



OPEN ACCESS

EDITED BY

Bin Liu,
University of Minnesota Twin Cities,
United States

REVIEWED BY

Yangbo Hu,
Wuhan Institute of Virology (CAS),
China
Ning Xu,
Tianjin Institute of Industrial Biotechnology
(CAS), China

*CORRESPONDENCE

Binghai Du
du_binghai@163.com
Yanqin Ding
dyq@sdau.edu.cn

SPECIALTY SECTION

This article was submitted to Microbial
Physiology and Metabolism,
a section of the journal
Frontiers in Microbiology

RECEIVED 08 September 2022

ACCEPTED 26 October 2022

PUBLISHED 22 November 2022

CITATION

Zhao D, Li H, Cui Y, Tang S, Wang C,
Du B and Ding Y (2022) MsmR1, a global
transcription factor, regulates polymyxin
synthesis and carbohydrate metabolism in
Paenibacillus polymyxa SC2.
Front. Microbiol. 13:1039806.
doi: 10.3389/fmicb.2022.1039806

COPYRIGHT

© 2022 Zhao, Li, Cui, Tang, Wang, Du and
Ding. This is an open-access article
distributed under the terms of the [Creative
Commons Attribution License \(CC BY\)](#). The
use, distribution or reproduction in other
forums is permitted, provided the original
author(s) and the copyright owner(s) are
credited and that the original publication in
this journal is cited, in accordance with
accepted academic practice. No use,
distribution or reproduction is permitted
which does not comply with these terms.

MsmR1, a global transcription factor, regulates polymyxin synthesis and carbohydrate metabolism in *Paenibacillus polymyxa* SC2

Dongying Zhao, Hui Li, Yanru Cui, Shengyue Tang,
Chengqiang Wang, Binghai Du* and Yanqin Ding*

College of Life Sciences and Shandong Engineering Research Center of Plant-Microbia Restoration for Saline-alkali Land and Shandong Key Laboratory of Agricultural Microbiology and National Engineering Laboratory for Efficient Utilization of Soil and Fertilizer Resources, Shandong Agricultural University, Tai'an, China

The multiple-sugar metabolism regulator (MsmR), a transcription factor belonging to the AraC/XylS family, participates in polysaccharide metabolism and virulence. However, the transcriptional regulatory mechanisms of MsmR1 in *Paenibacillus polymyxa* remain unclear. In this study, knocking out *msmR1* was found to reduce polymyxin synthesis by the SC2-M1 strain. Chromatin immunoprecipitation assay with sequencing (ChIP-seq) revealed that most enriched pathway was that of carbohydrate metabolism. Additionally, electromobility shift assays (EMSA) confirmed the direct interaction between MsmR1 and the promoter regions of *oppC3*, *sucA*, *sdr3*, *pepF*, *ycyN*, *PPSC2_23180*, *pppL*, and *ydfp*. MsmR1 stimulates polymyxin biosynthesis by directly binding to the promoter regions of *oppC3* and *sdr3*, while also directly regulating *sucA* and influencing the citrate cycle (TCA cycle). In addition, MsmR1 directly activates *pepF* and was beneficial for spore and biofilm formation. These results indicated that MsmR1 could regulate carbohydrate and amino acid metabolism, and indirectly affect biological processes such as polymyxin synthesis, biofilm formation, and motility. Moreover, MsmR1 could be autoregulated. Hence, this study expands the current knowledge of MsmR1 and will be beneficial for the application of *P. polymyxa* SC2 in the biological control against the certain pathogens in pepper.

KEYWORDS

Paenibacillus polymyxa, MsmR1, polymyxin, carbohydrate metabolism, ChIP-seq

Introduction

Paenibacillus polymyxa, proposed by Ash et al. (1993), is a plant growth-promoting rhizobacteria (PGPR) that contributes to disease prevention and growth promotion by inducing plant systemic resistance, resulting in generation of secondary metabolites (Li et al., 2019; Liu et al., 2021; Yin et al., 2022). *Paenibacillus polymyxa* is an important strain

resource in microbial medicine, microbial environmental remediation, microbial pesticides, and fertilizer research (Timmusk et al., 2005; Jeong et al., 2019). *Paenibacillus polymyxa* synthesizes polymyxin—an important antibiotic against gram-negative pathogens—via a multienzyme non-ribosomal peptide synthetase system (NRPS; Supplementary Figure S1A; Yu et al., 2015, 2018). Polymyxin is composed of 10 orderly assembled amino acid residues, five of which are L-2,4-diaminobutyric acid (L-Dabs) biosynthesized by 2,4-diaminobutyrate aminotransferase (EctB) (Choi et al., 2009). Moreover, polymyxins are primarily divided into seven types: A, B, C, D, E, M, and P, composed of a tripeptide side chain acylated by a fatty acid at the amino-terminus, and a cyclic heptapeptide (Supplementary Figure S1B). Indeed, carbon sources significantly impact the rate of polymyxin production and biosynthesis, for example, starch can promote polymyxin synthesis (Yu et al., 2018).

Carbohydrates exist in various forms, including monosaccharides, disaccharides, and polysaccharides. Energy released from carbohydrate catabolism is required for metabolic activity and growth (Warda et al., 2016). Pathways of carbohydrate catabolism involve complex networks that regulate gene expression. In bacteria, carbohydrate utilization is regulated by transcription factors (TFs) regulating the expression of genes which encodes transporters and enzymes (Khoroshkin et al., 2016). Efficient utilization of carbohydrates by bacteria is determined by various utilization systems and carbohydrate transporters (Warda et al., 2016).

Indeed, TFs have essential roles in regulating gene expression in all domains of life (Mejía-Almonte et al., 2020; Cortés-Avalos et al., 2021). More specifically, TFs can activate or repress gene transcription by binding to DNA in a manner dependent on environmental factors (Cortés-Avalos et al., 2021). In bacteria, TFs can be classified into at least 19 families based on their amino acid sequences. The largest TFs families are LysR, TetR/ArcR, and AraC/XylS, which together account for 30% of the total number of TFs identified in bacteria (Kotecka et al., 2021). The AraC/XylS family of TFs was originally described by Henikoff et al. (1990); members of this family act as positive transcriptional regulators or as both activators and repressors. Previous studies focusing on the TFs of RhaR (Kolin et al., 2008; Kettle, 2013), AraC (Schleif, 2010), UreR (Parra and Collins, 2012), and MelR (Belyaeva et al., 2000) in *Escherichia coli*, MsmR (Nakata et al., 2005) in *Streptococcus*, XylS (Zwick et al., 2013) in *Pseudomonas putida*, and ToxT (Lowden et al., 2010) in *Vibrio cholerae* revealed that they contain a highly conserved C-terminal DNA binding domain (DBD) and a variable ligand-binding domain (LBD) at the N-terminal (Bustos and Schleif, 1993; Cortés-Avalos et al., 2021). The DBD contains approximately 100 amino acids, with approximately 20% sequence identity in bacteria, and seven α -helices, forming two helix-turn-helix (HTH) DNA-binding motifs (Egan, 2002; Gahlot et al., 2021). While the DBD is in contact with RNA polymerase (RNAP) for DNA binding and transcription (Ibarra et al., 2008), the LBD is

involved in dimerization and effector/signal recognition (Egan, 2002). The AraC/XylS family transcriptional regulators control multiple functions, such as carbon metabolism, amino acid metabolism, stress response (e. g. Rob, SoxS, PliA, and OpiA were involved in stress response in *Erwinia amylovora*; Pletzer et al., 2014), and quorum sensing (Cortés-Avalos et al., 2021; Kotecka et al., 2021).

The MsmR of the AraC/XylS family is involved in polysaccharide metabolism (Russell et al., 1992). In *Streptococcus* mutants, MsmR positively regulates an operon for the uptake and catabolism of carbon sources (Russell et al., 1992). In *Streptococcus pyogenes*, MsmR positively regulates fibronectin- and collagen-binding T-antigen (FCT) region genes, including *nra*, which inhibit the expression of crucial virulence factors (Kreikemeyer et al., 2007). Meanwhile, the transcriptional regulatory mechanisms of MsmR1 in *P. polymyxa* remain unclear.

Paenibacillus polymyxa SC2 was isolated from the rhizosphere of pepper plants in Guizhou province, China (Ma et al., 2011). *Paenibacillus polymyxa* SC2-M1 is spontaneously mutated from *P. polymyxa* SC2 during strain cultivation, making it suitable for molecular manipulations (Hou et al., 2016). A previous study reported that the *msmR1* gene is upregulated by 2.17-fold during the interaction between *P. polymyxa* SC2 and pepper (Liu et al., 2021), indicating that *msmR1* may play an important role in this interaction. In the present study, we aimed to explore the regulatory mechanism of the transcription factor MsmR1 in polymyxin synthesis and related biological processes in *P. polymyxa* SC2. To this end, chromatin immunoprecipitation with sequencing (ChIP-seq) was performed and the results were verified by electrophoretic mobility shift assay (EMSA). The *oppC3*, *sucA*, *sdr3*, *pepF*, *yycN*, *PPSC2_23180*, *pppL*, and *ydfp* genes were found to be directly regulated by the global regulator MsmR1; the functions of these genes were further explored to delineate the regulatory mechanism of MsmR1. The results of this study provide a theoretical basis for the improved application of *P. polymyxa* SC2 in the biological control against the certain pathogens.

Materials and methods

Bacterial strains and culture conditions

The strains and plasmids used in this work are presented in Supplementary Table S1. *Paenibacillus polymyxa* SC2-M1 and its mutants, *Escherichia coli* DH5 α , *E. coli* BL21, and Trans 110 were cultured in Luria-Bertani (LB) broth at 37°C. When plasmids were presented in cells, antibiotics were added to LB medium as follows: chloramphenicol (6 μ g/ml for strain SC2-M1 and its mutants), kanamycin (50 μ g/ml), tetracycline (20 μ g/ml), ampicillin (100 μ g/ml), and erythromycin (12.5 μ g/ml). To measure carbon source utilization by the strains, basal medium (MgSO₄ 0.02%, NaCl 0.02%, (NH₄)₂SO₄ 0.8%, K₂HPO₄ 0.368%, KH₂PO₄ 0.132%) with a 1% sole carbon source was used.

Competent cells of *E. coli* were prepared using the transformation and storage solution (TSS) method and transformed by the heat pulse method (Chung et al., 1989). Competent cells were prepared and *P. polymyxa* SC2-M1 were electrotransformed as previously described (Hou et al., 2016).

Plasmid and strain construction

To construct knockout SC2-M1 strain mutants, 650–1,000 bp of homologous arm-flanking target genes were cloned from genomic DNA using PCR. The *cat* gene, encoding the chloramphenicol resistance cassette, was cloned from pDG1661 (Guérout-Fleury et al., 1996) using PCR to replace the target knockout genes. Two homologous arms and *cat* cassettes were fused by fusion PCR (Cha-Aim et al., 2012), and the fusion DNA fragments were ligated into the thermo-sensitive elimination plasmid pRN5101 to construct knockout vectors. The plasmid was demethylated by being further transformed into Trans 110. The demethylated knockout vectors were transferred into the SC2-M1 strain. Double-crossover recombinants were detected as previously reported (Hou et al., 2016), and the knockout strains were confirmed by PCR using the corresponding primers (Supplementary Table S2). For *in situ* complementation of the knockout genes, 650–1,000 bp of homologous arms fused with the corresponding complete target genes were cloned by PCR from genomic DNA (not involved in the resistance cassette), and ligated into pRN5101 to construct the complement vectors. Demethylated complementary vectors were introduced into the mutant strains. Double-crossover recombinants were detected as described above, and the complemented strains were confirmed by PCR using the corresponding primers (Supplementary Table S2). For ChIP-seq analysis, we obtained a Flag-tagged strain using homologous recombination. The Flag-tag was added in the front of termination codon of *msmR1* and a chloramphenicol resistance cassette was also added after the Flag tag using overlapping PCR. The homologous arms (650–1,000 bp) were cloned by PCR amplification, and ligated to pRN5101 to construct the plasmid pRN5101-*msmR1*-Flag. Demethylated pRN5101-*msmR1*-Flag were introduced into SC2-M1 and double-crossover recombinants were detected as described above, designated as strain SC2-M1-Flag. For overexpression of target genes, fragments carrying complete target genes were cloned from genomic DNA, fused with the promoter fragments, and ligated into pHY300PLK between *Bgl*II and *Sal*I sites by ClonExpress MultiS One Step Cloning Kit (Vazyme Biotech, Nanjing, China) for overexpression in SC2-M1. To evaluate the promoter activity of target DNA fragments, the fragments were cloned from genomic DNA, and the promoterless green fluorescent protein (gfp) fragment from pGFP4412 was cloned (Li et al., 2019). The two fragments were fused using PCR and ligated into pHY300PLK between *Bgl*II and *Sal*I sites as described above, thus generating the pHY300PLK-promoter-gfp plasmid.

Chromatin immunoprecipitation assay with sequencing (ChIP-seq)

The SC2-M1 and SC2-M1-Flag strains were cultured in 50 ml of LB medium at 37°C with shaking at 180 rpm for 10 h, inoculated with an initial OD₆₀₀ of 0.8 in 50 ml of LB broth by 2% (v/v), and cultured at 37°C with shaking at 180 rpm for 7 h. The two strains were then fixed with 1% formaldehyde for 10 min at 28°C and the reaction was stopped with 125 mM glycine for 5 min. The cells of the two strains were washed thrice with phosphate buffer solution (PBS) at 8000 rpm for 5 min at 4°C and stored at -80°C. ChIP was performed by Wuhan IGENEBOOK Biotechnology Co. Ltd., China. The manufacturer's instructions of Illumina TruSeq ChIP Sample Prep Set A were followed to construct sequencing libraries using chromatin immunoprecipitated DNA which was then sequenced on an Illumina Xten using the PE 150 method (Chen et al., 2021). The obtained reads were filtered by Trimmomatic (v 0.30). Clean reads were then mapped to the *P. polymyxa* SC2 genome using Bwa (v 0.7.15), which allowed for up to two mismatches (Pjanic, 2017). MsmR1 peaks were produced and visualized using MACS software and Integrative Genomics Viewer (IGV, v 2.3.91), respectively (Zhang et al., 2008; Thorvaldsdóttir et al., 2013). To understand the potential roles of genes, Kyoto Encyclopedia of Genes and Genomes (KEGG) and multiple EM for motif elicitation (MEME) were used for pathway enrichment analysis and motif analysis, respectively (Chen et al., 2021; Wang et al., 2022).

Quantitative real-time PCR

SteadyPure Universal RNA Extraction Kit (Accurate Biology, Hunan, China) was used to purify the total RNA from the target bacteria. cDNA was obtained from 1 µg of total RNA using the Evo M-MLV RT Kit with gDNA clean for qPCR II (Accurate Biology, Hunan, China). qRT-PCR analysis was performed on a Roter-Gene Q PCR system using the SYBR Green Pro Taq HS qPCR Kit (Accurate Biology, Hunan, China). The relative expression of target genes was calculated using the $2^{-\Delta\Delta Ct}$ method (Liu et al., 2021). The housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) was used as an internal control.

Purification of MsmR1 protein and electromobility shift assays

An 828 bp fragment (275 amino acids) of *msmR1* was cloned from the genomic DNA of strain SC2-M1 using primers MsmR1F and MsmR1R (Supplementary Table S2). The fragments of *msmR1* and plasmid pET-28b(+) were simultaneously cleaved with *Xho*I and *Sal*I, purified, ligated using T4 DNA ligase, and transferred into *E. coli* DH5α to obtain pET28b(+)-*msmR1*.

To overexpress the MsmR1 protein, the plasmid pET28b(+)-*msmR1* was transferred into *E. coli* BL21. The transformants were

cultured in kanamycin-resistant LB broth at 37°C with shaking at 180 rpm for 10 h. Next, the cells were inoculated into 50 ml of kanamycin-resistant LB broth at 2% (v/v) and cultured at 37°C with shaking at 180 rpm for approximately 1.2 h until the OD₆₀₀ reached 0.5–0.6. Then the cells were induced by 0.4 mM IPTG at 16°C for 20 h, harvested, washed, resuspended in PBS, and sonicated on ice. MsmR1-His6 protein was purified using BeyoGold™ His-tag Purification Resin (Beyotime, China) following the manufacturer's instructions. The purified protein was added to a final concentration of 10% (v/v) glycerol and stored at -80°C. EMSA was performed using a chemiluminescent EMSA kit (Beyotime, China; Zhang et al., 2021). The concentration of labeled probes and competitive probes were about 0.4 and 4 μM, respectively. The gradient concentration of protein were set up for the EMSA experiment.

Detection of the fluorescence intensity of promoters

Promoter reporter plasmids were transformed into *E. coli* DH5α cells. The strains were inoculated in LB plate containing 20 μg/ml tetracycline and fluorescence was observed with an OLYMPUS fluorescence microscope at 1000× (Li et al., 2019). The fluorescence intensity of the promoters was detected at different time points as previously reported (Li et al., 2019).

Antibacterial activity analysis

An Oxford cup assay was used to evaluate polymyxin production by the SC2-M1 strain, as previously described (Liu et al., 2021). Bacteria were inoculated in liquid LB medium and cultured at 37°C with shaking at 180 rpm for 10 h. Bacteria were then inoculated with an initial OD₆₀₀ of 0.8 in 50 ml fermentation medium (sucrose 40 g/l, (NH₄)₂SO₄ 8 g/l, CaCO₃ 5 g/l, KH₂PO₄ 0.2 g/l, MgSO₄ 0.2 g/l, NaCl 0.2 g/l) at 2% (v/v) and cultured at 37°C with shaking at 180 rpm for 48 h.

Motility assay

A motility assay was performed as previously reported (Guo et al., 2019). Bacteria were cultured on LB medium for approximately 12 h, after which the OD₆₀₀ was adjusted to 0.6. Diluted cell solutions (2 μl) were added to the center of the plate containing 0.5% glucose, 0.22% NaCl, 0.13% yeast extract, 0.45% tryptone, and 0.5% agar.

Growth curve assay

Bacteria were cultured overnight in liquid LB medium and adjusted to an OD₆₀₀ of 0.8. Diluted strain solutions were

inoculated at 2% in 50 ml of fresh LB medium. The bacteria were cultured at 37°C with shaking at 180 rpm. The OD₆₀₀ and number of live bacterial cells were measured every 3 h. A total of 100 μl of the strain solution was serially diluted with 900 μl of sterile water; 100 μl of the dilution was then spread on an LB plate. The plates were subsequently incubated for 24 h at 37°C.

The growth curves of bacteria on the carbon sources were determined using a basal medium. Bacteria were cultured overnight in LB medium. The cultures were centrifuged, washed thrice with basal medium, and resuspended to inoculate 50 ml of fresh basal medium with 1% sole carbon source; the original OD₆₀₀ value was approximately 0.1. Cultures were incubated at 37°C with shaking at 180 rpm, and growth curves were determined according to OD₆₀₀ every 3 h.

Statistical analysis

For data analysis, the analysis of variance (ANOVA) and Least Significant Difference (LSD) test ($p \leq 0.05$) were performed using statistical software SPSS (v 19.0, SPSS Inc., Chicago, IL, USA; Wang et al., 2020).

Results

Overview of the transcription factor MsmR1 from *Paenibacillus polymyxa* SC2

The MsmR1 of strain SC2, which is classified as an AraC/XylS family transcriptional regulator, contains two domains: an N-terminal heterologous dimerization domain and a C-terminal DNA-binding domain (Figure 1A). The C-terminus of MsmR1 shows high similarities to that of AraC family members: RhaR, AraC, UreR, MelR, MsmR, XylS, and Toxt. Seven α-helix were identified in the C-terminal domain of MsmR1, which combined to form two helix-turn-helix (HTH) motifs (Figure 1A).

AraC family TFs are often auto-regulated (Sun et al., 2016). Hence, the promoter activity of *msmR1* was evaluated using the pHY300PLK-*msmR1*-gfp plasmid. This plasmid was found to possess promoter activity in *E. coli* DH5α cells. In contrast, the control (pHY300PLK-gfp) showed no fluorescence (Supplementary Figure S2A). The pHY300PLK-*msmR1*-gfp plasmid was introduced into the strains SC2-M1 and SC2-M1-Δ*msmR1* which was a *msmR1* knock out strain obtained via homologous recombination (Supplementary Figure S2B). The fluorescence intensity of the strain SC2-M1-Δ*msmR1* was significantly higher than that of the SC2-M1 strain (Figure 1B), revealing that MsmR1 could auto-regulate its transcription. EMSA was further performed to determine whether MsmR1 is capable of direct auto-regulation. MsmR1 was found to specifically bind to its own promoter resulting in a retarded band (Figure 1C), indicating that MsmR1 auto-regulates its expression.

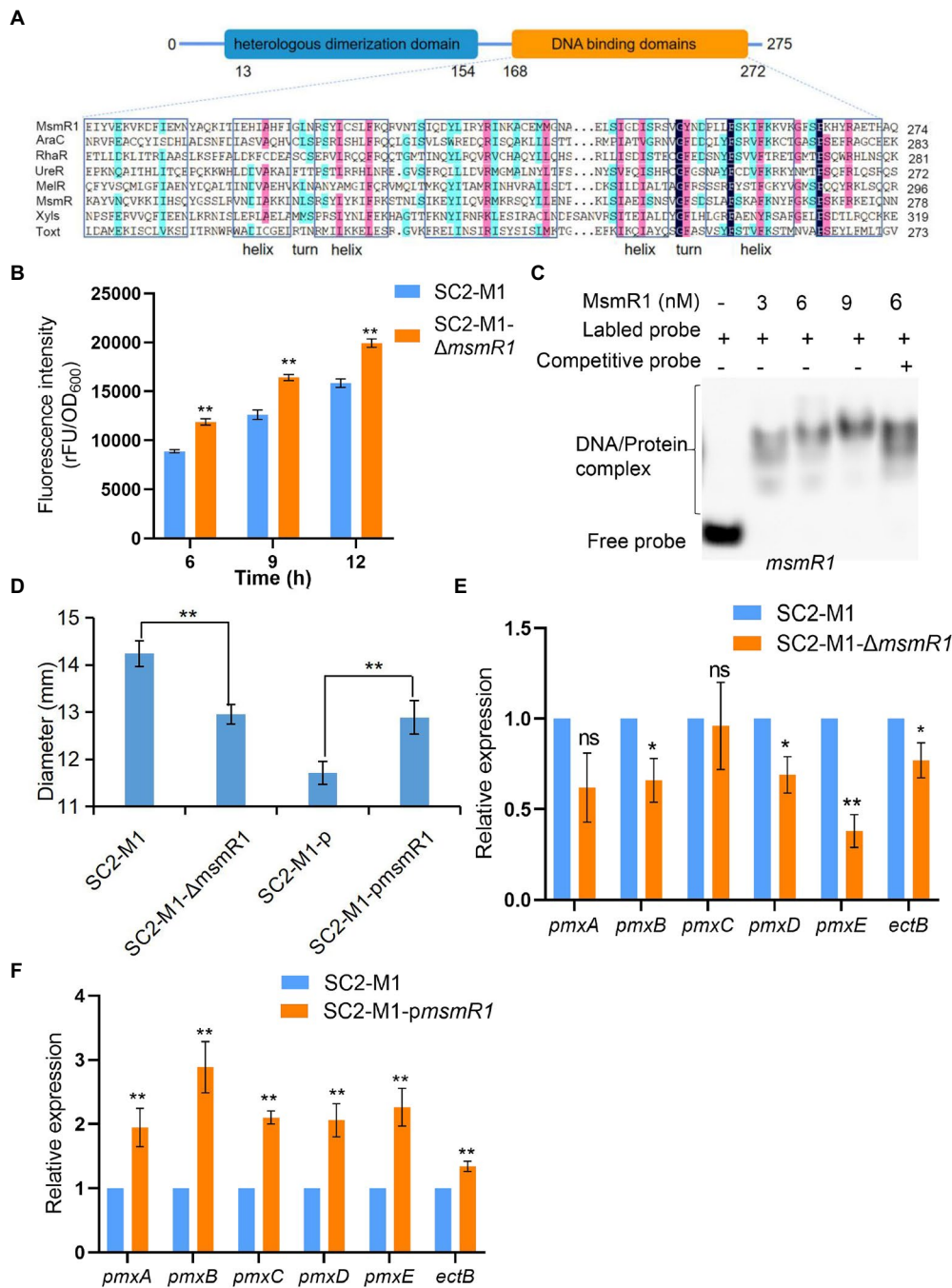


FIGURE 1
 Characteristics of MsmR1 protein in *Paenibacillus polymyxa* SC2-M1. **(A)** Homologous alignment of MsmR1 protein with several AraC family members: AraC (UniProtKB/Swiss-Prot: P0A9E0), RhaR (UniProtKB/Swiss-Prot: P09378), UreR (UniProtKB/Swiss-Prot: P32326), MelR (UniProtKB/Swiss-Prot: P0ACH8), MsmR (UniProtKB/Swiss-Prot: Q00753), XylS (UniProtKB/Swiss-Prot: P07859), and ToxT (UniProtKB/Swiss-Prot: A5F384). Sequences were aligned by DNAMAN software. **(B)** Fluorescence intensity of *msmR1* promoter in strains SC2-M1 and SC2-M1- Δ msmR1 detected at different time points. **(C)** EMSA results depicting MsmR1 binding to its own promoter. **(D)** Size of the inhibition zone between the SC2-M1, SC2-M1- Δ msmR1, SC2-M1-p, and SC2-M1-pmsmR1 strains was determined using the Oxford cup method. **(E)** Relative expression of genes related to polymyxin synthesis gene cluster in strain SC2-M1 and SC2-M1- Δ msmR1. **(F)** Relative expression of genes related to polymyxin synthesis gene cluster in the control strain SC2-M1-p and the overexpression strain SC2-M1-pmsmR1. * $p < 0.05$ and ** $p < 0.01$; ns, $p > 0.05$.

To investigate the function of MsmR1, *msmR1* knock out strain SC2-M1- Δ msmR1 and overexpression strain SC2-M1-pmsmR1 formed by ligation into pHY300PLK plasmid

(Supplementary Figure S2C), were used. Meanwhile, SC2-M1-p was set as the control. Notably, the amount of polymyxin in the mutant SC2-M1- Δ msmR1 was significantly less than that in the

SC2-M1 strain; the diameter of the inhibitory zone was decreased by 9.0% (Figure 1D; Supplementary Figure S2D). The qRT-PCR results revealed that the polymyxin synthetase genes were downregulated in the knockout strain compared with SC2-M1 (Figure 1E). In SC2-M1-*pmsmR1*, the inhibitory zone diameter was increased by 10.03% compared with SC2-M1-p (Figure 1D; Supplementary Figure S2D). The results were consistent with the qRT-PCR results which presented a significant increase in expression of polymyxin synthetase genes in SC2-M1-*pmsmR1* when compared with that in SC2-M1-p (Figure 1F).

Given that AraC family members are involved in carbohydrate metabolism (Cortés-Avalos et al., 2021), the growth curves of the SC2-M1 and SC2-M1- Δ *pmsmR1* strains were determined using LB and basal media with sole carbon sources. Although phenotypic differences were noted between the two strains using glucose, xylose, galactose, fructose, and lactose, the differences were not significant (Supplementary Figure S2E).

Identification of MsmR1 binding genes

To determine the target genes directly regulated by MsmR1 in *P. polymyxa* SC2, ChIP-seq experiments were performed using C-terminal FLAG-tagged *pmsmR1* in SC2-M1. The qRT-PCR results demonstrated that the expression of *pmsmR1* was high during the early stage and low during the decline phase (Supplementary Figure S3A). Based on the qRT-PCR results and the growth curve, a culture time of 7 h (mid-logarithmic phase) was selected for subsequent ChIP-seq analysis (Supplementary Figure S3).

Approximately 3.83–4.86 million reads in the ChIP-seq results were derived from the six samples, which were mapped to the *P. polymyxa* SC2 genome (Supplementary Table S3). Overall, 649 peaks were identified between the two strains, SC2-M1 and SC2-M1-Flag. Among these, 96.15% were located in the coding sequence (CDS) and 3.85% were located in the intergenic regions. To categorize the functions of MsmR1 target genes, KEGG enrichment analysis was performed. The target genes were enriched in various functions, including cellular processes (1.56%), environmental information processing (6.25%), genetic information processing (10.94%), and metabolism (81.25%; Supplementary Table S4). In the metabolism category, carbohydrate metabolism was the most enriched pathway, while amino acid metabolism, metabolism of cofactors and vitamins, lipid metabolism, energy metabolism, and nucleotide metabolism were also significantly enriched (Figure 2). Hence, MsmR1 primarily regulates basal metabolism in SC2 cells. According to the predicted results of MEME, we selected the fragments related to primary metabolism for further verification (Supplementary Table S5).

EMSA was performed to further validate the potential direct binding fragments with MsmR1. As shown in Figures 3A–K, MsmR1 could bind to the upstream fragments of *oppC3*, *sucA*, *sdr3*, *ycyN*, *pepF*, *pppL*, *ydfp*, *PPSC2_23180*, *gabD*, *araA*, and *bglX3*. The qRT-PCR results revealed that compared

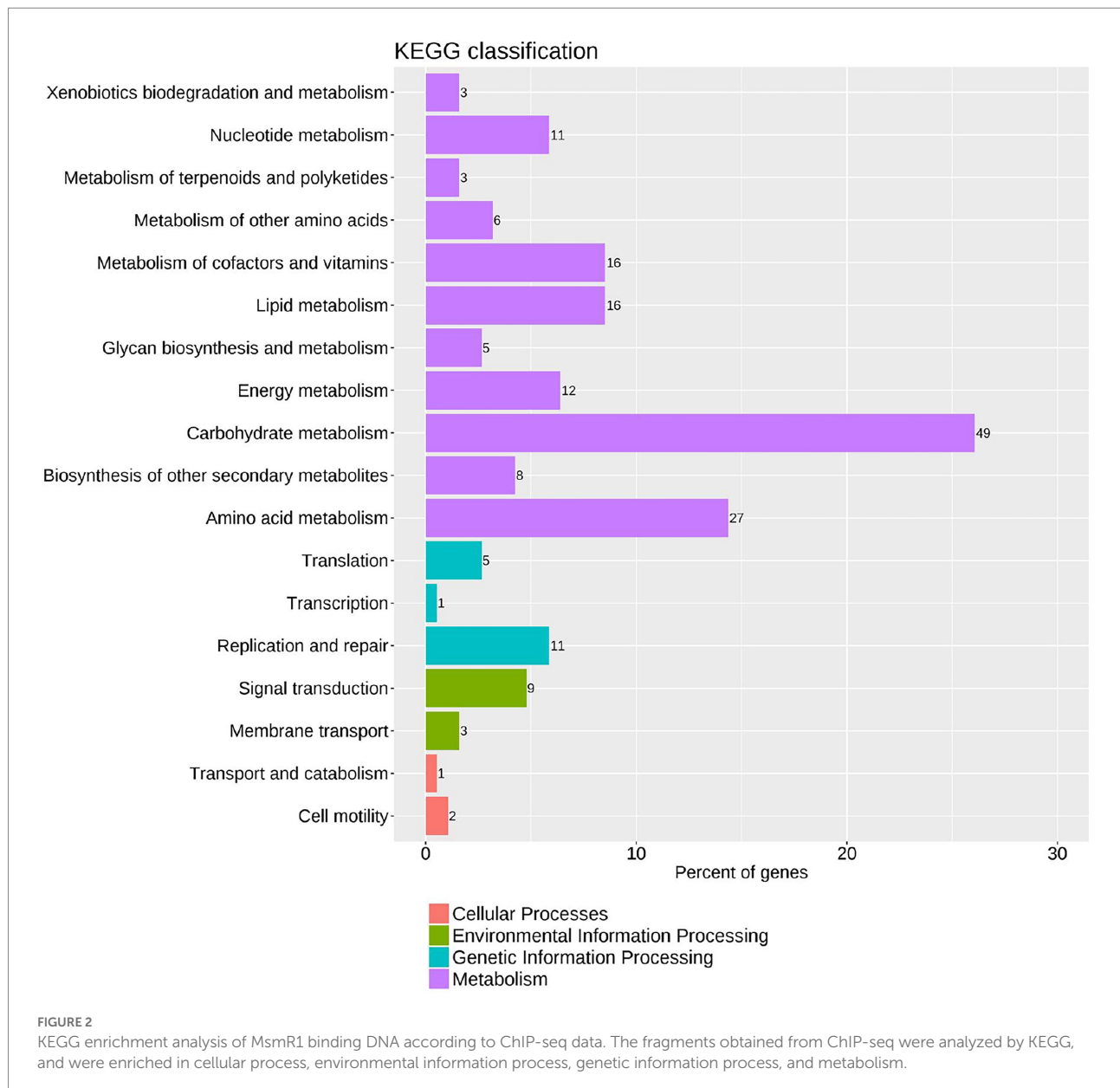
with strain SC2-M1, the relative expression of genes *oppC3*, *sucA*, *sdr3*, *ycyN*, *pepF*, *pppL*, *ydfp*, and *PPSC2_23180* were significantly decreased in *pmsmR1* mutant ($p < 0.05$, Figure 3L), which suggested that MsmR1 could positively regulate these genes. While no significant difference was observed in the expression of *gabD*, *araA*, and *bglX3* between SC2-M1 and SC2-M1- Δ *pmsmR1* ($p < 0.05$, Figure 3L). These results indicated that MsmR1 might not regulate the expression of *gabD*, *araA*, and *bglX3*, even though MsmR1 could bind to them. Additionally, we also determined two MsmR1-binding motif by MEME (Figure 3M).

MsmR1 positively regulates polymyxin synthesis by directly binding to the *oppC3* and *sdr3* promoters

As described previously, carbohydrate and amino acid could affect the biosynthesis of polymyxin (Yu et al., 2018). The *oppC3* gene encodes a peptide ABC transporter together with the OppB protein forming a transmembrane channel that can transport and release peptides and nickel into cells (Ge et al., 2018; Slamti and Lereclus, 2019). The gene *sdr3* whose predicted product belongs to the short-chain dehydrogenase/reductase (SDR) family is important for carbohydrate, amino acid, lipid metabolism, and redox sensor mechanisms (Kavanagh et al., 2008). *oppC3* and *sdr3* affected the growth state of strain SC2-M1 in LB medium (Figures 4A,B). Therefore, we determined the expression levels of primary metabolism-related genes, such as those involved in carbohydrate, amino acid, and fatty acid metabolism (Supplementary Table S2), and all of these genes were downregulated in the mutant strains compared with SC2-M1 (Figures 4C,D). The growth state of SC2-M1- Δ *oppC3* and SC2-M1- Δ *sdr3* in basal medium with the sole carbon source supported their roles in carbohydrate metabolism (Supplementary Figures S4, S5). In the present study, polymyxin synthesis was significantly reduced in the knockout strains SC2-M1- Δ *oppC3* and SC2-M1- Δ *sdr3* compared with SC2-M1, and was recovered in the complement/overexpression strain (Figures 4E,F). Moreover, the qRT-PCR results confirmed that polymyxin synthetase genes were downregulated in the knockout strain compared with SC2-M1 (Figures 4C,D). The *oppC3* and *sdr3* genes indirectly influence polymyxin synthesis by reducing the rate of primary metabolism and nutrient uptake, and further reduce precursors and energy for polymyxin synthesis.

MsmR1 positively regulates the TCA cycle by directly binding to the *sucA* promoter

The *sucA* gene encodes 2-oxoglutarate dehydrogenase (E1), part of the 2-oxoglutarate dehydrogenase complex (Gayán et al., 2019). In the TCA cycle, α -ketoglutarate is converted to succinate, which requires the catalytic action of the 2-oxoglutarate

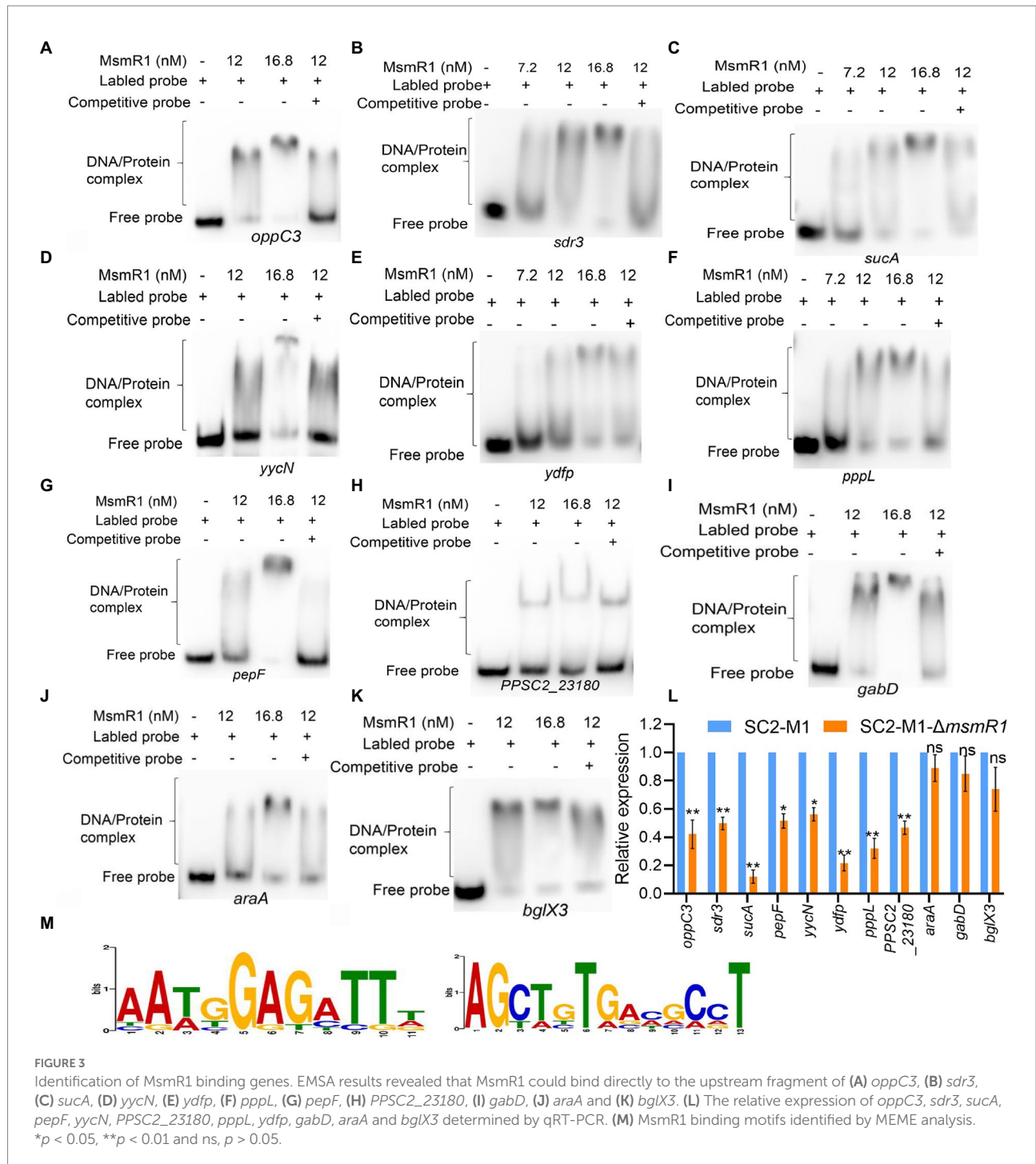


dehydrogenase complex (*sucAB*) and succinate dehydrogenase (*sucCD*; Walshaw et al., 1997). To assess the function of *sucA* in SC2, a knockout mutant strain SC2-M1- Δ *sucA* was generated. The colony morphology on LB plates appeared flat and transparent following *sucA* knockout, whereas the original morphology was recovered in the complemented strain SC2-M1- Δ *sucA*/*sucA* (Figure 5A).

The growth curve in LB medium was determined to evaluate the growth status, and showed that growth of the mutant strain SC2-M1- Δ *sucA* was markedly reduced compared to SC2-M1 and SC2-M1- Δ *sucA*/*sucA* (Figure 5B). Simultaneously, live bacterial counts were determined every 3 h; those of SC2-M1- Δ *sucA* were greatly reduced compared with those of SC2-M1 and SC2-M1- Δ *sucA*/*sucA*, which was consistent with the OD₆₀₀ results (Figure 5C). Moreover, expression of the related gene *sucB* was

markedly downregulated, while other genes involved in the TCA cycle, such as *sucC*, *sucD*, *citC*, *lpdV*, and *pdhC* (Supplementary Table S2) were markedly upregulated (Figure 5D). Due to disruption of the TCA cycle, bacteria may metabolize carbohydrate sources through the pentose phosphate pathway and glyoxylate cycle. Therefore, the energy decreased and the bacterial mass was further reduced.

During carbon source (xylose, arabinose, glucose, galactose, fructose, mannose, sucrose, maltose, trehalose, lactose and raffinose) utilization, the SC2-M1- Δ *sucA* strain presented a longer lag phase compared with the SC2-M1 strain. For the utilization of xylose, galactose, fructose, lactose, and raffinose, SC2-M1- Δ *sucA* presented a markedly lower OD₆₀₀ value during the stationary phase compared with SC2-M1 (Supplementary Figure S6). Hence, *sucA* have essential roles in carbon source utilization.

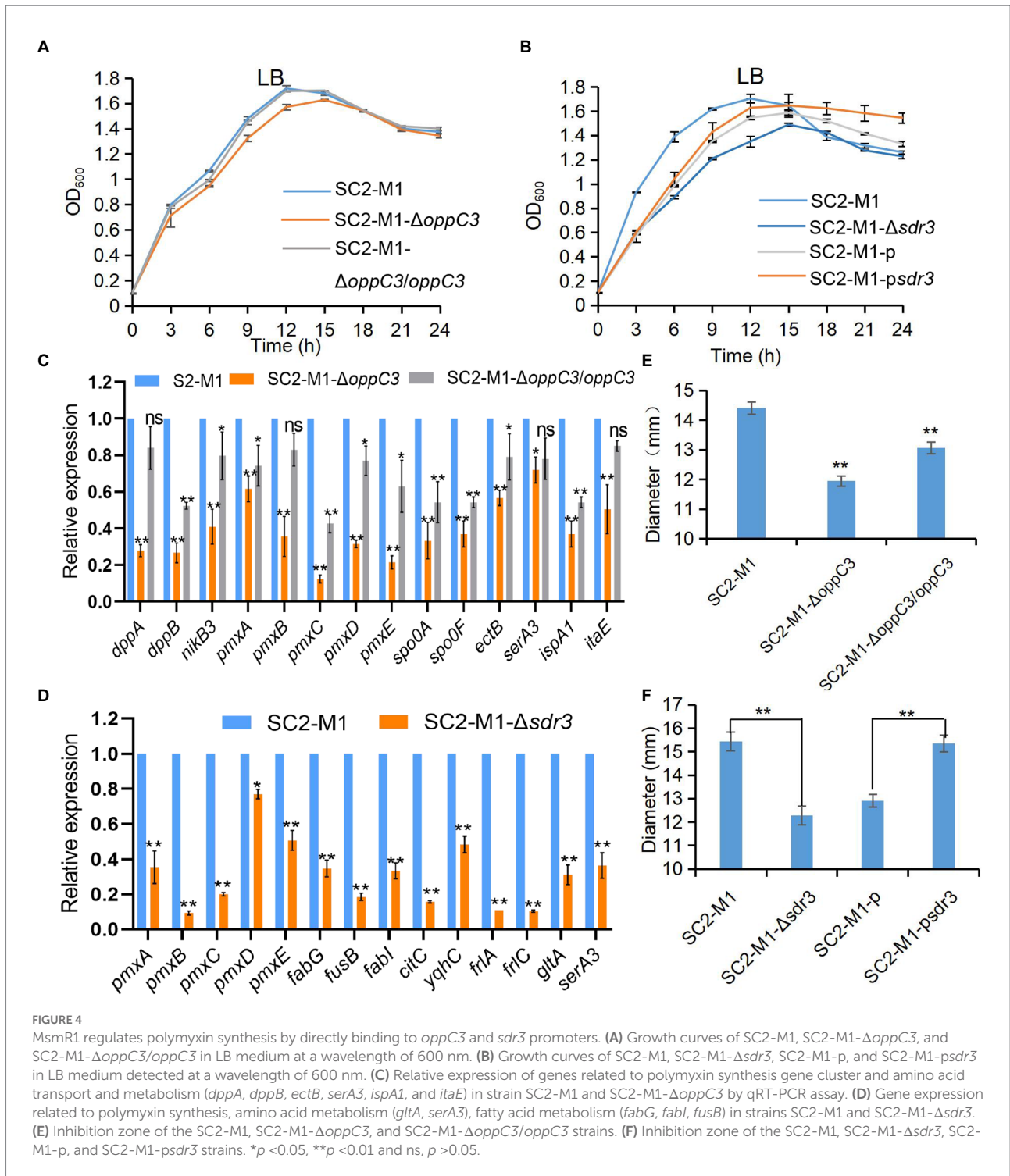


MsmR1 regulates motility, biofilm, and spore synthesis, as well as other processes

The *pepF* gene encodes an oligoendopeptidase F. In *Bacillus subtilis*, the *pepF* homolog *phrA* can regulate spore formation (Kanamaru et al., 2002), and in *Aeromonas hydrophila*, *pepF* can regulate biofilm formation (Du et al., 2016). qRT-PCR results revealed that *pepF* regulated spore and biofilm formation. In other

words, the relative expressions of genes related to biofilm and spore formation were downregulated (Figure 6A). The relative expressions of these genes were also downregulated in strain SC2-M1- Δ *msmR1* compared with SC2-M1 (Supplementary Figure S7). Overall, MsmR1 could positively regulate spore and biofilm formation.

Chemotaxis enables microbes to sense environmental signals and move to more favorable environments, which is important for bacterial colonization (Ud-Din and Roujeinikova, 2017). We found that motility was lost following *sdr3* deletion



and was increased in the SC2-M1-*psdr3* strain compared with that in the SC2-M1-p strain (Figure 6B). Moreover, the relative expression levels of genes encoding chemotaxis and flagellum (Supplementary Table S2) were downregulated in the SC2-M1- $\Delta sdr3$ strain compared with those in the SC2-M1 strain (Figure 6C). Taken together, these results indicate that MsmR1

may positively regulate cell motility by directly binding to the *sdr3* promoter.

The *yycN* gene encodes GNAT family acetyltransferase. The *ydfp* gene acts as a DoxX family protein, combined with SodA and SseA, forming a membrane-associated oxidoreductase complex (Nambi et al., 2015). The *pppL* gene encodes a PPM family protein

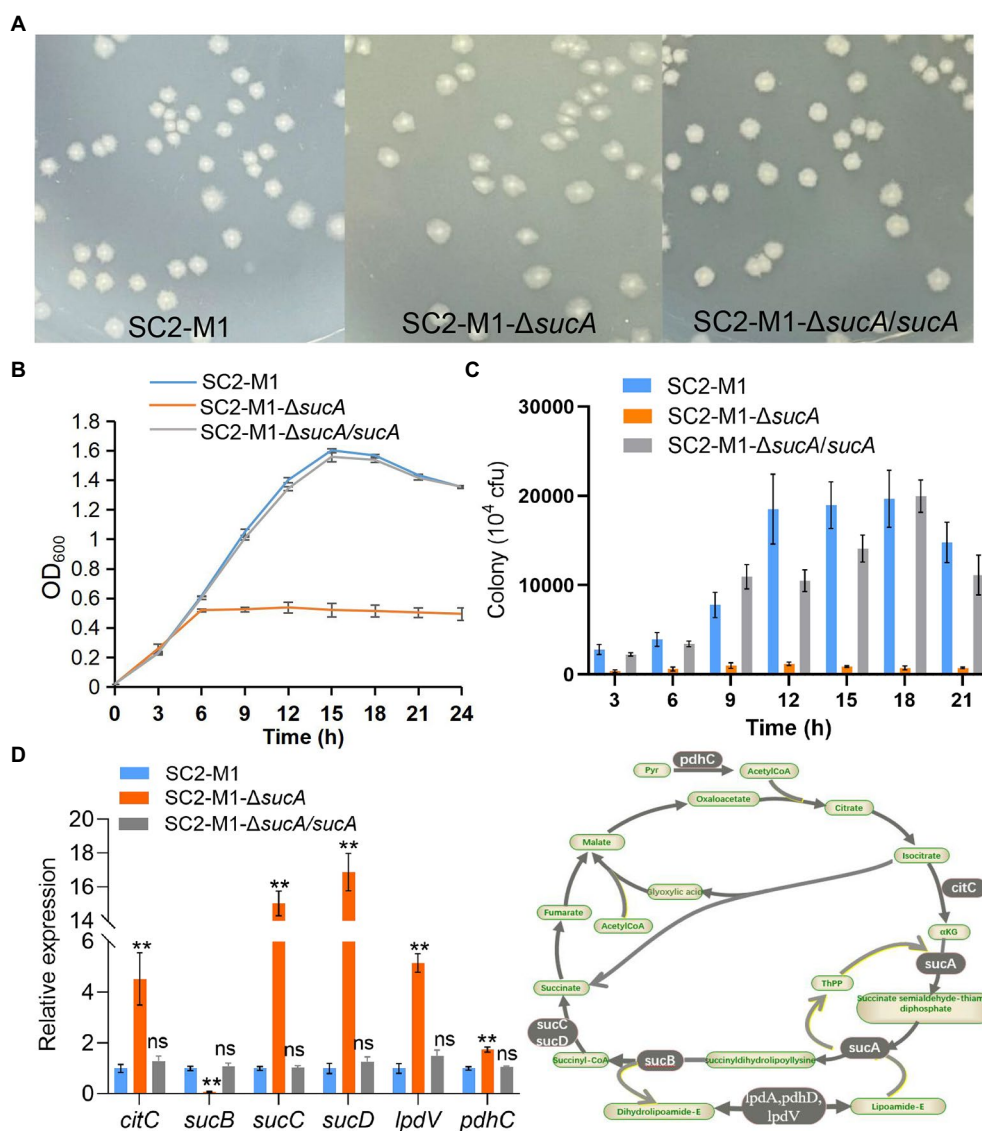


FIGURE 5 MsmR1 regulates carbohydrate metabolism by directly binding to the *sucA* promoter. **(A)** Colony morphology of the SC2-M1, SC2-M1-Δ*sucA*, and SC2-M1-Δ*sucA/sucA* strains in LB plates. **(B)** Growth curves of SC2-M1, SC2-M1-Δ*sucA*, and SC2-M1-Δ*sucA/sucA* in LB broth determined by OD₆₀₀. **(C)** SC2-M1, SC2-M1-Δ*sucA*, and SC2-M1-Δ*sucA/sucA* colonies in LB broth determined by the live bacterial count method every 3h. **(D)** Relative expression of genes related to the TCA cycle determined by qRT-PCR assay. ***p* < 0.01 and ns, *p* > 0.05.

phosphatase, which participates in environmental stresses (Shi et al., 1998). Hence, MsmR1 in *P. polymyxa* SC2 may also be involved in other biological processes.

Discussion

The purpose of this study was to elucidate the transcriptional regulatory mechanism of MsmR1 in *P. polymyxa* SC2. Sequence analysis revealed that MsmR1 comprises seven α-helices forming two HTH motifs at the C-terminal domain, which is the DNA binding domain, as well as a sensing or oligomerization domain of approximately 140 amino acids at the N-terminal domain.

MsmR1 is a typical AraC/XylS family transcriptional regulator (Egan, 2002), which can act as activators, repressors, or both, and are involved in various biological processes ranging from metabolism to stress response and virulence (Gahlot et al., 2021). In this study, a ChIP-seq experiment combined with EMSA was performed, the regulatory network of the global regulator MsmR1 was proposed (Figure 7), and the role of MsmR1 in *P. polymyxa* was described.

MsmR1 directly and positively regulates the expression of *oppC3*, *sucA*, *sdr3*, *pepF*, *ycyN*, *PPSC2_23180*, *pppL*, and *ydfp*. In particular, MsmR1 directly regulates *sucA* and influences the TCA cycle. OppC3, an ABC transporter, transports extracellular oligopeptides and oligosaccharides into cells, affecting primary

metabolism and polymyxin synthesis. Meanwhile, as a member of the short-chain dehydrogenases/reductases (SDRs) family, Sdr3 can influence fatty acid, carbohydrate, amino acid metabolism, and motility, which in turn reduces energy and precursors for polymyxin synthesis. PepF affects spore and biofilm formation. Due to the fact that spontaneous mutant strain SC2-M1 lost the ability to form endospores and biofilm (Hou et al., 2016), we could not detect the physiological or biochemical characteristics of endospores and biofilm in the test strains. However, since the genes were not successfully knocked out, the functions of *PPSC2_23180*, *ydfp*, *pppL*, and *yycN* required further investigation. Nevertheless, the results of this study revealed the mechanism through which MsmR1 regulates polymyxin synthesis, carbohydrate metabolism, and other biological processes.

We obtained 649 peaks by ChIP-seq. KEGG enrichment analysis of these sequences revealed that the genes were involved in various functions, including cellular processes, environmental information processing, genetic information processing, and metabolism. Among these, the proportion of genes enriched in metabolism was the highest, reaching 81.25%, of which carbohydrate metabolism was the most enriched, followed by amino acid metabolism. These results support previous findings indicating that MsmR is primarily involved in polysaccharide metabolism (Russell et al., 1992). In addition, only 3.85% of the

identified fragments were located in intergenic regions, whereas 96.15% were located in coding regions. This may be due to differences in the DNA-binding properties of MsmR1 *in vivo* and *in vitro*. Meanwhile, in *B. subtilis*, 643 binding sites for AbrB and 411 binding sites for Abh were identified, and approximately half of these binding sites were located in the gene coding region. Although half of all binding sites are located in intergenic regions, this does not affect transcription, even with strong binding intensities (Chumsakul et al., 2011). RutR is a regulator of pyrimidine catabolism in *E. coli*, and possesses 20 binding sites primarily bound to the gene coding regions with minimal effects on transcription levels (Shimada et al., 2008). Hence, MsmR1 may be a transcriptional regulator with binding site preferences that are similar to those of RutR.

Additionally, EMSA experiments revealed that MsmR1 could also bind its own promoter, however, this was not observed in the ChIP-seq results. In fact, most genes from the ChIP-seq results were not found to bind to MsmR1. There are several possible reasons for this. First, strains in the middle of the logarithmic growth phase were selected for ChIP-seq experiments; MsmR1 may interact weakly with these genes, and some binding sites may not have been detected (Galagan et al., 2013). Second, other cofactors may be required for MsmR1 to bind target genes. Transcription factors are

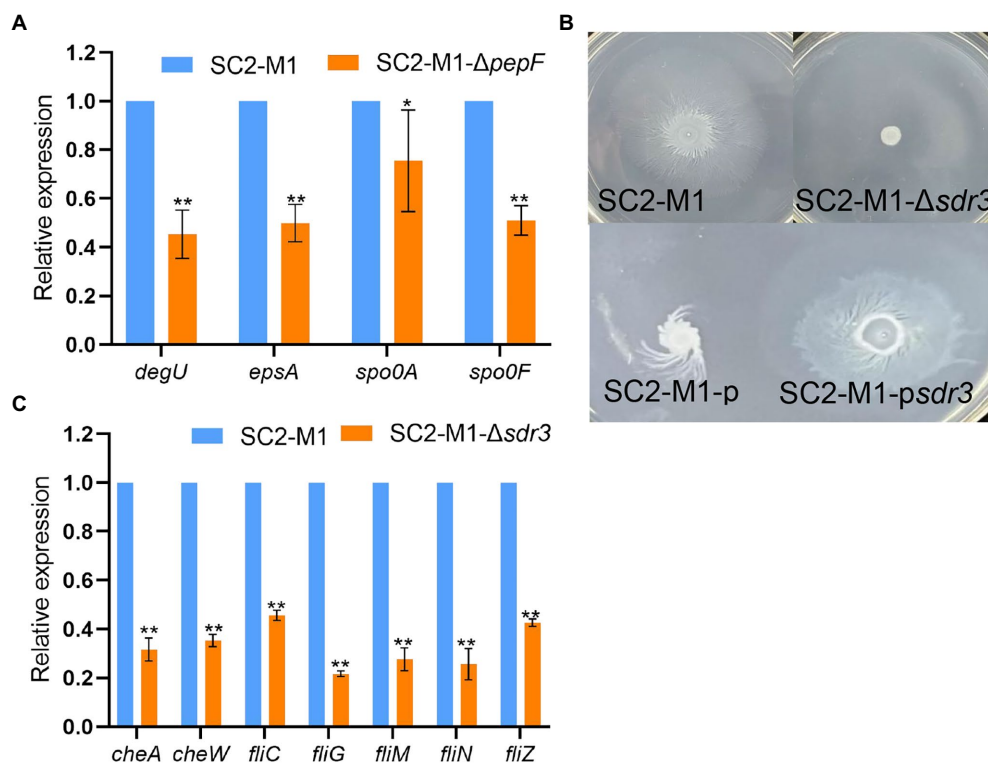


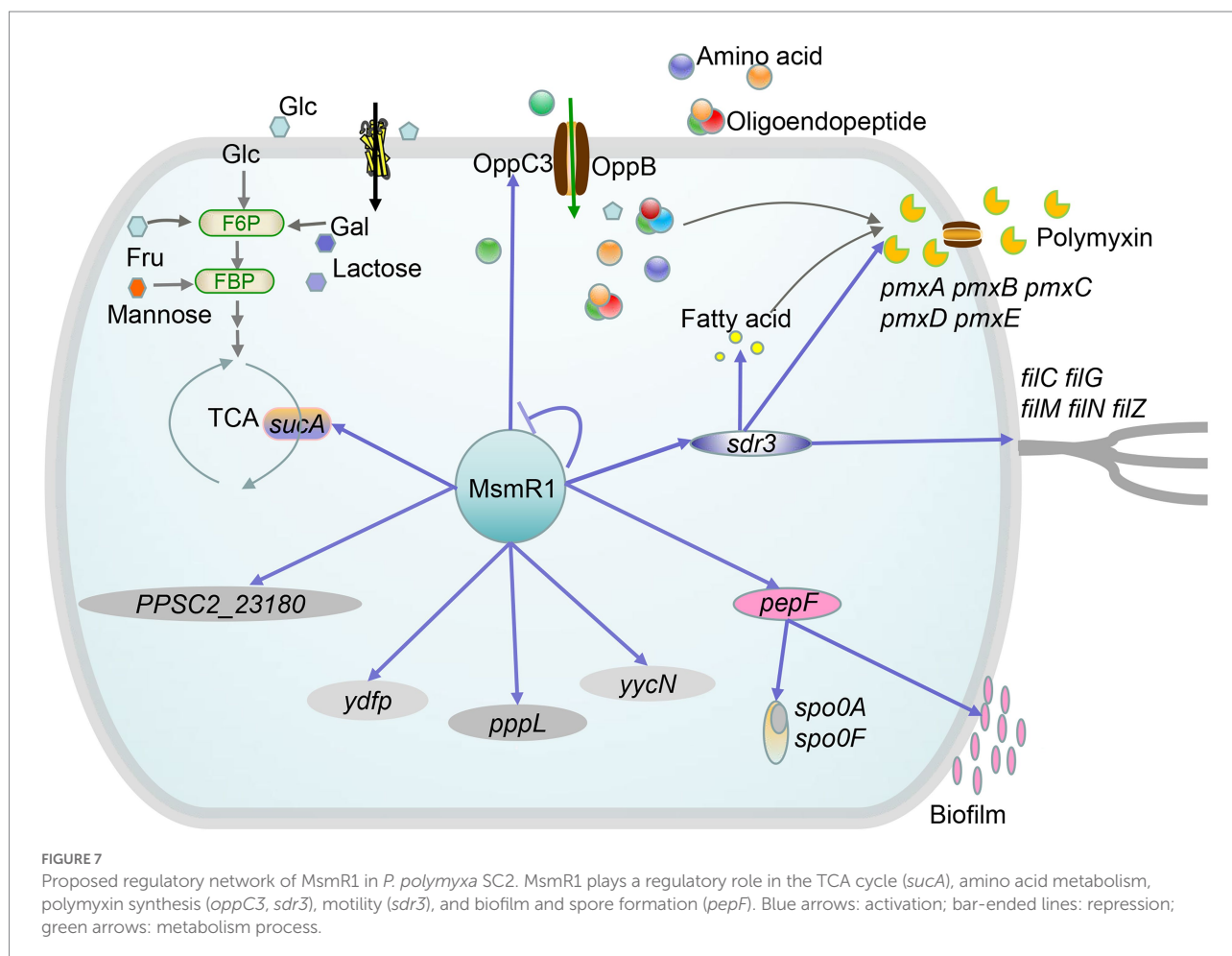
FIGURE 6

MsmR1 positively regulates motility, biofilm, and spore synthesis. (A) The expression of genes related to spore and biofilm formation in strain SC2-M1- $\Delta pepF$ and SC2-M1. (B) Motility of the SC2-M1, SC2-M1- $\Delta sdr3$, SC2-M1-p, and SC2-M1-psdr3 strains in a specific plate. (C) The expression of genes related to chemotaxis and flagellum (*cheA*, *cheW*, *fliC*, *fliG*, *fliM*, *fliN*, *fliZ*) determined by qRT-PCR. * $p < 0.05$ and ** $p < 0.01$.

typically coordinated by two or more transcription factors (Zhang et al., 2021). Third, adding a Flag tag to the C-terminus of MsmR1 with a *cat* resistance gene in the genome of SC2-M1 may interfere with the DNA binding ability and result in false-positive or -negative results. ChIP results reported for *Salmonella enterica* did not reveal previously described OmpR-binding targets but revealed that binding to some OmpR-regulated genes was affected by the C-terminal Flag tag (Perkins et al., 2013; Zhang et al., 2021). Finally, the EMSA conditions may influence the binding process. In the EMSA experiment, some retarded bands were relatively clear at low or high protein concentrations, while other DNA-MsmR1 binding processes presented clearer retarded bands only at high protein concentrations and smeared bands at low concentrations. This might be due to the different binding abilities of MsmR1 to DNA or the stability of the MsmR1-DNA complex *in vitro* during electrophoresis. As reported previously, a weak interaction occurs between the regulator VjbR and the *virB* (*PvirB*) promoter; however, no retarded signal was observed in the EMSA experiment, indicating that VjbR and *PvirB* cannot withstand the electrophoretic conditions of EMSA (Arocena

et al., 2010). Therefore, in ChIP-seq, some DNA may have bound weakly to MsmR1 causing it to not be detected during EMSA electrophoresis.

MsmR is a regulator of polysaccharide metabolism, hence the growth curve of the *msmR1* knockout strain was determined. The results revealed no significant change in the LB medium or minimal medium with different carbon sources (Supplementary Figure S2E). However, its role in carbohydrate metabolism cannot be ruled out, as most of the identified target genes are related to carbon source metabolism. We hypothesized that this may be due to the functional redundancy of MsmR1 homologs in the SC2 strain. For genome sequence analysis, three genes were annotated as *msmR* (*msmR5*, *msmR7*, and *msmR9*), and 44 AraC family transcription factors were identified in the genome of *P. polymyxa* SC2, most of which were related to carbon source metabolism, such as arabinose metabolism and L-rhamnose metabolism (Supplementary Table S6). This possibility was verified using qRT-PCR, which revealed that *msmR5*, *msmR7*, and *msmR9* were all upregulated after *msmR1* knockout (Supplementary Figures S8A–C). Since *msmR5* was the most upregulated, we then assessed the impact of knocking out *msmR5*. The results showed no significant difference in SC2-M1, single mutant of *msmR5* (SC2-M1- Δ *msmR5*) and double mutant



(SC2-M1- Δ *msmR1/msmR5*; [Supplementary Figure S8D](#)). In *Pseudomonas aeruginosa* PAO1161, PA3027 is an AraC-type transcriptional regulator with no effect on growth or carbon source utilization due to the functional redundancy of *glpD* homologs ([Kotecka et al., 2021](#)). Moreover, in *Ustilaginoidea virens*, due to the functional redundancy of other cutinase genes, cutinase activity was not affected after knockout of the cutinase G-box-binding protein UvCGBP1 ([Chen et al., 2021](#)). As a result of gene functional redundancy, growth status and carbon source utilization could not be considered the main markers of the *msmR1* knockout mutant.

AraC family transcription factors often form symmetric dimers and bind to palindromic DNA sequences ([Kato et al., 2005](#); [Sun et al., 2016](#)). A 5-nt inverted repeat was identified by binding site analysis of the AraC family transcription factor SAV742, separated by 10 or 12 nt (GCCGA-n10/n12-TCGGC). Mutating the probe revealed that the 5-nt inverted repeat was essential for SAV742 binding ([Sun et al., 2016](#)). In *E. coli*, the TF RhaS could bind to the promoter region of *rhaI*; ATCTTCCCTGGTTGCC ([Egan and Schleif, 1994](#)). In *Pseudomonas putida*, Xyls could bind to two 15 bp direct repeats (TGCA-N6-GGNTA; [Domínguez-Cuevas et al., 2008](#)). In *Vibrio cholerae*, ToxT binds to a “toxbox” (yrTTTtwTwAwW). In the *tcpA*, *ctxAB*, and *tcpI-2* promoters, two “toxboxes” are direct repeats, while in the *acfD/acfA*, *tagA*, and *tcpI-1* promoters, two “toxboxes” are inverted repeats ([Weber and Klose, 2011](#)). In *Streptomyces griseus*, the AdpA regulon consensus binding sequence is 5'-TGGCSNGWWY-3', serving as inverted repeats at both ends, separated by 13–14 bp loops ([Yamazaki et al., 2004](#)). In contrast, *Streptococcus pyogenes* possesses two identical MsmR-binding motifs, ACTGTGACCATAA and GAATAGCTATTC, without palindromic sequences or inverted repeats ([Nakata et al., 2005](#)). In a study on the binding sequences (*emm*, *mrp*, *arp*, *enn*, and *scpA*) of Mga in *Streptococcus pyogenes*, a 45-bp binding site was identified by DNase I protection experiments, containing three conserved nucleotide regions and 29 nucleotides, separated by several unrelated nucleotides ([Almengor and McIver, 2004](#)). This consensus sequence failed to identify the location of the Mga-binding sites in the *mga*-promoter ([McIver et al., 1999](#)), suggesting that Mga can bind with different sequences ([Almengor and McIver, 2004](#)). In a study on the DNA-binding site of MsmR1 in the SC2 strain, two motifs were preliminary identified, while no palindromic sequences or inverted repeats were found. At the same time, no similar motifs were identified with those of other homologue MsmR1 proteins.

Oligopeptide permease (Opp) is primarily involved in importing short-chain peptides, such as dipeptide and tripeptide, from the extracellular environment ([Solomon et al., 2003](#); [Ge et al., 2018](#)). The Opp system play an important role in the uptake of limiting nutrients like nickel, proline and betaine, especially under starvation and stress conditions ([Alloing et al., 2006](#); [Ge et al., 2018](#)). Additionally, the Opp system plays a crucial role in various processes controlled by

signal peptides, including sporulation, virulence, and binding ([Slamti and Lereclus, 2019](#)). In fact, OppC is required for peptide uptake but not for cell viability ([Solomon et al., 2003](#)). In this study, construction of an *oppC3*-knockout mutant revealed that *oppC3* also has an important role in amino acid metabolism. Simultaneously, the expression levels of genes related to nickel and sporulation were significantly downregulated following *oppC3* knockout. Polymyxin synthesis requires various amino acids as precursors. Owing to the downregulation of amino acid metabolism-related genes, precursor substances for polymyxin biosynthesis were reduced, further inhibiting the synthesis of polymyxin. In addition, *oppC3* may play an important role in oligosaccharide uptake by detecting the utilization of a sole carbon source. More specifically, *oppC3* plays a crucial role in the uptake of lactose and sucrose, but not maltose and trehalose. Meanwhile, with raffinose, a trisaccharide composed of glucose, galactose, and fructose, strains with *oppC3* knockout did not exhibit significant growth, suggesting that *oppC3* is critical for raffinose uptake. Hence, given that the role of *oppC* in the uptake of oligosaccharides has not yet been reported, the findings of this study report an important novel role of *oppC3* in the uptake of oligosaccharides.

During the interaction between *P. polymyxa* SC2 and pepper, the biosynthetic genes of polymyxin and fusaricidin were upregulated, which induced systemic resistance in pepper and enhanced its resistance to certain pathogens. Simultaneously, the upregulation of genes related to chemotaxis, motility, and biofilm formation is beneficial for the colonization of strains in the rhizosphere of plants ([Liu et al., 2021](#)). MsmR1, a global transcriptional regulator, regulates biological processes, including polymyxin synthesis, carbohydrate metabolism, biofilm formation, chemotaxis, and motility, which are beneficial for the interaction between *P. polymyxa* SC2 and pepper. The findings of this study provide a theoretical basis for polymyxin production and the application of strain *P. polymyxa* SC2.

Conclusion

In the study, we investigated the transcriptional regulatory mechanism of MsmR1 in *P. polymyxa* SC2. MsmR1, a AraC/XylS family transcriptional regulator, is involved in polymyxin synthesis. In particular, MsmR1 was found to be highly associated with carbohydrate metabolism pathways. Indeed, MsmR1 affects polymyxin synthesis by directly regulating *oppC3* and *sdr3*, affecting basal metabolism and precursors for polymyxin synthesis, while also directly regulating *sucA* and influencing the TCA cycle. In addition, MsmR1 could influence spore and biofilm formation and other biological processes. These results provide a theoretical basis for the application of *P. polymyxa* SC2 in the biological control of various pathogenic bacteria in pepper.

Data availability statement

The data presented in the study are deposited in the NCBI Sequence Read Archive (SRA) repository, accession number PRJNA885964.

Author contributions

DZ performed the laboratory work, analyzed the data, and wrote the manuscript. HL, YC, and ST analyzed the data. CW, BD, and YD provided insights on the manuscript. YD and BD supported the study. All authors contributed to the article and approved the submitted version.

Funding

This work was funded by the National Natural Science Foundation of China (31770115), Key Research and Development Program of Shandong Province (2021CXGC010804), and Tai-Shan Scholar Program from the Shandong Provincial Government.

References

- Alloung, G., Travers, I., Sagot, B., Le Rudulier, D., and Dupont, L. (2006). Proline betaine uptake in *Sinorhizobium meliloti*: characterization of Prb, an Opp-like ABC transporter regulated by both proline betaine and salinity stress. *J. Bacteriol.* 188, 6308–6317. doi: 10.1128/JB.00585-06
- Almengor, A. C., and McIver, K. S. (2004). Transcriptional activation of *sclA* by Mga requires a distal binding site in *Streptococcus pyogenes*. *J. Bacteriol.* 186, 7847–7857. doi: 10.1128/JB.186.23.7847-7857.2004
- Arocena, G. M., Sieira, R., Comerici, D. J., and Ugalde, R. A. (2010). Identification of the quorum-sensing target DNA sequence and N-Acyl homoserine lactone responsiveness of the *Brucella abortus virB* promoter. *J. Bacteriol.* 192, 3434–3440. doi: 10.1128/JB.00232-10
- Ash, C., Priest, F., and Collins, M. (1993). Molecular identification of rRNA group 3 bacilli using a PCR probe test. *Anton. Leeuw. Int. J. G.* 64, 253–260. doi: 10.1007/BF00873085
- Belyaeva, T. A., Wade, J. T., Webster, C. L., Howard, V. J., Thomas, M. S., Hyde, E. I., et al. (2000). Transcription activation at the *Escherichia coli melAB* promoter: the role of MeIR and the cyclic AMP receptor protein. *Mol. Microbiol.* 36, 211–222. doi: 10.1046/j.1365-2958.2000.01849.x
- Bustos, S. A., and Schleif, R. F. (1993). Functional domains of the AraC protein. *Proc. Natl. Acad. Sci. U. S. A.* 90, 5638–5642. doi: 10.1073/pnas.90.12.5638
- Cha-Aim, K., Hoshida, H., Fukunaga, T., and Akada, R. (2012). Fusion PCR via novel overlap sequences. *Methods Mol. Biol.* 852, 97–110. doi: 10.1007/978-1-61779-564-0_8
- Chen, X., Li, P., Liu, H., Chen, X., Huang, J., Luo, C., et al. (2021). A novel transcription factor UvCGBP1 regulates development and virulence of rice false smut fungus *Ustilaginoida virens*. *Virulence* 12, 1563–1579. doi: 10.1080/21505594.2021.1936768
- Choi, S.-K., Park, S.-Y., Kim, R., Kim, S.-B., Lee, C.-H., Kim, J. F., et al. (2009). Identification of a polymyxin synthetase gene cluster of *Paenibacillus polymyxa* and heterologous expression of the gene in *Bacillus subtilis*. *J. Bacteriol.* 191, 3350–3358. doi: 10.1128/JB.01728-08
- Chumsakul, O., Takahashi, H., Oshima, T., Hishimoto, T., Kanaya, S., Ogasawara, N., et al. (2011). Genome-wide binding profiles of the *Bacillus subtilis* transition state regulator AbrB and its homolog Abh reveals their interactive role in transcriptional regulation. *Nucleic Acids Res.* 39, 414–428. doi: 10.1093/nar/gkq780
- Chung, C., Niemela, S. L., and Miller, R. H. (1989). One-step preparation of competent *Escherichia coli*: transformation and storage of bacterial cells in the

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.2022.1039806/full#supplementary-material>

- same solution. *Proc. Natl. Acad. Sci. U. S. A.* 86, 2172–2175. doi: 10.1073/pnas.86.7.2172
- Cortés-Avalos, D., Martínez-Pérez, N., Ortiz-Moncada, M. A., Juárez-González, A., Baños-Vargas, A. A., Estrada-de Los Santos, P., et al. (2021). An update of the unceasingly growing and diverse AraC/XylS family of transcriptional activators. *FEMS Microbiol. Rev.* 45:fuab020. doi: 10.1093/femsre/fuab020
- Domínguez-Cuevas, P., Marín, P., Marqués, S., and Ramos, J. L. (2008). XylS–Pm promoter interactions through two helix–turn–helix motifs: identifying XylS residues important for DNA binding and activation. *J. Mol. Biol.* 375, 59–69. doi: 10.1016/j.jmb.2007.10.047
- Du, H., Pang, M., Dong, Y., Wu, Y., Wang, N., Liu, J., et al. (2016). Identification and characterization of an *Aeromonas hydrophila* oligopeptidase gene *pepF* negatively related to biofilm formation. *Front. Microbiol.* 7:1497. doi: 10.3389/fmicb.2016.01497
- Egan, S. M. (2002). Growing repertoire of AraC/XylS activators. *J. Bacteriol.* 184, 5529–5532. doi: 10.1128/JB.184.20.5529-5532.2002
- Egan, S. M., and Schleif, R. F. (1994). DNA-dependent renaturation of an insoluble DNA binding protein: identification of the RhaS binding site at rhaBAD. *J. Mol. Biol.* 243, 821–829. doi: 10.1006/jmbi.1994.1684
- Gahlot, D. K., Ifill, G., and MacIntyre, S. (2021). Optimised heterologous expression and functional analysis of the *Yersinia pestis* F1-Capsular antigen regulator Caf1R. *Int. J. Mol. Sci.* 22:9805. doi: 10.3390/ijms22189805
- Galagan, J. E., Minch, K., Peterson, M., Lyubetskaya, A., Azizi, E., Sweet, L., et al. (2013). The *Mycobacterium tuberculosis* regulatory network and hypoxia. *Nature* 499, 178–183. doi: 10.1038/nature12337
- Gayán, E., Rutten, N., Van Impe, J., Michiels, C. W., and Aertsen, A. (2019). Identification of novel genes involved in high hydrostatic pressure resistance of *Escherichia coli*. *Food Microbiol.* 78, 171–178. doi: 10.1016/j.fm.2018.10.007
- Ge, Y., Lee, J. H., Hu, B., and Zhao, Y. (2018). Loss-of-function mutations in the Dpp and Opp permeases render *Erwinia amylovora* resistant to kasugamycin and blasticidin S. *Mol. Plant Microbe Interact.* 31, 823–832. doi: 10.1094/MPMI-01-18-0007-R
- Guérout-Fleury, A.-M., Frandsen, N., and Stragier, P. (1996). Plasmids for ectopic integration in *Bacillus subtilis*. *Gene* 180, 57–61. doi: 10.1016/s0378-1119(96)00404-0
- Guo, Q.-Q., Zhang, W.-B., Zhang, C., Song, Y.-L., Liao, Y.-L., Ma, J.-C., et al. (2019). Characterization of 3-oxacyl-acyl carrier protein reductase homolog genes in *Pseudomonas aeruginosa* PAO1. *Front. Microbiol.* 10:1028. doi: 10.3389/fmicb.2019.01028

- Henikoff, S., Wallace, J. C., and Brown, J. P. (1990). Finding protein similarities with nucleotide sequence databases. *Methods Enzymol.* 183, 111–132. doi: 10.1016/0076-6879(90)83009-x
- Hou, X., Yu, X., Du, B., Liu, K., Yao, L., Zhang, S., et al. (2016). A single amino acid mutation in Spo0A results in sporulation deficiency of *Paenibacillus polymyxa* SC2. *Res. Microbiol.* 167, 472–479. doi: 10.1016/j.resmic.2016.05.002
- Ibarra, J. A., Pérez-Rueda, E., Segovia, L., and Puente, J. L. (2008). The DNA-binding domain as a functional indicator: the case of the AraC/XylS family of transcription factors. *Genetica* 133, 65–76. doi: 10.1007/s10709-007-9185-y
- Jeong, H., Choi, S.-K., Ryu, C.-M., and Park, S.-H. (2019). Chronicle of a soil bacterium: *Paenibacillus polymyxa* E681 as a tiny guardian of plant and human health. *Front. Microbiol.* 10:467. doi: 10.3389/fmicb.2019.00467
- Kanamaru, K., Stephenson, S., and Perego, M. (2002). Overexpression of the PepF oligopeptidase inhibits sporulation initiation in *Bacillus subtilis*. *J. Bacteriol.* 184, 43–50. doi: 10.1128/JB.184.1.43-50.2002
- Kato, J.-y., Ohnishi, Y., and Horinouchi, S. (2005). Autorepression of AdpA of the AraC/XylS family, a key transcriptional activator in the A-factor regulatory cascade in *Streptomyces griseus*. *J. Mol. Biol.* 350, 12–26. doi: 10.1016/j.jmb.2005.04.058
- Kavanagh, K., Jörnvall, H., Persson, B., and Oppermann, U. (2008). Medium- and short-chain dehydrogenase/reductase gene and protein families: the SDR superfamily: functional and structural diversity within a family of metabolic and regulatory enzymes. *Cell. Mol. Life Sci.* 65, 3895–3906. doi: 10.1007/s00018-008-8588-y
- Kettle, B. C. (2013). *Genetic Analysis of allosteric signaling in RhaR from Escherichia coli and characterization of the VirF protein from Shigella flexneri*. [Doctoral dissertation thesis]. Kansas (IL): University of Kansas.
- Khoroshkin, M. S., Leyn, S. A., Van Sinderen, D., and Rodionov, D. A. (2016). Transcriptional regulation of carbohydrate utilization pathways in the *Bifidobacterium* genus. *Front. Microbiol.* 7:120. doi: 10.3389/fmicb.2016.00120
- Kolin, A., Balasubramaniam, V., Skredenske, J. M., Wickstrum, J. R., and Egan, S. M. (2008). Differences in the mechanism of the allosteric r-rhamnose responses of the AraC/XylS family transcription activators RhaS and RhaR. *Mol. Microbiol.* 68, 448–461. doi: 10.1111/j.1365-2958.2008.06164.x
- Kotecka, K., Kawalek, A., Kobylecki, K., and Bartosik, A. A. (2021). The AraC-type transcriptional regulator GliR (PA3027) activates genes of glycerolipid metabolism in *Pseudomonas aeruginosa*. *Int. J. Mol. Sci.* 22:5066. doi: 10.3390/ijms22105066
- Kreikemeyer, B., Nakata, M., Köller, T., Hildisch, H., Kourakos, V., Standar, K., et al. (2007). The *Streptococcus pyogenes* serotype M49 Nra-Ralp3 transcriptional regulatory network and its control of virulence factor expression from the novel eno ralp3 epf sagA pathogenicity region. *Infect. Immun.* 75, 5698–5710. doi: 10.1128/IAI.00175-07
- Li, H., Ding, Y., Zhao, J., Ge, R., Qiu, B., Yang, X., et al. (2019). Identification of a native promoter P_{LH-77} for gene expression in *Paenibacillus polymyxa*. *J. Biotechnol.* 295, 19–27. doi: 10.1016/j.jbiotec.2019.02.002
- Liu, H., Li, Y., Ge, K., Du, B., Liu, K., Wang, C., et al. (2021). Interactional mechanisms of *Paenibacillus polymyxa* SC2 and pepper (*Capsicum annuum* L.) suggested by transcriptomics. *BMC Microbiol.* 21, 70–16. doi: 10.1186/s12866-021-02132-2
- Lowden, M. J., Skorupski, K., Pellegrini, M., Chiorazzo, M. G., Taylor, R. K., and Kull, F. J. (2010). Structure of *Vibrio cholerae* ToxT reveals a mechanism for fatty acid regulation of virulence genes. *Proc. Natl. Acad. Sci. U. S. A.* 107, 2860–2865. doi: 10.1073/pnas.0915021107
- Ma, M., Wang, C., Ding, Y., Li, L., Shen, D., Jiang, X., et al. (2011). Complete genome sequence of *Paenibacillus polymyxa* SC2, a strain of plant growth-promoting rhizobacterium with broad-spectrum antimicrobial activity. *J. Bacteriol.* 193, 311–312. doi: 10.1128/JB.01234-10
- Mclver, K. S., Thurman, A. S., and Scott, J. R. (1999). Regulation of mga transcription in the group A streptococcus: specific binding of Mga within its own promoter and evidence for a negative regulator. *J. Bacteriol.* 181, 5373–5383. doi: 10.1128/JB.181.17.5373-5383.1999
- Mejía-Almonte, C., Busby, S. J., Wade, J. T., van Helden, J., Arkin, A. P., Stormo, G. D., et al. (2020). Redefining fundamental concepts of transcription initiation in bacteria. *Nat. Rev. Genet.* 21, 699–714. doi: 10.1038/s41576-020-0254-8
- Nakata, M., Podbielski, A., and Kreikemeyer, B. (2005). MsmR, a specific positive regulator of the *Streptococcus pyogenes* FCT pathogenicity region and cytolysin-mediated translocation system genes. *Mol. Microbiol.* 57, 786–803. doi: 10.1111/j.1365-2958.2005.04730.x
- Nambi, S., Long, J. E., Mishra, B. B., Baker, R., Murphy, K. C., Olive, A. J., et al. (2015). The oxidative stress network of *Mycobacterium tuberculosis* reveals coordination between radical detoxification systems. *Cell Host Microbe* 17, 829–837. doi: 10.1016/j.chom.2015.05.008
- Parra, M. C., and Collins, C. M. (2012). Mutational analysis of the N-terminal domain of UreR, the positive transcriptional regulator of urease gene expression. *Microbiol. Res.* 167, 433–444. doi: 10.1016/j.micres.2012.03.005
- Perkins, T. T., Davies, M. R., Klemm, E. J., Rowley, G., Wileman, T., James, K., et al. (2013). ChIP-seq and transcriptome analysis of the OmpR regulon of *Salmonella enterica* serovars Typhi and Typhimurium reveals accessory genes implicated in host colonization. *Mol. Microbiol.* 87, 526–538. doi: 10.1111/mmi.12111
- Pjanic, M. (2017). ChIPSeqFPro, a pipeline for sequential processing of ChIP-Seq fastq to bigwig files. *bioRxiv*:118281. doi: 10.1101/118281
- Pletzer, D., Schweizer, G., and Weingart, H. (2014). AraC/XylS family stress response regulators Rob, SoxS, PliA, and OpiA in the fire blight pathogen *Erwinia amylovora*. *J. Bacteriol.* 196, 3098–3110. doi: 10.1128/JB.01838-14
- Russell, R. R., Aduse-Opoku, J., Sutcliffe, I. C., Tao, L., and Ferretti, J. J. (1992). A binding protein-dependent transport system in *Streptococcus mutans* responsible for multiple sugar metabolism. *J. Biol. Chem.* 267, 4631–4637. doi: 10.1016/S0021-9258(18)42880-3
- Schleif, R. (2010). AraC protein, regulation of the l-arabinose operon in *Escherichia coli*, and the light switch mechanism of AraC action. *FEMS Microbiol. Rev.* 34, 779–796. doi: 10.1111/j.1574-6976.2010.00226.x
- Shi, L., Potts, M., and Kennelly, P. J. (1998). The serine, threonine, and/or tyrosine-specific protein kinases and protein phosphatases of prokaryotic organisms: a family portrait. *FEMS Microbiol. Rev.* 22, 229–253. doi: 10.1111/j.1574-6976.1998.tb00369.x
- Shimada, T., Ishihama, A., Busby, S. J., and Grainger, D. C. (2008). The *Escherichia coli* RutR transcription factor binds at targets within genes as well as intergenic regions. *Nucleic Acids Res.* 36, 3950–3955. doi: 10.1093/nar/gkn339
- Slamti, L., and Lereclus, D. (2019). The oligopeptide ABC-importers are essential communication channels in Gram-positive bacteria. *Res. Microbiol.* 170, 338–344. doi: 10.1016/j.resmic.2019.07.004
- Solomon, J., Su, L., Shyn, S., and Grossman, A. D. (2003). Isolation and characterization of mutants of the *Bacillus subtilis* oligopeptide permease with altered specificity of oligopeptide transport. *J. Bacteriol.* 185, 6425–6433. doi: 10.1128/JB.185.21.6425-6433.2003
- Sun, D., Zhu, J., Chen, Z., Li, J., and Wen, Y. (2016). SAV742, a novel AraC-family regulator from *Streptomyces avermitilis*, controls avermectin biosynthesis, cell growth and development. *Sci. Rep.* 6, 1–14. doi: 10.1038/srep36915
- Thorvaldsdóttir, H., Robinson, J. T., and Mesirov, J. P. (2013). Integrative genomics viewer (IGV): high-performance genomics data visualization and exploration. *Brief. Bioinform.* 14, 178–192. doi: 10.1093/bib/bbs017
- Timmusk, S., Grantcharova, N., and Wagner, E. G. H. (2005). *Paenibacillus polymyxa* invades plant roots and forms biofilms. *Appl. Environ. Microb.* 71, 7292–7300. doi: 10.1128/AEM.71.11.7292-7300.2005
- Ud-Din, A. I. M. S., and Roujeinikova, A. (2017). Methyl-accepting chemotaxis proteins: a core sensing element in prokaryotes and archaea. *Cell. Mol. Life Sci.* 74, 3293–3303. doi: 10.1007/s00018-017-2514-0
- Walshaw, D. L., Wilkinson, A., Mundy, M., Smith, M., and Poole, P. S. (1997). Regulation of the TCA cycle and the general amino acid permease by overflow metabolism in *Rhizobium leguminosarum*. *Microbiology* 143, 2209–2221. doi: 10.1099/0021287-143-7-2209
- Wang, P.-H., Sai, W., NIE, W.-H., Yan, W., Iftikhar, A., Ayizekeranmu, Y., et al. (2022). A transferred regulator that contributes to *Xanthomonas oryzae* pv. *Oryzicola* oxidative stress adaptation and virulence by regulating the expression of cytochrome bd oxidase genes. *J. Integr. Agric.* 21, 1673–1682. doi: 10.1016/S2095-3119(21)63801-7
- Wang, C., Zhao, D., Qi, G., Mao, Z., Hu, X., Du, B., et al. (2020). Effects of *Bacillus velezensis* FKM10 for promoting the growth of *Malus hupehensis* Rehd and inhibiting *Fusarium verticillioides*. *Front. Microbiol.* 10:2889. doi: 10.3389/fmicb.2019.02889
- Warda, A. K., Siezen, R. J., Boekhorst, J., Wells-Bennik, M. H., de Jong, A., Kuipers, O. P., et al. (2016). Linking *Bacillus cereus* genotypes and carbohydrate utilization capacity. *PLoS One* 11:e0156796. doi: 10.1371/journal.pone.0156796
- Weber, G. G., and Klose, K. E. (2011). The complexity of ToxT-dependent transcription in *Vibrio cholerae*. *Indian J. Med. Res.* 133:201.
- Yamazaki, H., Tomono, A., Ohnishi, Y., and Horinouchi, S. (2004). DNA-binding specificity of AdpA, a transcriptional activator in the A-factor regulatory cascade in *Streptomyces griseus*. *Mol. Microbiol.* 53, 555–572. doi: 10.1111/j.1365-2958.2004.04153.x
- Yin, Z., Wang, X., Hu, Y., Zhang, J., Li, H., Cui, Y., et al. (2022). *Metabacillus dongyingensis* sp. nov. is represented by the plant growth-promoting bacterium BY2G20 isolated from saline-alkaline soil and enhances the growth of *Zea mays* L. under salt stress. *mSystems* 7:e01426-21. doi: 10.1128/mSystems.01426-21
- Yu, Z., Guo, C., and Qiu, J. (2015). Precursor amino acids inhibit polymyxin E biosynthesis in *Paenibacillus polymyxa*, probably by affecting the expression of

polymyxin E biosynthesis-associated genes. *Biomed. Res. Int.* 2015, 1–11. doi: 10.1155/2015/690830

Yu, Z., Sun, Z., Yin, J., and Qiu, J. (2018). Enhanced production of polymyxin E in *Paenibacillus polymyxa* by replacement of glucose by starch. *Biomed. Res. Int.* 2018, 1–10. doi: 10.1155/2018/1934309

Zhang, Y., Liu, T., Meyer, C. A., Eeckhoutte, J., Johnson, D. S., Bernstein, B. E., et al. (2008). Model-based analysis of ChIP-Seq (MACS). *Genome Biol.* 9, R137–R139. doi: 10.1186/gb-2008-9-9-r137

Zhang, Q., Xing, C., Kong, X., Wang, C., and Chen, X. (2021). ChIP-seq analysis of the global regulator Vfr reveals novel insights into the biocontrol agent *Pseudomonas protegens* FD6. *Front. Microbiol.* 12:1156. doi: 10.3389/fmicb.2021.667637

Zwick, F., Lale, R., and Valla, S. (2013). Regulation of the expression level of transcription factor XylS reveals new functional insight into its induction mechanism at the Pm promoter. *BMC Microbiol.* 13, 1–12. doi: 10.1186/1471-2180-13-262