OmpR and EnvZ.

Based on the above results, we proposed that the regulatory mechanism of the two-component EnvZ/OmpR system on prodigiosin biosynthesis was probably as follows (Figure 4): Some unknown factors induced the expression of EnvZ and OmpR, and OmpR was then phosphorylated by EnvZ (Cai and Inouye, 2002). Phosphorylated OmpR activated the expression of more EnvZ and OmpR by binding with the envZ/ompR promoter. When the concentration of OmpR reached a certain level, the pig gene cluster promoter persistently bound with OmpR, and genes involved in prodigiosin biosynthesis were highly expressed at the transcriptional level.

Although we have previously found that when ompR was knocked out, S. marcescens FZSF02 lost the prodigiosin biosynthesis ability (Jia et al., 2021), in this study, the proposed regulatory mechanism of OmpR on prodigiosin biosynthesis was demonstrated. EnvZ/OmpR was demonstrated to be a new two-compound system that can directly positively regulate prodigiosin production in S. marcescens FZSF02.

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 author.
FIGURE 4
Proposed model of OmpR involvement in the regulatory mechanism of prodigiosin biosynthesis in *S. marcescens* FZSF02.

**Author contributions**

XJ and JC designed the study, wrote the manuscript, and analyzed the results. XJ, FL, JL, CL, and KZ performed the experiments. All authors contributed to the article and approved the submitted version.

**Funding**

This study was supported by the Exploration Program of Fujian Academy of Agricultural Sciences (ZYTS202217), Scientific Research in the Public Interest of Fujian Province (2020R1025003 and 2021R1025002), Natural Science Foundation of Fujian Province of China (2021J01480), Chinese National Natural Science Foundation (31800068), the Special Program for Extension Research of National Natural Science Foundation of Fujian Academy of Agricultural Sciences (AGY2018-1), and Science and Technology Innovation Team Program of Fujian Academy of Agricultural Sciences (CXTD2021002-3).
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.

Publisher’s note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2022.1041146/full#supplementary-material

References

Bang, I. S., Audia, J. P., Park, Y. K., and Foster, J. W. (2002). Autoinduction of the ompR response regulator by acid shock and control of the Salmonella enterica acid tolerance response. Mol. Microbiol. 44, 1235–1250. doi: 10.1046/j.1365-2958.2002.02937.x

Beier, D., and Gross, R. (2006). Regulation of bacterial virulence by two-component systems. Curr. Opin. Microbiol. 9, 143–152. doi: 10.1016/j.mib.2006.01.003

Bhapkarth, A. Y., Li, Y., Patidar, R., Yere, K., Ma, X., Kumar, A., et al. (2019). Two component regulatory systems and antibiotic resistance in gram-negative pathogens. Int. J. Mol. Sci. 20:1781. doi: 10.3390/ijms20071781

Boyce, K. J., Cao, C., and Andrianopoulos, A. (2016). Two-component signaling regulates osmotic stress adaptation via SkxA and the high osmolality glycerol MAPK pathway in the human pathogen Talonomyces marneffei. mSphere 1:e00886-e00915. doi: 10.1128/mSphere.00086-15

Cai, S. J., and Inouye, M. (2002). EnvZ-OmpR interaction and osmoregulation in Escherichia coli. J. Biol. Chem. 277, 24155–24161. doi: 10.1074/jbc.M110715200

Cameron, A. D., and Dorman, C. J. (2012). A fundamental regulatory mechanism operating through OmpR and DNA topology controls expression of salmonella pathogenicity islands SPI-1 and SPI-2. PLoS Genet. 8:e1002615. doi: 10.1371/journal.pgen.1002615

Darshan, N., and Manonmani, H. K. (2015). Prodigiosin and its potential applications. J. Food Sci. Technol. 52, 5393–5407. doi: 10.1007/s13197-015-1740-4

Dhiman, A., Gopalani, M., and Bhattacharjya, R. (2015). WalRK two component system of Bacillus anthracis responds to temperature and antibiotic stress. Biochem. Biophys. Res. Commun. 459, 623–628. doi: 10.1016/j.bbrc.2015.02.159

Dixon, R. (1998). The oxygen-responsive NIFL-NIFA complex: a novel two-component system in nitrogen metabolism. J. Biol. Chem. 273, 18680–18683. doi: 10.1074/jbc.273.26.18680

Du, Z., Zhang, M., Qin, Y., Zhao, L., Huang, L., Xu, X., et al. (2022). The role and importance of the LrpR response regulator by acid shock and control of the prodigiosin production by transcription factor engineering and promoter engineering in Serratia marcescens FZSF02. Front. Microbiol. 12:705853. doi: 10.3389/fmicb.2021.705853

Jia, X., Liu, F., Zhao, K., Liu, J., Fang, Y., Cai, S., et al. (2021). Identification of essential genes associated with prodigiosin production in Serratia marcescens FZSF02. Front. Microbiol. 12:705853. doi: 10.3389/fmicb.2021.705853

Ko, D., and Choi, S. H. (2022). Mechanistic understanding of antibiotic resistance mediated by EnvZ/OmpR two-component system in salmonella enterica serovar Enteritidis. J. Antonimicrob. Chemother. 77, 2419–2428. doi: 10.1093/jac/dkaa223

Kruppa, M., Krom, B. P., Chauhan, N., Bambah, A. V., Ciblar, R. L., and Calderone, R. A. (2004). The two-component signal transduction protein Chk1p regulates quorum sensing in Candida albicans. Eukaryot. Cell 3, 1062–1065. doi: 10.1128/EC.3.4.1062-1065.2004

Lai, H. C., Soo, P. C., Wei, J. R., Yi, W. C., Liaw, S. J., Horng, Y. T., et al. (2005). The RasAB two-component signal transduction system in Serratia marcescens regulates swarming motility and cell envelope architecture in response to exogenous saturated fatty acids. J. Bacteriol. 187, 3407–3414. doi: 10.1128/JB.187.11.3407-3414.2005

Maeda, S., Takayanagi, K., Nishimura, Y., Maruyama, T., Sato, K., and Mizuno, T. (1991). Activation of the osmoregulated ompC gene by the OmpK protein in Escherichia coli: a study involving synthetic OmpK binding sequences. J. Biochem. 110, 324–327. doi: 10.1093/oxfordjournals.jbchem.a213579

Najnin, T., Siddiqui, K. S., Tah, T., Elkaid, N., Kornfeld, G., Curmi, P. M., et al. (2016). Characterization of a temperature-responsive two component regulatory system from the Antarctic archaean, Methanothermococcus burtonii. Sci. Rep. 6:24278. doi: 10.1038/srep24278

Nieckarz, M., Kaczer, P., Javorska, K., Raczkowska, A., and Brzostek, K. (2020). Urease expression in pathogenic Yersinia enterocolitica strains of bio-serotypes 2/O9 and 18/O8 is differentially regulated by the OmpR regulator. Front. Microbiol. 11:607. doi: 10.3389/fmicb.2020.00607

Ochman, H., and Wilson, A. C. (1987). Evolution in bacteria: evidence for a universal substitution rate in cellular genomes. J. Mol. Evol. 26, 74–86. doi: 10.1007/BF0011283

Pan, X., Tang, M., You, J., Liu, F., Sun, C., Oestre, T., et al. (2021). Regulator RsCF controls prodigiosin synthesis and various cellular processes in Serratia marcescens INBS-1. Appl. Environ. Microbiol. 87, e02052-e02020. doi: 10.1128/AEM.02052-20

Pan, X., You, J., Tang, M., Zhang, X., Xu, M., Yang, T., et al. (2022). Improving prodigiosin production by transcription factor engineering and promoter engineering in Serratia marcescens. Front. Microbiol. 13:977337. doi: 10.3389/fmicb.2022.977337

Papen, N., and Stock, A. M. (2019). Two component systems. Curr. Biol. 29, R724–R725. doi: 10.1016/cub.2019.06.010

Pardoe, A. B., Jacob, E., and Monod, J. (1959). The genetic control and cytoplasmic expression of “Inducibility” in the synthesis of β-galactosidase by E. coli. J. Mol. Biol. 1, 165–178. doi: 10.1016/S0022-2836(59)80045-0

Paul, T., Bandyopadhyay, T. K., Mondal, A., Tiwari, O. N., and Bhusan, B. (2020). A comprehensive review on recent trends in production, purification, and applications of prodigiosin. Biomass Convers. Biofuel 12, 1409–1413. doi: 10.1016/j.bioc.2020.02.00928-2

Prüß, B. M. (2017). Involvement of two-component signaling on bacterial motility and biofilm development. J. Bacteriol. 199, e00259–e00217. doi: 10.1128/JB.00259-17

Qin, L., Yoshida, T., and Inouye, M. (2001). The critical role of DNA in the intracellular survival of Aeromona hydrophila. J. Fish Dis. 25, 1659–1662. doi: 10.1111/j.1364-1508.2001.t01-1-01147.00020.x

Qiu, S., Jia, S., Zhang, F., Liu, X., Ran, T., Wang, W., et al. (2021). Two component system CprRa regulates the prodigiosin biosynthesis by negative control in Serratia

Frontiers in Microbiology
Sarwar, Z., and Garza, A. G. (2015). Two-component signal transduction systems that regulate the temporal and spatial expression of Myxococcus xanthus sporulation genes. *J. Bacteriol.* 198, 377–385. doi: 10.1128/JB.00474-15

Scharf, B. E. (2010). Summary of useful methods for two-component system research. *Curr. Opin. Microbiol.* 13, 246–252. doi: 10.1016/j.mib.2010.01.006

Shi, K., Fan, X., Qiao, Z., Han, Y., McDermott, T. R., Wang, Q., et al. (2017). Arsenite oxidation regulator AioR regulates bacterial chemotaxis toward arsenite in Agrobacterium tumefaciens GW4. *Sci. Rep.* 7:43252. doi: 10.1038/srep43252

Sola-Landa, A., Moura, R. S., and Martín, J. F. (2003). The two-component PhoR-PhoP system controls both primary metabolism and secondary metabolite biosynthesis in Streptomyces lividans. *Proc. Natl. Acad. Sci. U. S. A.* 100, 6133–6138. doi: 10.1073/pnas.0931429100

Stock, A. M., Robinson, V. L., and Goudreau, P. N. (1999). Two-component signal transduction. *Annu. Rev. Biochem.* 69, 183–215. doi: 10.1146/annurev.biochem.69.1.183

Sun, D., Zhou, X., Liu, C., Zhu, J., Ru, Y., Liu, W., et al. (2021). Fnr negatively regulates prodigiosin synthesis in Serratia sp. ATCC 39006 during aerobic fermentation. *Front. Microbiol.* 12:734854. doi: 10.3389/fmicb.2021.734854

Tierney, A. R., and Rather, P. N. (2019). Roles of two-component regulatory systems in antibiotic resistance. *Future Microbiol.* 14, 533–552. doi: 10.2217/fmb-2019-0002

Tipton, K. A., and Rather, P. N. (2017). An ompR-envZ two-component system ortholog regulates phase variation, osmotic tolerance, motility, and virulence in *Acinetobacter baumannii* strain ABS075. *J. Bacteriol.* 199, e00705–e00716. doi: 10.1128/JB.00705-16

Wang, S. T., Kuo, C. J., Huang, C. W., Lee, T. M., Chen, J. W., and Chen, C. S. (2021). OmpR coordinates the expression of virulence factors of Enterohemorrhagic *Escherichia coli* in the alimentary tract of *Caenorhabditis elegans*. *Mol. Microbiol.* 116, 168–183. doi: 10.1111/mmi.14698

Williamson, N. R., Fineran, P. C., Leeper, F. J., and Salmond, G. P. (2006). The biosynthesis and regulation of bacterial prodiginines. *Nat. Rev. Microbiol.* 4, 887–899. doi: 10.1038/nrmicro1531

Wright, G., Saeki, A., Hikima, T., Nishizono, Y., Hisano, T., Kamaya, M., et al. (2018). Architecture of the complete oxygen-sensing FixL-FixJ two-component signal transduction system. *Sci. Signal.* 11.eaaq0825. doi: 10.1126/scisignal.aaaq0825

Yuchuan, M., Sayak, B., Tatats., and Banerjee, (2019). Wave patterns organize cellular protrusions and control cortical dynamics. *Mol. Syst. Biol.* 15:e8585. doi: 10.15252/msb.20188585