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ORIGINAL PAPER

A Novel LysR Family Factor STM0859 is Associated with The Responses of *Salmonella* Typhimurium to Environmental Stress and Biofilm Formation

ZHONGMEI MA[1#], NA LI[1#], CHENGCHENG NING[1#], YUCHENG LIU[2], YUN GUO[1], CHUNHUI JI[1], XIAOZHEN ZHU[1], QINGLING MENG[1], XIANZHU XIA[1], XINGXING ZHANG[2], XUEPENG CAI[3], KUOJUN CAI[4] and QIAO JUN[1*]

[1] College of Animal Science and Technology, Shihezi University, Shihezi, China
[2] Institute of Animal Science and Veterinary Research, Xinjiang Academy of Agricultural and Reclamation Science, Shihezi, China
[3] State Key Lab of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, China
[4] Center for Animal Disease Prevention and Control, Urumqi, China

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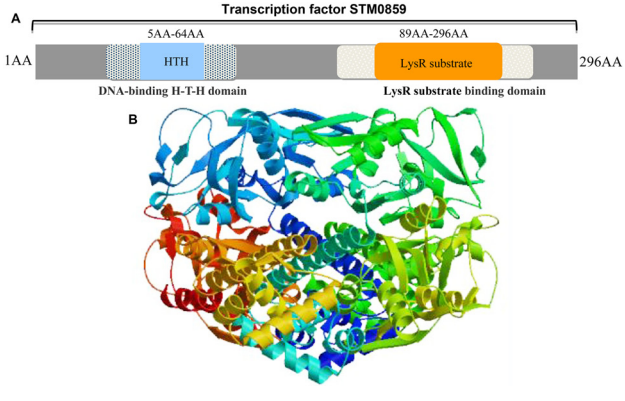
Abstract

Salmonella enterica subsp. *enterica* serovar Typhimurium (ST) is an intracellularly parasitic bacterium. This zoonotic pathogen causes food poisoning and thus imposes a severe threat to food safety. Here, to understand the regulatory roles of the novel transcription factor STM0859 on the response of ST to environmental stress and biofilm formation, the *STM0859* gene-deficient strain and the complementation strain *ΔSTM0859/STM0859* were generated, respectively. Then, its capacity of responding to environmental stresses and biofilm (BF) formation ability under different stresses, including acid, alkali, high salt, cholate, and oxidative stresses was tested. We further analyzed the interaction between the STM0859 protein and the promoter of the acid stress response-related gene *rcsB* by performing an electrophoresis mobility shift assay (EMSA). The results showed that acid resistance and BF formation capacities of ST-*ΔSTM0859* strain were significantly weaker, as compared with those of *Salmonella* Typhimurium SL1344 (ST-SL1344) wild strain ($p < 0.01$). Quantitative qRT-PCR analysis showed that the expression levels of acid stress and BF formation-related genes, *rcsB* and *rpoS*, of ST-*ΔSTM0859* strain were significantly reduced at the transcription levels, while the transcription levels of these genes were fully restored in complementation strain ST-*ΔSTM0859/STM0859*. The results of EMSA showed that STM0859 was capable of binding the promoter DNA fragments of the *rcsB* gene, suggesting that STM0859 can promote the transcription of the *rcsB* gene through interaction with its promoter, thereby exerting an indirectly regulatory role on the adaptive responses to acid stress and BF formation of ST. This study provided new insights into the regulatory mechanisms of the LysR family factors on the tolerances of ST under adverse environmental stresses.



Key words: *Salmonella* Typhimurium, biofilm, environmental stress, *STM0859* gene

Introduction

Salmonella is a gram-negative intracellular pathogenic bacterium (Richardson et al. 2018), widely present in contaminated animal-derived foods or drinking water. This bacterium frequently infects humans due to ingestion of contaminated foods (Jajere 2019). The infection of this zoonotic pathogen will result in various clinical symptoms, which are characterized by diarrhea, abdominal pain, and vomiting. Especially in immunocompromised individuals (e.g., infants, children, or the elderly), *Salmonella* can cause serious syndromes and

* Corresponding author: Q. Jun, College of Animal Science and Technology, Shihezi University, Shihezi, China; **e-mail: qj710625@shzu.edu.cn**
# Zhongmei Ma, Na Li and Chengcheng Ning contributed equally to this study.
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even death, leading to serious global food safety and public health issues (Zishiri et al. 2016).

As a representative strain of *Salmonella enterica* subsp. *enterica*, *Salmonella* Typhimurium (ST) can survive under unfavorable environmental conditions, such as dryness, extreme temperatures, antibiotics, and disinfectants. What is more, it can form a biofilm (BF) (Borges et al. 2018), which enables ST to acquire the capability of long-term survival in the contaminated eggs, meat, milk, and other animal-derived food sources, causing frequent occurrences of human food-borne salmonellosis (Lamas et al. 2018; Rohren et al. 2019). Currently available studies have shown that ST needs several transcriptional factors to regulate the expression of specific genes to adapt to the complex and changeable environments to withstand various adverse environmental stresses (Suar and Ryan 2015).

Among the transcriptional factors found in prokaryotes, LysR is an important transcription factor family, which was proved to be related to bacterial quorum sensing, response to environmental stress, biofilm (BF) formation, and regulation of virulence (Gebhardt et al. 2020). Through bioinformatics analyses, we have revealed that STM0859 protein is a novel member of LysR transcription factor family of ST. However, so far, the regulatory roles of STM0859 in ST response to environmental stress and BF formation remain unclear.

The main purpose of this study was to clarify the regulatory role of a novel transcription factor STM0859 of the LysR family in response to environmental stress and BF formation. Using λ-Red homologous recombination technology, we knocked out the *STM0859* gene and carried out the detection of responses to environmental stress, BF formation capability, and the expression levels of related genes of *STM0859* gene-deficient strains, aiming to provide new insights into the regulatory mechanism of STM0859 for the response to environmental stress in ST.

Experimental

Materials and Methods

Plasmids, strains and growth conditions. The plasmids pKD3, pKD46, pCP20, and pBR322 (The Key Laboratory of Preventive Veterinary, Shihezi University) were employed to construct recombinant vectors. *Salmonella* Typhimurium SL1344 was used as wild-type strain (The Key Laboratory of Preventive Veterinary, Shihezi University) to generate deletion and complementation strains of ST, while *Escherichia coli* TOP10 strain was used for all plasmid construction. These strains were routinely cultured in Luria-Bertani (LB) broth at 37°C with vigorous shaking or on LB agar plates containing 1.5% (WT/Vol) agar.

Design of primers. Based on ST-*STM0859* gene sequence (Accession number: FQ312003.1) deposited in GenBank, by using the Primer 5.0 software, we designed and constructed the *STM0859* gene-deficient strain and complementation strain. The related specific primers are listed in Table I.

Analysis of molecular characterization of STM0859 protein. The amino acid sequence of STM0859 protein encoded by *STM0859* gene was deduced, and then the molecular characterization of STM0859 was analyzed using online software such as Signal P 5.0 (http://www.cbs.dtu.dk/services/SignalP/), Blast, TMHMM 2.0, Prosite scan and Swiss-model software (http://www.expasy.org/), respectively.

Generation of ST-*ΔSTM0859* gene deletion mutant and complementation strains. Briefly, ST-SL1344 was cultured with LB (Aoboxing, China) broth, and then pKD46 plasmid was introduced into ST-SL1344 competent cells via electroporation. The positive transformants were screened out on LB culture containing Ampicillin (Amp) (100 mg/ml) (Sigma, Germany). The positive bacterial cells were verified by PCR with primer pair R1/R2. The positive bacteria were used as the homogeneous recombinant bacterial host. With pKD3 as the template, the target fragment being used to knock out the *STM0859* gene was amplified by PCR with primer pair R3/R4 targeting the left and the right arms of the *STM0859* gene, which were homogeneous to right and left ends of the *STM0859* gene and contains 50 bp in length. The amplified target fragment was recovered with DNA gel recovery kits (Omega, USA) and then introduced into the host bacterial SL1344-pKD46 competent cells via electroporation. The recombinant bacterial ST-*ΔSTM0859* cat was screened on LB plates containing Amp and chloramphenicol (Cm) (Sigma, Germany) (100 mg/ml Amp and 34 mg/ml Cm), verified by PCR, and sequenced. The ST-*ΔSTM0859* cat competent cells that were used to eliminate the pKD46 plasmid were prepared. After that, the pCP20 plasmid was introduced into these cells via electroporation. The transformed bacterial solution was sprayed evenly on a LB plate-containing Amp. The plate was placed in incubator at 30°C and cultured overnight. The positive clones were verified by PCR. The ST-*ΔSTM0859* gene-deficient strain was obtained and verified by sequencing. For the construction of the ST-*ΔSTM0859*/*STM0859*, the full-length *STM0859* gene with flank sequences was amplified using primers *STM0859*-F and *STM0859*-R and then cloned into pBR322 vector to generate pBR322-*STM0859*. Then, pBR322-*STM0859* was transferred into the ST-*ΔSTM0859* competent cells to obtain the complementation strain ST-*ΔSTM0859*/*STM0859*.

Effects of *STM0859* gene deficiency on ST responses to environmental stresses. The tolerance capacities of ST-SL1344, ST-*ΔSTM0859* and ST-*ΔSTM0859*/

Table I
The primers used in the study.

Primer name	Primer sequence (5'→3')	Target gene/DNA fragment	Product size (bp)
R1	TCTATGACAAGGATGAAGCC	pKD46	1,046
R2	GTATTTCCCGGCCTTTCTGT		
R3	ACTACGCCTGAAGGTGGCAACGGACCAAATATACAGGGATGTAACGCTATTAGAGCCTCTCAAAGCAAT	pKD3	1,198
R4	TCTATAAAAAAGCAGCACACTGTATTATACGTTAATTATGAGCCACAACGGTCTTGAGCGATTGTGTAGG		
R5	ACAACTCAAAGGAAAAGGAC	pCP20	1,202
R6	CACCAAAAACTCGTAAAAGC		
R7	GACCAAATTATACAGGGATGT	ST-*ΔSTM0859*cat/ ST-SL1344	220/1,146
R8	ATACGTTAATTATGAGCCACA		
R9	GGAATTCATGCACTTTGATATAAAAGATTTA	*STM0859*	905
R10	CCTCGAGTCACTCAGTCTGAGCTGTGAG		
rcsB F	AAAATGCCGGATGGCGG	*rcsB* promoter	102
rcsB R	TCGCTTTTATGTTACCCAGCC		
16*s RNA* F	GCGGTAATACGGAGGGTG	16*s RNA*	167
16*s RNA* R	CACCGCTACACCTGGAAT		
rcsB F	AGCGTCATTTTCCGAGC	*rcsB*	193
rcsB R	TCCAGCAGACGAGAAACG		
rpoS F	GGTGGATTCGCCAGACAA	*rpoS*	132
rpoS R	CGTGGTCCAGTTTATGCG		
Fur F	TTTTCCGCAATCAAGGC	*Fur*	158
Fur R	TGGGTGAAGAAATCGGTC		

STM0859 in environmental stress were determined by reference to the method reported in the literature (Peng et al. 2016). Briefly, the bacterial solutions were transferred in 1:100 ratio into 50 ml of BHI liquid medium with pH values of 4 and 10, 4% NaCl, 0.3% cholate, and 1 mM H_2O_2 and incubated for 2 h, respectively. Then $OD_{600\,nm}$ values of cultured bacterial solutions were measured hourly, and growth curves were plotted to analyze the effects of *STM0859* gene deficiency on ST's capacity to respond to environmental stresses. At the same time, the $OD_{600\,nm}$ values of the overnight cultured (LB broth) bacterial solutions were adjusted to 1.0 and then sprayed at 0.3% semi-solid LB medium with pH values of 4, and 7, respectively, and cultured in a stationary culture system for 16 h at 30°C, each experiment was repeated three times. And then, the diameters of the bacterial colonies in the Petri dish were measured with a ruler, and its motion ability was analyzed under different pH conditions.

Effects of *STM0859* gene deficiency on biofilm formation of ST. The BF was measured referencing the method described in the literature (Peng et al. 2016). Briefly, ST-SL1344, ST-*ΔSTM0859*, and ST-*ΔSTM0859*/*STM0859* were cultured in LB broth overnight and inoculated 200 μl into the 96-well microplate and cultured for 30 h and 48 h, respectively. Each sample was divided into two groups. For each group, 9 parallel repetitions were set. The cells were stained with 1.0% crystal violet solution. After being dried at room temperature, the microplate was placed under an inverted microscope and visualized. 95% ethanol was added to each well and decolored for 30 min. The $OD_{595\,nm}$ values were measured with a full-wave length microplate reader (Multiskan GO, USA).

Effects of *STM0859* gene deficiency on expression of environmental stress related genes. Using the 16*sRNA* gene as an internal reference control, we measured the expression levels of genes *rcsB*, *fur* and *rpoS* related to acid stress and biofilm formation by real-time quantitative RT-PCR (qRT-PCR) with those primers (Table I) at the transcription levels. Briefly, ST-SL1344, ST-*ΔSTM0859*, and ST-*ΔSTM0859*/*STM0859* were cultured in the LB medium to $OD_{600\,nm}$ value of about 1.0, and the total RNA was extracted according to Trizol (Invitrogen, USA) instructions. Reverse transcription was performed with the AMV reverse transcription kit (TaKaRa, Japan) according to the operation procedure provided. Then, the qRT-PCR assay was carried out with the LightCycler 480 instrument (Roche, Switzerland) according to the SYBR Premix Ex TaqTM kit's operation procedure (TaKaRa, Japan) with three repetitions per sample. The relative transcription level was calculated according to the method of $2^{-\Delta\Delta CT}$ for statistical analysis (Livak and Schmittgen 2001).

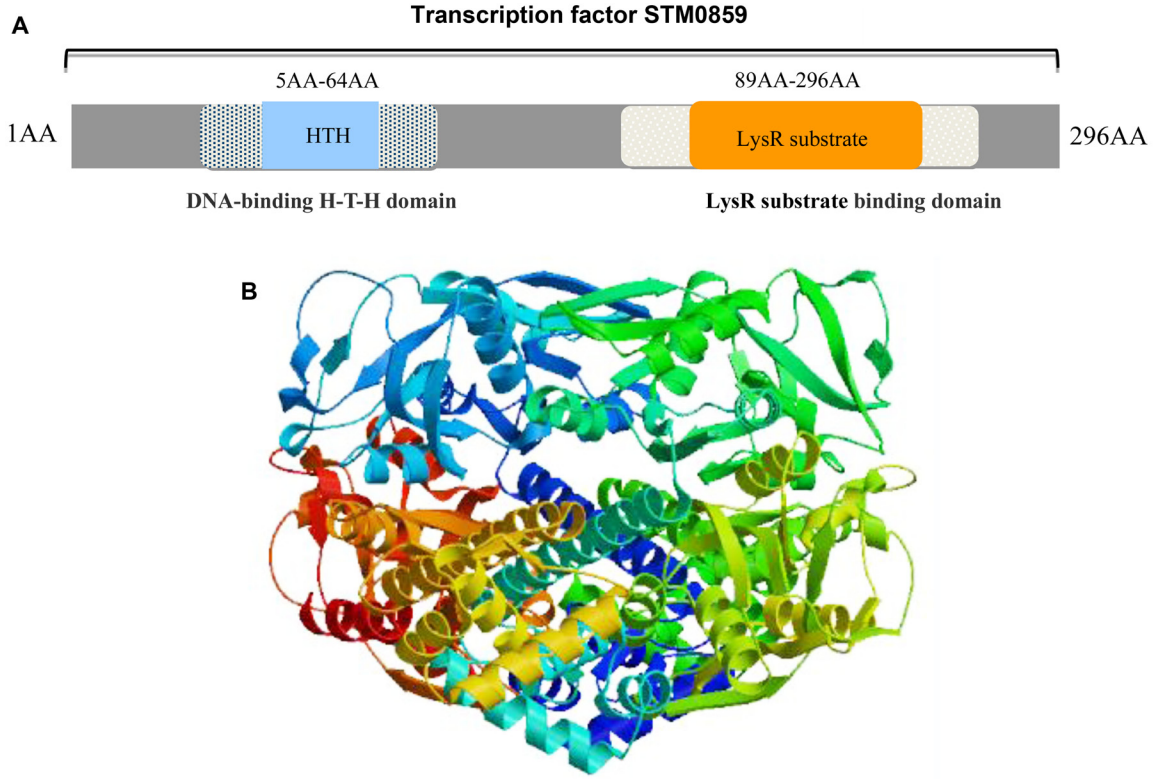

Fig. 1. Molecular characteristics of STM0859 of *Salmonella* Typhimurium; A) the H-T-H DNA binding and LysR substrate binding domains in STM0859 protein, B) prediction of 3D structure model of STM0859 protein.

Expression and purification of recombinant protein STM0859. Recombinant protein STM0859 was prepared using a prokaryotic expression system. In brief, the *STM0859* gene was cloned into pMD19-T (TaKaRa, Japan) to generate recombinant plasmid pT-STM0859. Then, pT-STM0859 and pET-32a (Invitrogen, USA) were simultaneously double digested with *Eco*R I and *Xho* I (TaKaRa, Japan). The digested products were separated via agarose gel electrophoresis. After gel electrophoresis, the target fragments and vectors were recovered and ligated with T4 ligase (TaKaRa, Japan) to generate pET-STM0859, which was correctly identified by PCR and restriction enzyme digestion and transformed into BL21 (DE3) strain of *E. coli* (Invitrogen, USA). After being induced with isopropyl β-D-1-thiogalactopyranoside (IPTG) (Sigma, Germany) for 8 h, the expressed protein was analyzed by SDS-PAGE and western blot. Then, the recombinant protein was purified according to the Ni-NTA protein purification system (Invitrogen, USA).

Analysis of the interaction between STM0859 and the promoter of *rcsB* gene. The interaction between the STM0859 protein and the *rcsB* promoter was analyzed using electrophoresis mobility shift assay (EMSA). Briefly, the *rcsB* promoter fragment was obtained by using the PCR method. After that, the *rcsB* promoter DNA fragment and STM0859 protein were co-incubated at room temperature for 30 min in the gel-shift buffer (Beyotime, China), with bovine serum protein (BSA) (Sigma, Germany) as the control protein. The *rcsB* promoter DNA fragment-STM0859 protein mixture was subjected to electrophoresis with 8% non-polyacrylamide gel, and the migration of *rcsB*-promoter DNA complex was observed in the gel imaging system after being stained.

Statistical analysis of data. The data were analyzed by SPSS 18 software (IBM SPSS, USA). The continuous variables were compared using the analysis of variance (ANOVA), while the Chi-square test was used to analyze categorical variables. *p*-Value less than 0.05 was considered significantly different, while *p* value less than 0.01 was considered highly significantly different.

Results

As shown in Fig. 1, STM0859 protein had a characteristic helix-turn-helix (HTH) DNA-binding and LysR substrate-binding domains (Fig. 1A). Besides, STM0859 protein was composed of α-helices, β-turns, and random coils, which formed a cage-like shape in 3D structure (Fig. 1B).

PCR showed that the homogeneous recombination strain SL1344-pKD46 was successfully constructed (Fig. S1A). The target fragment was amplified with R3/R4 primer and transferred into SL1344-pKD46 to

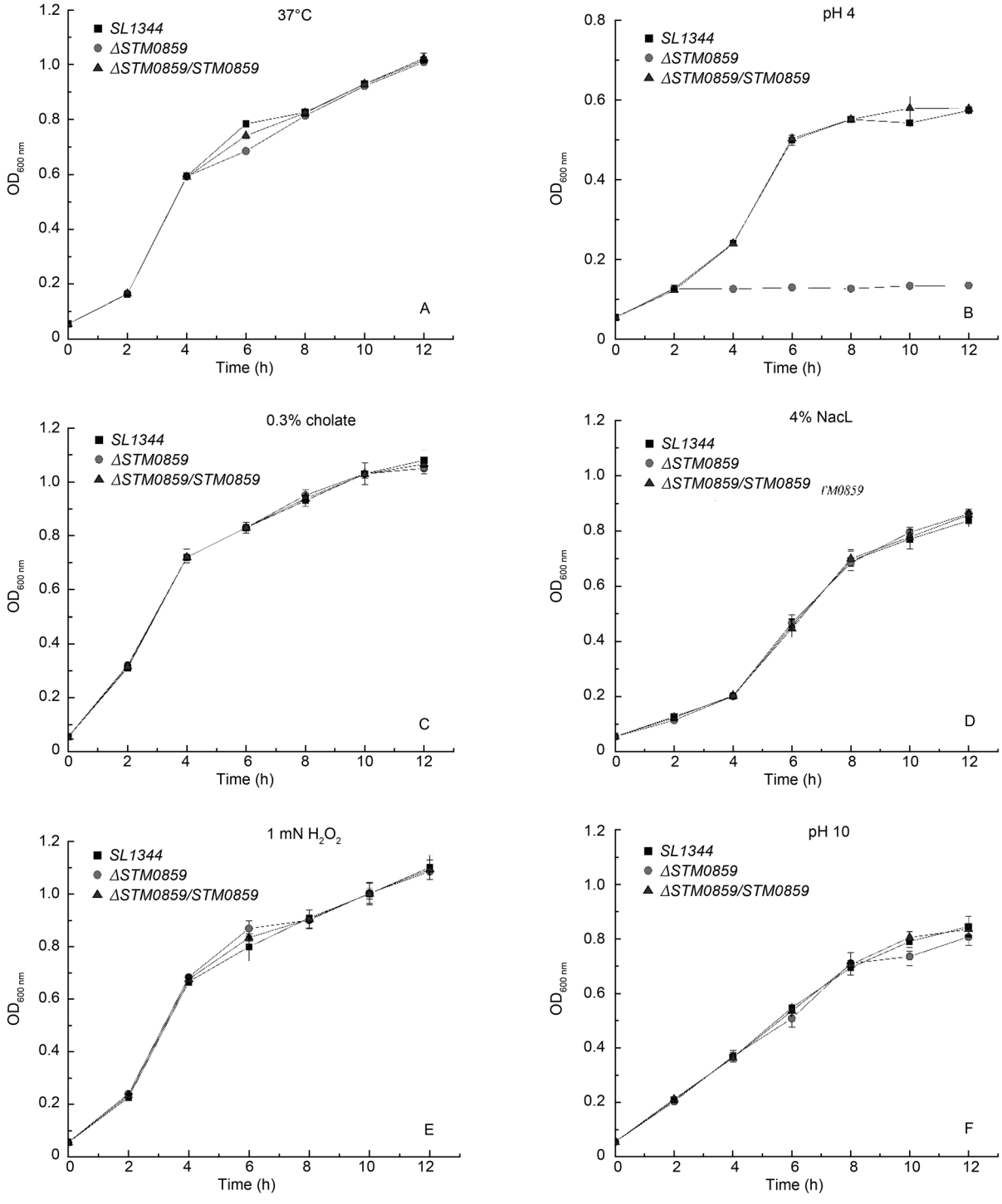

Fig. 2. Growth curves of SL1344, *ΔSTM0859* and *ΔSTM0859*/*STM0859* under different conditions; A-F) growth curves of SL1344, *ΔSTM0859* and *ΔSTM0859*/*STM0859* at 37°C, pH 4, 0.3% cholate, 4% NaCl, 1 mM H_2O_2 and pH 10, respectively.

generate ST-*ΔSTM0859* strain (Fig. S1B). PCR verification of ST-*ΔSTM0859* strain carrying pCP20 plasmid was performed with the primer pair R7/R8 (Fig. S2A). The fragments of 1,146 bp and 220 bp were amplified from ST-SL1344 and ST-*ΔSTM0859* strains, respectively (Fig. S2B). The constructed ST-*ΔSTM0859* gene-deficient strain was verified by DNA sequencing, and the complementation strain ST-*ΔSTM0859*/*STM0859* was confirmed by PCR (Fig. S3).

The growth of ST-SL1344, ST-*ΔSTM0859* and ST-*ΔSTM0859*/*STM0859* were consistent under 37°C culture condition. There was no significant difference in growth rate between the three strains in the stress environment of 4% NaCl, 1 mM H_2O_2, 0.3% cholate, and pH 10 (Fig. 2), indicating that the deficiency of *STM0859* gene did not affect ST growth under the stressful environmental conditions, such as alkaline, high salt, oxidation and ethanol stresses. However, as

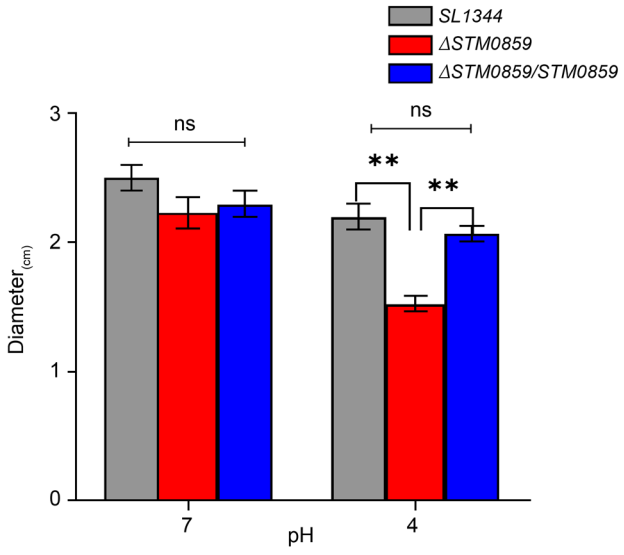

Fig. 3. Assay of motility of SL1344, *ΔSTM0859* and *ΔSTM0859/STM0859* in semi-solid medium with different pH; ns – not significant, while ** – $p < 0.01$.

compared with that of ST-SL1344, the growth rate of ST-*ΔSTM0859* in the acidic stress environment of pH 4 (Fig. 2B), and the movement ability (Fig. S4) of the semi-solid culture base were significantly reduced ($p < 0.01$, Fig. 3), indicating that the *STM0859* gene deficiency can lead to a decrease in ST's acid resistance.

BF detection indicated that ST-SL1344, ST-*ΔSTM0859,* and ST-*ΔSTM0859/STM 0859* strains could form BF at 30 h and 48 h. However, the BF growth ability of ST-*ΔSTM0859* was significantly declined than that of ST-SL1344 and ST-*ΔSTM0859/STM0859* when observed under the inverted microscope (LEICA, Germany) (Fig. 4A). There was a significant difference in BF formation between ST-*ΔSTM0859* and ST-SL1344 ($p < 0.01$, Fig. 4B), indicating that *STM0859* can promote BF formation.

The transcription levels of the genes *rcsB* and *rpoS* in ST-*ΔSTM0859* were significantly reduced when compared with those of ST-SL1344, while the transcription levels of these genes mentioned above were restored in ST-*ΔSTM0859/STM0859* (Fig. 5), indicating that the *STM0859* gene deficiency reduced the expression levels of genes (*rcsB* and *rpoS*) related acid stress and BF formation in ST.

The pET-STM0859 recombinant vector was successfully constructed by double enzymatic digestion

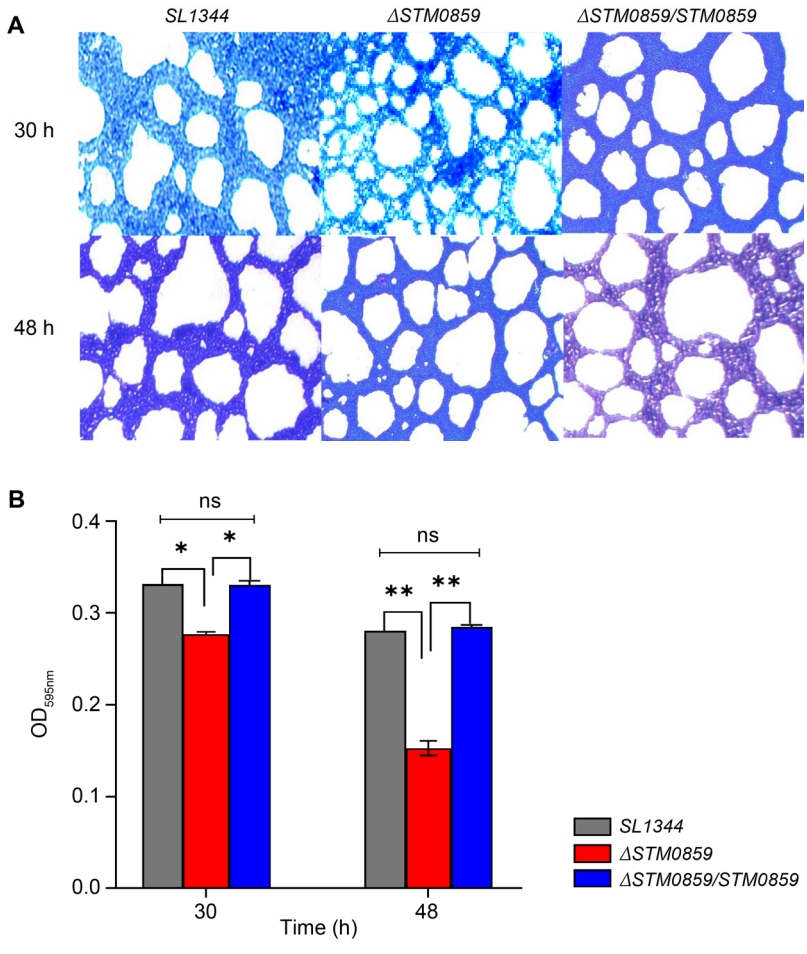

Fig. 4. Determination of formatting capability of biofilm of SL1344, *ΔSTM0859* and *ΔSTM0859/STM0859*; A) formation of biofilm by SL1344, *ΔSTM0859* and *ΔSTM0859/STM0859* at 30 h, and 48 h, respectively; B) biofilm of SL1344, *ΔSTM0859* and *ΔSTM0859/STM0859* biofilm determined by $OD_{595\,nm}$ at 30 h, and 48 h, respectively; ns – not significant, * – $p < 0.05$, while ** – $p < 0.01$.

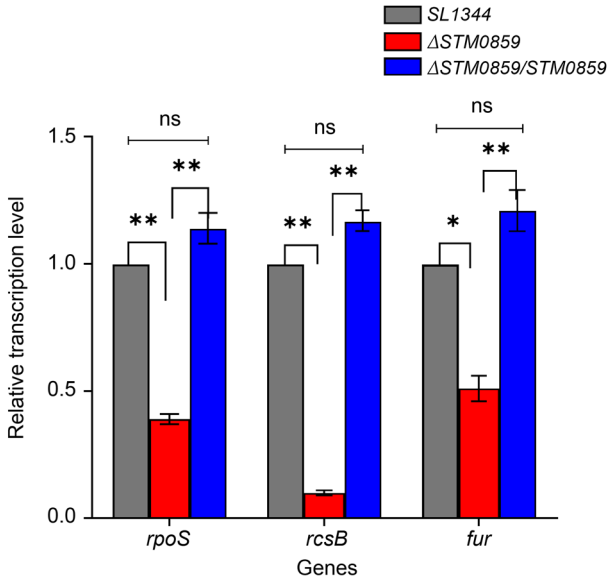

Fig. 5. Determination of the relative transcriptional levels of *rpoS*, *rcsB* and *fur* gene; ns – indicates not significant, * – $p < 0.05$, while ** – $p < 0.01$.

with *Eco*R I. and *Xho* I (Fig. S5). After being induced by IPTG, SDS-PAGE and Western blot confirmed that the recombination protein STM0859 with a molecular weight of 48.6 kDa was obtained (Fig. S6). The results of the gel retardation assay (EMSA) showed that the DNA fragment of the *rcsB* gene promoter (Fig. 6A and 6B) displayed apparent band retardation after being bound by STM0859 protein (Fig. 6C). In contrast, the migration of the DNA fragment of the *rcsB* gene promoter with the unrelated protein BSA (control group) was not retarded (Fig. 6D), indicating that the STM0859 protein could specifically bind with the promoter of *rcsB* gene.

Discussions

As an important foodborne pathogen, ST can infect humans through animal food and cause poisoning, posing a serious threat to food safety and public health. Moreover, ST can survive in stressful environments such as low temperature, hyperosmolarity, and acidity, and form biofilms under certain conditions to resist unfavorable internal and external environments (Kim et al. 2013; Ramachandran et al. 2016). It has been proven that many factors are associated with the responses of *Salmonella* Typhimurium to environmental stress and biofilm formation (Eran et al. 2020).

Available studies have confirmed that the transcription factor is an essential regulatory molecule in ST, regulating virulence and environmental stress-related gene expression (Song et al. 2020). Among them, the members of LysR family are the transcription factors widely found in prokaryotes (Yang et al. 2020), playing important regulatory roles in the life activities of bacteria. As a LysR transcription factor family member, ST-STM0859 contains a DNA binding domain and

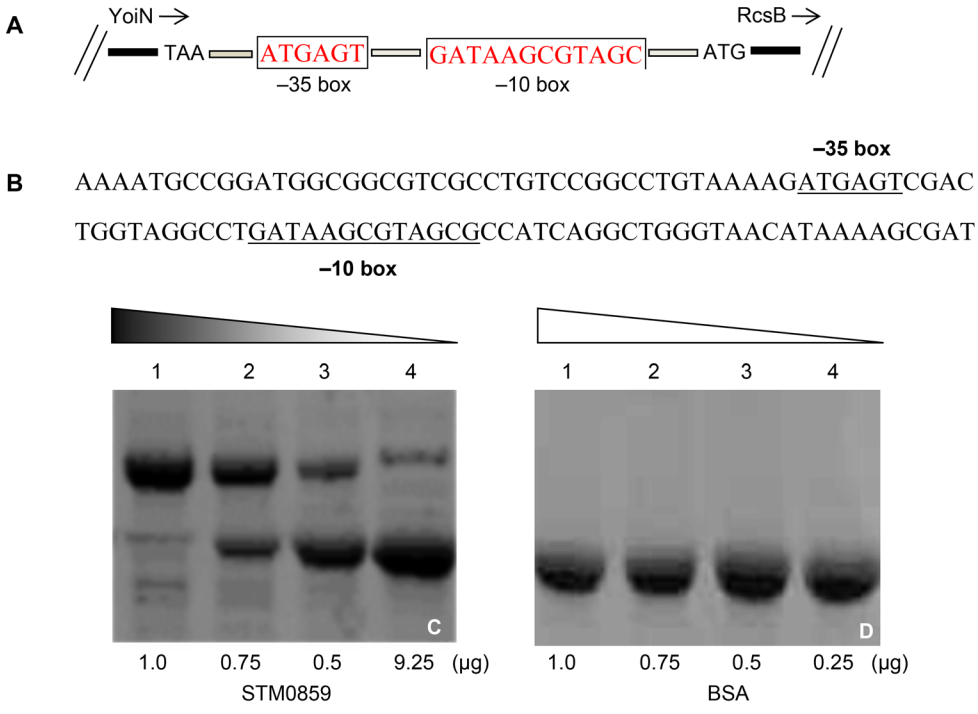

Fig. 6. Analysis of the interaction between STM0859 and the promoter sequence of *rcsB* gene using EMSA; A) the relevant features and localization in genomic DNA of ST, B) the promoter sequence of *rcsB* gene used in this study, C) interaction between STM0859 and the promoter of *rcsB* gene, D) interaction between BSA and the promoter of *rcsB* gene (negative control).

an HTH-18 domain consisting of a helix-turn-helix in molecular structure, similar to that of *E. coli* and *Acinetobacter*. Moreover, sequence analysis of the *STM0859* gene showed that this gene is highly conservative among ST strains (data not shown).

It has been shown that there are significant differences in the expression levels of LysR family proteins in different growth environments, suggesting that they are involved in the regulation of the bacterial responses to environmental stresses (Zhang et al. 2019). Srinivasan et al. (2013) revealed that oxyRKP was involved in regulating the response to oxidative stresses, drug resistance, and virulence of *Klebsiella pneumoniae*. Fragel et al. (2019) confirmed that ST LysR transcription factors OxyR, SpvR, LeuO, CysB were involved in the regulation of oxidative stress and toxicity. Fu et al. (2019) revealed that four LysR-type transcriptional regulator family proteins (LTTRs) were related to antibiotic resistance in *Aeromonas hydrophila*. Herein, we confirmed that the acid stress tolerance and BF formation ability were significantly reduced after deleting the ST *STM0859* gene, suggesting that *STM0859* was involved in regulating acid stress tolerance and BF formation of ST.

The current researches have found that *Salmonella* has evolved a complex acid-resistant mechanism involving the regulation of transcription factors and the synthesis of acid shock proteins in the acid stress environments (Lang et al. 2020). When ST infects host cells, the first pressure is the host's stomach acid (Lee and Kim 2017). Therefore, sensing and responding to pH changes is critical to the survival of ST. It has been proven that *fur*, *rcsB* and *rpoS* play important roles in modulating the adaptability of *Salmonella* Typhimurium to environmental stresses such as low temperature, hyperosmolarity or acidity, and biofilm formation (Knudsen et al. 2014; Leclerc et al. 2017). Here, we confirmed that the transcription levels of genes *rcsB* were significantly reduced in the *STM0859* gene deficiency strain.

Based on the analysis of the transcription levels of those genes related to acid response and biofilm formation using qRT-PCR, we further explored the interaction between the STM0859 protein and the promoter DNA fragment of *rcsB* gene whose expression was significantly reduced. It was confirmed that the STM0859 protein could modulate the mRNA level of the *rcsB* gene by binding with its promoter. Given that rcsB plays an important role in the adaptive response to acid stress (Lehti et al. 2012); thus, it is reasonable to speculate that the STM0859 protein can indirectly regulate the response to acid stress and BF formation through the interaction with the promoter of the *rcsB* gene.

However, the acid stress response is a highly complex process in *Salmonella*, which is involved acid resistance systems and a complicated regulation network (Kenney 2019). The mechanism insights into the regulation of acid stress response at the molecular level will contribute to developing novel antibiotics to deal with *Salmonella* Typhimurium infection (Zhao and Houry 2010).

Taken together, this study confirmed that the novel *LysR* family factor STM0859 was involved in the acid stress tolerance and BF formation of ST. Moreover, the STM0859 could bind the promoter of the *rcsB* gene and alter its expression, thus indirectly modulating the response to acid stress and BF formation of ST.

ORCID

Zhongmei Ma https://orcid.org/0000-0002-9621-4315
Na Li https://orcid.org/0000-0002-5762-3140
Chengcheng Ning https://orcid.org/0000-0002-7329-6661
Yucheng Liu https://orcid.org/0000-0001-8633-974X
Yun Guo https://orcid.org/0000-0002-3261-0829
Qiao Jun https://orcid.org/0000-0001-5210-4379

Ethical statement

The experiments were carried out in accordance with the guidelines issued by the Ethical Committee of Shihezi University.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

Literature

Borges KA, Furian TQ, Souza SN, Menezes R, Lima DA, Fortes FB, Salle CT, Moraes HL, Nascimento VP. Biofilm formation by *Salmonella* Enteritidis and *Salmonella* Typhimurium isolated from avian sources is partially related with their *in vivo* pathogenicity. Microb Pathog. 2018 May;118:238–241. https://doi.org/10.1016/j.micpath.2018.03.039

Eran Z, Akçelik M, Yazıcı BC, Özcengiz G, Akçelik N. Regulation of biofilm formation by marT in *Salmonella* Typhimurium. Mol Biol Rep. 2020 Jul;47(7):5041–5050. https://doi.org/10.1007/s11033-020-05573-6

Fragel SM, Montada A, Heermann R, Baumann U, Schacherl, MSchnetz K. Characterization of the pleiotropic LysR-type transcription regulator Leu0 of *Escherichia coli*. Nucleic Acids Res. 2019 Aug 22;47(14):7363–7379. https://doi.org/10.1093/nar/gkz506

Fu Y, Cai Q, Wang Y, Li W, Yu J, Yang G, Lin W, Lin X. Four LysR-type transcriptional regulator family proteins (LTTRs) involved in antibiotic resistance in *Aeromonas hydrophila*. World J Microbiol Biotechnol. 2019 Aug 2;35(8):127. https://doi.org/10.1007/s11274-019-2700-3

Gebhardt MJ, Czyz DM, Singh S, Zurawski DV, Becker L, Shuman HA. GigC, a LysR family transcription regulator, is required for cysteine metabolism and virulence in *Acinetobacter baumannii*. Infect Immun. 2020 Dec 15;89(1):e00180-20. https://doi.org/10.1128/IAI.00180-20

Jajere SM. A review of *Salmonella enterica* with particular focus on the pathogenicity and virulence factors, host specificity and antimicrobial resistance including multidrug resistance. Vet World. 2019; 12(4): 504–521. https://doi.org/10.14202/vetworld.2019.504-521

Kim SI, Ryu S, Yoon H. Roles of YehZ, a putative osmoprotectant transporter, in tempering growth of *Salmonella enterica* serovar Typhimurium. J Microbiol Biotechnol. 2013 Nov 28;23(11):1560–1568. https://doi.org/10.4014/jmb.1308.08006

Knudsen GM, Nielsen MB, Thomsen LE, Aabo S, Rychlik I, Olsen JE. The role of ClpP, RpoS and CsrA in growth and filament formation of *Salmonella enterica* serovar Typhimurium at low temperature. BMC Microbiol. 2014 Aug 14;14:208. https://doi.org/10.1186/s12866-014-0208-4

Kenney LJ. The role of acid stress in *Salmonella* pathogenesis. Curr Opin Microbiol. 2019 Feb;47:45–51. https://doi.org/10.1016/j.mib.2018.11.006

Leclerc JM, Dozois CM, Daigle F. *Salmonella enterica* serovar Typhi siderophore production is elevated and Fur inactivation causes cell filamentation and attenuation in macrophages. FEMS Microbiol Lett. 2017 Aug 15;364(15). https://doi.org/10.1093/femsle/fnx147

Lehti TA, Heikkinen J, Korhonen TK, Westerlund-Wikström B. The response regulator RcsB activates expression of Mat fimbriae in meningitic *Escherichia col*. J Bacteriol. 2012 Jul;194(13):3475–3485. https://doi.org/10.1128/JB.06596-11

Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001 Dec;25(4):402–408. https://doi.org/10.1006/meth.2001

Lang C, Zhang Y, Mao Y, Yang X, Wang X, Luo X, Dong P, Zhu L. Acid tolerance response of *Salmonella* during simulated chilled beef storage and its regulatory mechanism based on the PhoP/Q system. Food Microbiol. 2021 May;95:103716. https://doi.org/10.1016/j.fm.2020.103716

Peng Y, Meng Q, Qiao J, Xie K, Chen C, Liu T, Hu Z, Ma Y, Cai X, Chen C. The regulatory roles of ncRNA *Rli60* in adaptability of *Listeria monocytogenes* to environmental stress and biofilm formation. Curr Microbiol. 2016 Jul;73(1):77–83. https://doi.org/10.1007/s00284-016-1028-6

Richardson KE, Cox NA, Cosb DE, Berrang ME. Impact of desiccation and heat exposure stress on *Salmonella* tolerance to acidic conditions. *J Environ Sci Health B.* 2018 Feb;53(2):141–144. https://doi.org/10.1080/03601234.2017.1397467

Rohren M, Xie Y, O'Leary C, Kongari R, Gill J, Liu M. Complete genome sequence of *Salmonella enterica* serovar Typhimurium siphophage Skate. Microbiol Resour Announc. 2019 Jul;8(27): e00541-19. https://doi.org/10.1128/MRA.00541-19

Ramachandran G, Aheto K, Shirtliff ME, Tennant SM. Poor biofilm-forming ability and long-term survival of invasive *Salmonella* Typhimurium ST313. Pathog Dis. 2016 Jul;74(5):ftw049. https://doi.org/10.1093/femspd/ftw049

Suar M, Ryan D. Small RNA in the acid tolerance response of *Salmonella* and their role in virulence. Virulence. 2015;6(2):105–106. https://doi.org/10.4161/21505594.2014.988543

Song X, Zhang H, Liu X, Yuan J, Wang P, Lv R, Yang B, Huang D, Jiang L. The putative transcriptional regulator STM14_3563 facilitates *Salmonella* Typhimurium pathogenicity by activating virulence-related genes. Int Microbiol. 2020 Aug; 23(3):381–390. https://doi.org/10.1007/s10123-019-00110-3

Srinivasan VB, Mondal A, Venkataramaiah M, Chauhan NK, Rajamohan G. Role of *oxyR*^{KP}, a novel LysR-family transcriptional regulator in antimicrobial resistance and virulence in *Klebsiella pneumoniae*. Microbiology. 2013 Jul; 159(Pt_7):1301–1314. https://doi.org/10.1099/mic.0.065052-0

Yang W, Wang WY, Zhao W, Cheng JG, Wang Y, Yao XP, Yang ZX, Yu D, Luo Y. Preliminary study on the role of novel LysR family gene *kp05372* in *Klebsiella* pneumoniae of forest musk deer. J. Zhejiang Univ Sci B. 2020 Feb;21(2):137–154. https://doi.org/10.1631/jzus.B1900440

Zhang H, Song X, Lv R, Liu X, Wang P, Jiang L. The LysR-type transcriptional regulator STM0030 contributes to *Salmonella* Typhimurium growth in macrophages and virulence in mice. J Basic Microbiol. 2019 Nov;59(11):1143–1153. https://doi.org/10.1002/jobm.201900315

Zhao B, Houry WA. Acid stress response in enteropathogenic gammaproteobacteria: an aptitude for survival. Biochem Cell Biol. 2010 Apr;88(2):301–314. https://doi.org/10.1139/o09-182

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