

Phosphorylation residue T175 in RsbR protein is required for efficient induction of sigma B factor and survival of *Listeria monocytogenes* under acidic stress*

Ke HE^{†1,2}, Yong-ping XIN², Ying SHAN², Xian ZHANG¹, Hou-hui SONG¹, Wei-huan FANG^{†‡1,2}

¹College of Animal Science and Technology, Zhejiang A&F University, Lin'an 311300, China

²Zhejiang University Institute of Preventive Veterinary Medicine, Zhejiang Provincial Key Laboratory of Preventive Veterinary Medicine, Hangzhou 310058, China

[†]E-mail: heke@zafu.edu.cn; whfang@zju.edu.cn

Received Oct. 31, 2018; Revision accepted Feb. 17, 2019; Crosschecked June 13, 2019

Abstract: *Listeria monocytogenes* is an important zoonotic foodborne pathogen that can tolerate a number of environmental stresses. RsbR, an upstream regulator of the sigma B (SigB) factor, is thought to sense environmental challenges and trigger the SigB pathway. In *Bacillus subtilis*, two phosphorylation sites in RsbR are involved in activating the SigB pathway and a feedback mechanism, respectively. In this study, the role of RsbR in *L. monocytogenes* under mild and severe stresses was investigated. Strains with genetic deletion (Δ rsbR), complementation (C- Δ rsbR), and phosphorylation site mutations in the *rsbR* (RsbR-T175A, RsbR-T209A, and RsbR-T175A-T209A) were constructed to evaluate the roles of these RsbR sequences in listerial growth and survival. SigB was examined at the transcriptional and translational levels. Deletion of *rsbR* reduced listerial growth and survival in response to acidic stress. Substitution of the phosphorylation residue RsbR-T175A disabled RsbR complementation, while RsbR-T209A significantly upregulated SigB expression and listerial survival. Our results provide clear evidence that two phosphorylation sites of RsbR are functional in *L. monocytogenes* under acidic conditions, similar to the situation in *B. subtilis*.

Key words: *Listeria monocytogenes*; RsbR; Sigma B (Sig B) factor; Phosphorylation
<https://doi.org/10.1631/jzus.B1800551>

CLC number: S855.1


1 Introduction

Listeria monocytogenes can cause septicemia, meningitis, and abortion in pregnant women with mortality rates as high as 30% (Varma et al., 2007; Okpo et al., 2015). As a foodborne pathogen, *L. monocytogenes* encounters acidic environments such

as silage, fermented food, stomach, and phagolysosomes within host cells (Nicaogáin and O'Byrne, 2016). Its ability to adapt to relatively harsh environmental conditions is one of the important features enabling its persistence in food processing environments and for establishing infection in the host. *L. monocytogenes* can maintain its intracellular pH (pH_i) homeostasis when exposed to an environmental pH (pH_{ex}) of 4.5, and survives well at pH_{ex} 3.5 (Cotter et al., 2000). Pre-exposure of *L. monocytogenes* cells to mild acid stress (pH_{ex} 4.5) may induce an acid tolerance response (ATR) that can render them more resistant to fatal acidic stress (O'Byrne and Karatzas, 2008). Sigma B (SigB or σ^B) is known as a positive

[‡] Corresponding author

* Project supported by the National Natural Science Foundation of China (Nos. 31702250 and 31600292) and the Open Fund Project by Key Laboratory of Animal Preventive Medicine of Zhejiang Province (No. ZPKLPVM2017KFKT001), China

 ORCID: Ke HE, <https://orcid.org/0000-0001-6446-9439>

© Zhejiang University and Springer-Verlag GmbH Germany, part of Springer Nature 2019

regulator of ATR enabling bacteria to cope better with environmental stresses.

SigB was first identified in *Bacillus subtilis*, and is also present in *L. monocytogenes* and *Staphylococcus aureus* (Hecker et al., 2007, 2009; Price, 2011). It plays a pivotal role in resistance to environmental stresses in these bacteria. The SigB operons, consisting of *rsbR-rsbS-rsbT-(rsbU)-rsbV-rsbW-sigB-rsbX*, are highly conserved between *L. monocytogenes* and *B. subtilis* (O'Byrne and Karatzas, 2008), suggesting existence of similar regulatory mechanisms. In *B. subtilis*, partner switching through serine phosphorylation and dephosphorylation is the main regulatory mechanism of this operon in response to stress (Hecker et al., 2007; Eymann et al., 2011; Hengge, 2011). The operon is composed of the co-antagonist RsbR, antagonist RsbS, and serine-threonine kinase RsbT as the upstream regulators (Delumeau et al., 2006). The phosphorylation of RsbR-RsbT-RsbS could be the initial crucial step to trigger activation of the SigB pathway. There are three important phosphorylation sites in this complex: residues T171 and T205 of RsbRA (formerly RsbR), and residue S59 of RsbS (Chen et al., 2004; Kim et al., 2004a; Liebal et al., 2013). RsbRA-T171 is a prerequisite and related to stress-induced phosphorylation of RsbS-S59, while RsbRA-T205 phosphorylation may function as a second feedback mechanism to limit σ^B activation (Eymann et al., 2011).

In *L. monocytogenes*, upstream genes in this operon, *rsbT*, *rsbV*, and *rsbU*, have been proved to play roles in activating σ^B in response to nutritional and environmental stresses (Chaturongakul and Boor, 2004; Shin et al., 2010; Zhang et al., 2013). Martinez et al. (2010) expressed the RsbR protein of *L. monocytogenes* in the *B. subtilis* Δ *rsbR* mutant, and detected σ^B induction following nutritional stress. In our previous study (Xia et al., 2016), we showed that RsbX was a negative regulator of *L. monocytogenes* σ^B during the recovery period after the primary stress or in the stationary phase, thus affecting its survival under secondary stress. However, the detailed roles of RsbR, RsbT, and RsbS in the resistance of *L. monocytogenes* to environmental stresses remain unclear. This study was attempted to examine whether RsbR is vital in *L. monocytogenes* under acid stress, and if the two putative phosphorylation sites are functional.

2 Materials and methods

2.1 Bacterial strains, plasmids, and culture conditions

L. monocytogenes reference strain 10403S, a serotype 1/2a isolate from a human skin lesion, was provided by Dr. Martin Wiedmann (Cornell University, USA) and used as the wild-type strain. The plasmids pMD19-T (TaKaRa, Dalian, China), pERL3 (a gift from Dr. Qin LUO, Huazhong Normal University, Wuhan, China), and pKSV7 (a gift from Dr. M. WIEDMANN) were maintained in *Escherichia coli* DH5 α as the host strain. *L. monocytogenes* was cultured in brain heart infusion (BHI) medium (Dingguo, Beijing, China), and *E. coli* was grown in Luria Bertani (LB) broth (Oxoid, Hampshire, UK), all at 37 °C.

2.2 Bioinformatics analysis

Alignment of amino acid sequences was performed with MegAlign using the DNASTar software package (Version 5; Madison, USA). Four RsbR paralogs of *B. subtilis*, BS-RsbRA (GenBank: P42409), BS-RsbRB (GenBank: O34860), BS-RsbRC (GenBank: O31856), and BS-RsbRD (GenBank: P54504), were used to align with *L. monocytogenes* RsbR (LM-RsbR; GenBank: CP002002). The three homologous RsbRs in *L. monocytogenes* were from the genome of 10403S (GenBank: CP002002). The phosphorylation sites of BS-RsbRs were searched in PhosphositePlus (<https://www.phosphosite.org>), and the phosphorylation sites of LM-RsbR were predicted by DISPHOS (<http://www.dabi.temple.edu/disphos>).

2.3 Construction of the *rsbR* deletion mutant

The temperature-sensitive pKSV7 shuttle vector was used for generating the Δ *rsbR* mutant from the wild-type strain *L. monocytogenes* 10403S. Gene splicing using the splice-overlap extension PCR (SOE-PCR) procedure was used to construct the homologous arms of *rsbR* (Table 1), and a homologous recombination strategy was used to construct the Δ *rsbR* mutant as described by Xia et al. (2016). The Δ *sigB* mutant kept in our laboratory was constructed using the same approach.

2.4 Complementation of the *rsbR* deletion mutant

Expression of *rsbR* was complemented based on previous methods (Chen et al., 2011; Xia et al., 2016).

Table 1 PCR primers used in this study

Name	Primer sequence (5'→3') ^a	Purpose
rsbR-a	CCGGGATCCATACGGCCTGTTCTCATCATTC	Construction of <i>rsbR</i> null mutant
rsbR-b	ACGCGTTGTTTGTAGGTTTCTG	
rsbR-c	CAGAAACCTACAAACAACGCGTTACAGCCAGCAGTTGCGATTACAC	
rsbR-d	CCCAAGCTTTACAGATTTCTCCTCGTCCAG	
rsbR-up	GCGAGTGTACCCATGTGCAAGCAG	Screening of positive clones of <i>rsbR</i> null mutant
rsbR-dn	CCCCAATTCCTGTTTAAGTTTTTCCAG	
rsbR-W	ACGCGTCCGACCAATCCAAGCAAAATAGCTAGGTAGAAA	Complementation of <i>rsbR</i> deletion
rsbR-X	CAATCCAAGCAAAATAGCTAGGTAGAAA	
rsbR-Y	CTAGCTATTTTGCTTGGATTGATGTATAAAGATTTTGCAAACCT	
rsbR-Z	CGAGCTCTCACCCCTCTTTTCTACT	
rsbR175m-F	TGTAATGCCGTTAATTGGAACGATTGACGCAGAAAGAGCCAAG	
rsbR175m-R	AGTAAGTTTTCTATGATTAACCTGGCTCTTTCTGCGTCAATCGT	
rsbR209m-F	TGATTGATATTACGGGAGTTCCTGTTGTTGATGCAATGGTTGCG	qRT-PCR
rsbR209m-R	GACGCTTGAATAATATGGTGCGCAACCATTGCATCAACAACAGG	
rsbR-RT-F	CGCGGATACGTGGGAAAAG	qRT-PCR
rsbR-RT-R	TGCCTGACAGCCGACAAGTCT	

^a Nucleotides introduced to create restriction sites and point mutations are underlined and in bold, respectively. qRT-PCR: quantitative reverse transcription PCR

SOE-PCR was used with the primer pairs rsbR-W/X and rsbR-Y/Z to fuse the *rsbR* open reading frame (ORF) with the *dlt* promoter. The product was ligated to pERL3 and confirmed by sequencing. The recombinant plasmid pERL3-Pdlt-rsbR was electroporated into the *L. monocytogenes* Δ rsbR strain and transformants were identified by PCR using the primer pair rsbR-Y/Z.

2.5 Site-directed mutagenesis

Residues T175 and T209 in RsbR of *L. monocytogenes* might be the vital phosphorylation sites (Fig. 1) corresponding to residues T171 and T205 of RsbR in *B. subtilis*. These two sites were chosen for site-directed mutagenesis of three expression plasmids containing single mutations (T175A=M1 or T209A=M2) or double mutations (T175A and T209A=M3). The plasmids were generated from the recombinant vector pERL3-Pdlt-rsbR using the primers listed in Table 1. All three mutated fragments were ligated with pERL3, confirmed by sequencing and electroporated into the *L. monocytogenes* Δ rsbR strain, as described above.

2.6 Growth and survival assays

All strains (*L. monocytogenes* 10403S, the Δ rsbR mutant and Δ rsbR complemented (C- Δ rsbR) strains;

(Table 2)) were grown to stationary phase with shaking at 37 °C. For the growth assay, the bacteria were adjusted to similar optical density at 620 nm (OD₆₂₀). Each calibrated strain was inoculated at a 1:20 ratio in BHI at pH 7.4 or 5.0 and then transferred into wells (triplicate wells per strain) on a microplate. The bacterial cultures were incubated at 37 °C for 8 h, and the OD₆₂₀ was then captured as the end-point measurement to indicate their relative growth potential. For the survival assay, bacterial cells at exponential phase were transferred at a 1:20 ratio in BHI at pH 7.4 and cultured with shaking at 37 °C for 4 h (to early exponential phase, OD₆₂₀ was approximately 0.3). One milliliter of each culture was centrifuged, resuspended with 1 mL BHI at pH 2.5 and incubated at 37 °C for 30 min. The treated cultures were subjected to immediate dilution in 10 mmol/L phosphate-buffered saline (PBS) at pH 7.4 at the end of 30-min incubation, followed by serial 10-fold dilutions. The diluted samples were plated onto BHI agar for the enumeration of viable bacterial cells. Relative survival was calculated by dividing the colony forming units (CFUs) of the mutants by those of the wild-type strain. The experiments were carried out three times.

2.7 Acidic stress for analysis of SigB expression

For transcriptional analysis of *sigB* after acid stress, all the strains were grown to stationary phase in

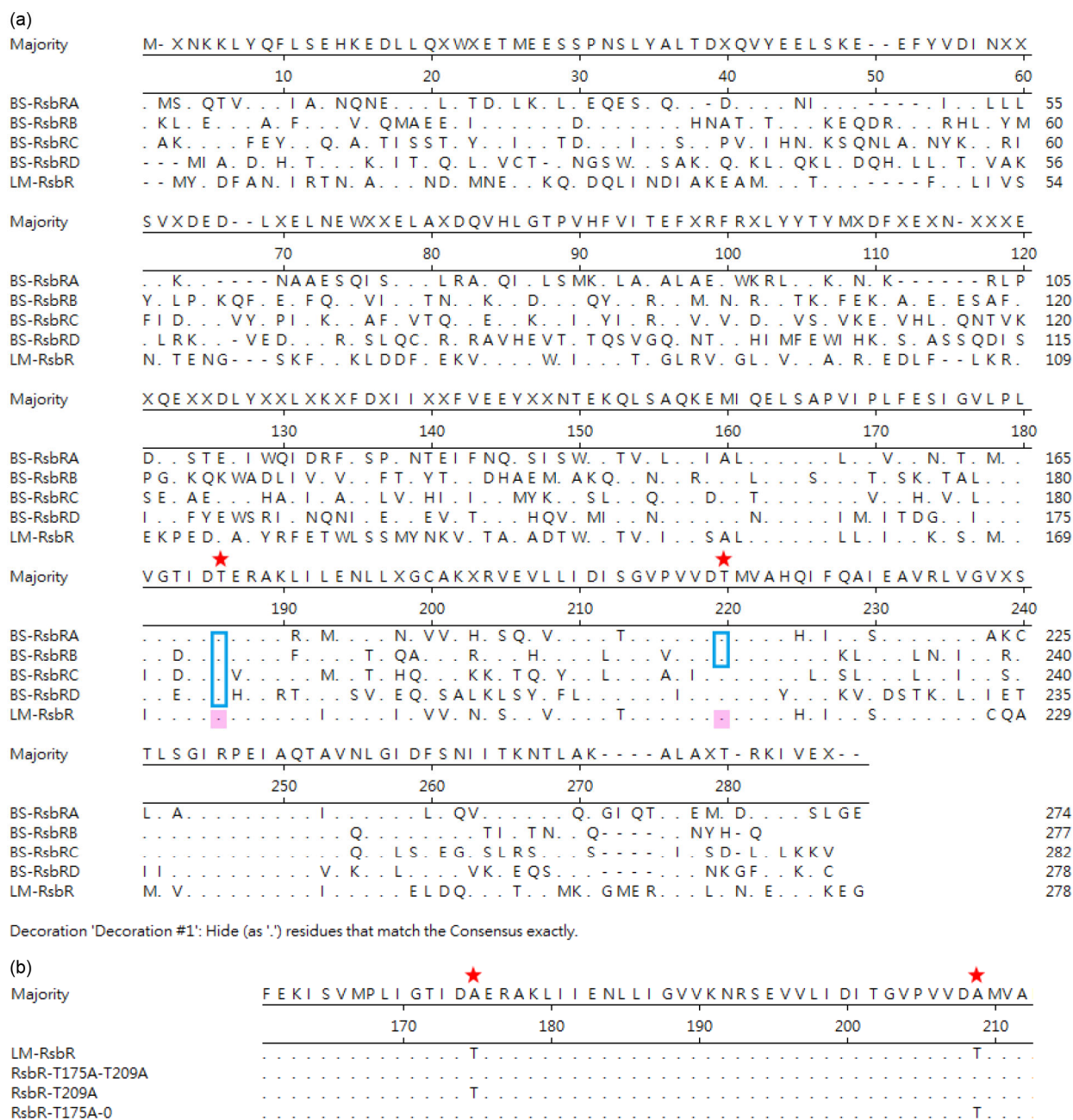


Fig. 1 Sequence alignment of RsbR homologs of *Bacillus subtilis* (BS) and *Listeria monocytogenes* (LM) (a) and alanine substitutions in the recombinant complementary plasmids (b)

Blue squares indicate the phosphorylation sites in PhosphositePlus (<https://www.phosphosite.org>); pink shading indicates the phosphorylation sites predicted by DISPHOS (<http://www.dabi.temple.edu/disphos>); red stars indicate the conservative phosphorylation sites in both BS and LM (Note: for interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

BHI, diluted 1:20 in 40 mL BHI at pH 7.4 and cultured at 37 °C for 6 h (to late exponential phase, OD₆₂₀ was approximately 0.45). The cultures were then divided into two parts (each in a different sterile tube) and were centrifuged at 3000 r/min for 5 min,

and then resuspended in 20 mL BHI at pH 5.0 (stressed) or BHI at pH 7.4 (as unstressed control) for 1 h. At the end of the treatment, 1 mL of the culture was taken for total RNA isolation, and the rest for extraction of total proteins.

Table 2 *Listeria monocytogenes* strains

<i>L. monocytogenes</i> strain	Genotype
WT	10403S
Δ rsbR	rsbR null
C- Δ rsbR	P _{dlt} ::rsbR
C- Δ rsbR-M1	P _{dlt} ::rsbR-T175A
C- Δ rsbR-M2	P _{dlt} ::rsbR-T209A
C- Δ rsbR-M3	P _{dlt} ::rsbR-T175A-T209A

P_{dlt}::rsbR means that the promoter of *dlt* and open reading frame (ORF) of *rsbR* are connected together

2.8 qRT-PCR and western blotting

Total RNAs from the treatments as described above were prepared using an RNAPrep Pure Cell/Bacteria kit (Tiangen Biotech, Beijing, China) and complementary DNAs (cDNAs) were synthesized with ReverTra Ace[®] reverse transcriptase (Toyobo, Japan). For quantification of *sigB* and *rsbR* mRNAs, quantitative reverse transcription PCR (qRT-PCR) was performed as described by Xia et al. (2016). The *gyrB* gene was used as a reference. Primers for *sigB* and *gyrB* were as listed elsewhere (Xia et al., 2016), and primers for *rsbR* were rsbR-RT-F/R (Table 1). Each treatment was tested in triplicate and the relative expression of each strain was calculated using the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001), and transcriptional level of *sigB* was normalized as Shan et al. (2018).

For western blotting, total proteins were extracted from the bacterial cells treated as above following a previous procedure (Xia et al., 2016). The protein concentration was adjusted to the same level for different strains and separated by 12% (0.12 g/mL) sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The remaining procedures for

western blotting and image capture were followed as described (Zhou et al., 2017), using rabbit polyclonal antibodies against SigB and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Xia et al., 2016) as well as antibodies against RsbR prepared in this study by immunization of rabbits with RsbR protein expressed in *E. coli* strain BL21. Horseradish peroxidase (HRP)-conjugated anti-rabbit IgG (Sangon, China) was used at 1:2000 (v/v).

2.9 Statistical analysis

All data are expressed as mean±standard deviation (SD) of three independent experiments. Two-tailed Student's *t*-tests were employed to extract statistical significance among or between treatments or strains.

3 Results

3.1 Identification of the *rsbR* deletion mutant and complemented strains

PCR, qRT-PCR, and western blotting were used to confirm successful construction of the *rsbR* deletion mutant and complemented strain. Fig. 2 shows that the fragments were shorter with the primer pair rsbR-up/dn in Δ rsbR and C- Δ rsbR, suggesting deletion of *rsbR* in the bacterial genome (Fig. 2a). With the primer pair rsbR-Y/Z, fragments showing the size of the *rsbR* ORF (687 bp) were present in C- Δ rsbR and the wild-type strain. All strains, including those complemented strains having substitutions of single or double amino acid residues, were confirmed by sequencing. Expression of *rsbR* was significantly higher in all complemented strains than in their parent strains (Fig. 2b). Western blotting showed visible

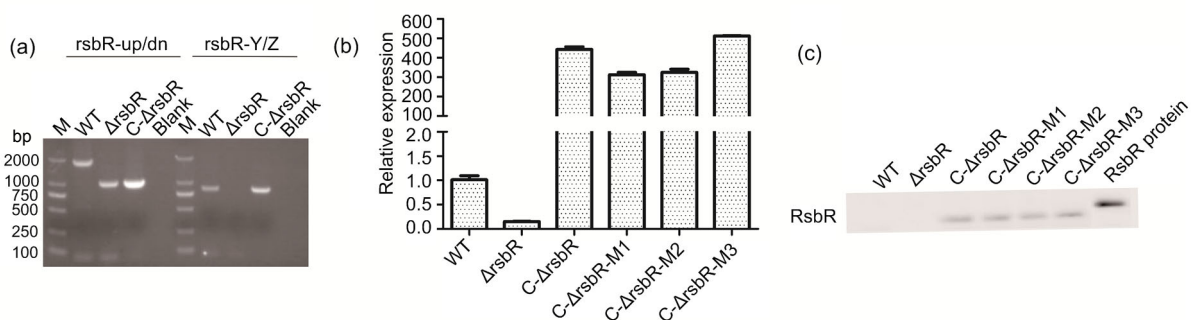


Fig. 2 Identification of *Listeria monocytogenes* *rsbR* deletion and complementation strains containing single or double alanine substitutions by PCR (a), qRT-PCR (b), and western blotting (c)

Data are expressed as mean±SD from three independent experiments

bands in complemented strains (Fig. 2c). The RsbR band in the wild-type strain was barely visible, possibly because its expression was below a detectable level (the concentration of prokaryotic RsbR protein was 0.27×10^{-5} mg/mL and we used super sensitive exposure).

3.2 Effects of *L. monocytogenes* *rsbR* on growth and survival under acidic conditions

To study whether the *rsbR* deletion would affect resistance to acidic stress, the Δ *rsbR* mutant strain and its parent strain 10403S were cultured in BHI at pH 5.0 with pH 7.4 as control. In the control medium, the growth of Δ *rsbR* and C- Δ *rsbR* was similar to that of the wild-type strain ($P=0.515$; Fig. 3a). However, Δ *rsbR* showed significantly slower growth than its parent strain ($P=0.044$; Fig. 3b) at acidic pH 5.0 shown as the end-point OD₆₂₀ at 8 h. Survival of the *rsbR* deletion mutant was also significantly lower than that of the wild-type strain upon brief exposure at pH 2.5 (Fig. 3c). These effects could be reversed to a significant degree (growth at mild stress, $P=0.032$; and survival at pH 2.5, $P<0.001$) by genetic complementation using the strain C- Δ *rsbR*.

3.3 Role of residue T175 contributing to RsbR function in listerial survival under acidic stress

To investigate the key phosphorylated amino acid residues of RsbR, we constructed complement plasmids with a single mutation at T175 or T209, and double mutations at both sites by alanine substitution. In normal and mildly acidic media, there were no significant differences between the growth of the complemented strains with mutations and that of the

C- Δ *rsbR* without alanine substitution (Figs. 3a and 3b). When treated with severe stress at pH 2.5 in BHI, C- Δ *rsbR*-M1 and C- Δ *rsbR*-M3 had lower survival ($P<0.001$), while C- Δ *rsbR*-M2 showed significantly higher survival ($P<0.001$) than the non-mutated C- Δ *rsbR* (Fig. 3c). These findings indicate that T175 (in M1 or M3) is critical for survival.

3.4 Regulation of RsbR and its residue T175 of SigB expression under stress conditions

A mild acid stress was introduced to the bacteria (in BHI at pH 5.0) to examine the response of SigB expression to acidic stress. Expression of SigB was 2.0–2.5-fold higher in stressed bacterial cells than in unstressed controls at the mRNA and protein levels. Both qRT-PCR and western blotting revealed that deletion of *rsbR* led to a significant reduction in SigB expression compared with that in the wild-type strain ($P=0.001$ and $P<0.001$ at the mRNA and protein levels, respectively), while complementation (C- Δ *rsbR*) improved SigB expression significantly ($P=0.003$ and $P<0.001$ at the mRNA and protein levels, respectively), though not to the level of the wild-type strain (Fig. 4).

Comparison of the complemented strains with or without mutations of T175 and T209 reveals that C- Δ *rsbR*-M1 and C- Δ *rsbR*-M3 down-regulated SigB expression ($P<0.01$ for *sigB* mRNA and $P<0.001$ for SigB protein), while C- Δ *rsbR*-M2 increased SigB expression ($P=0.012$ for *sigB* mRNA and $P=0.11$ for SigB protein; Fig. 4) compared with that of the non-mutated C- Δ *rsbR*, suggesting that T175 (M1 or M3) is involved in regulating SigB expression, but not T209 (M2).

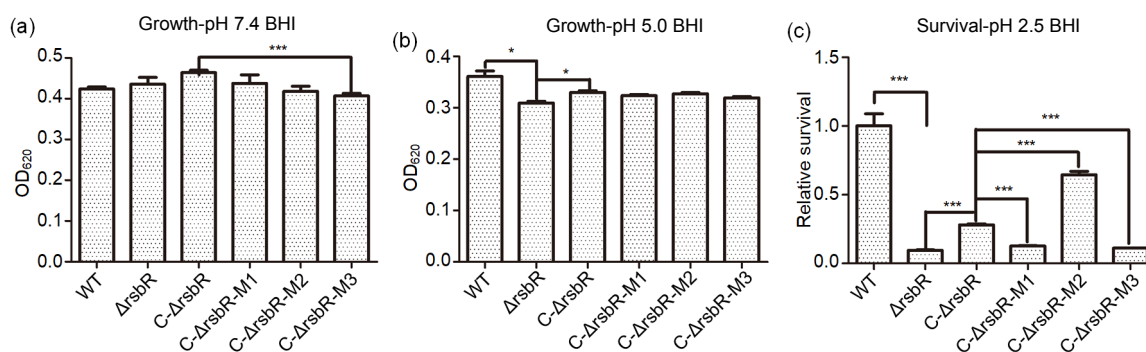


Fig. 3 Growth of *Listeria monocytogenes* wild-type (WT) strain and related mutants in BHI medium at pH 7.4 (a) and pH 5.0 (b), and survival in BHI at pH 2.5 (c)

Data are expressed as mean \pm SD from three independent experiments. * $P<0.05$, *** $P<0.001$

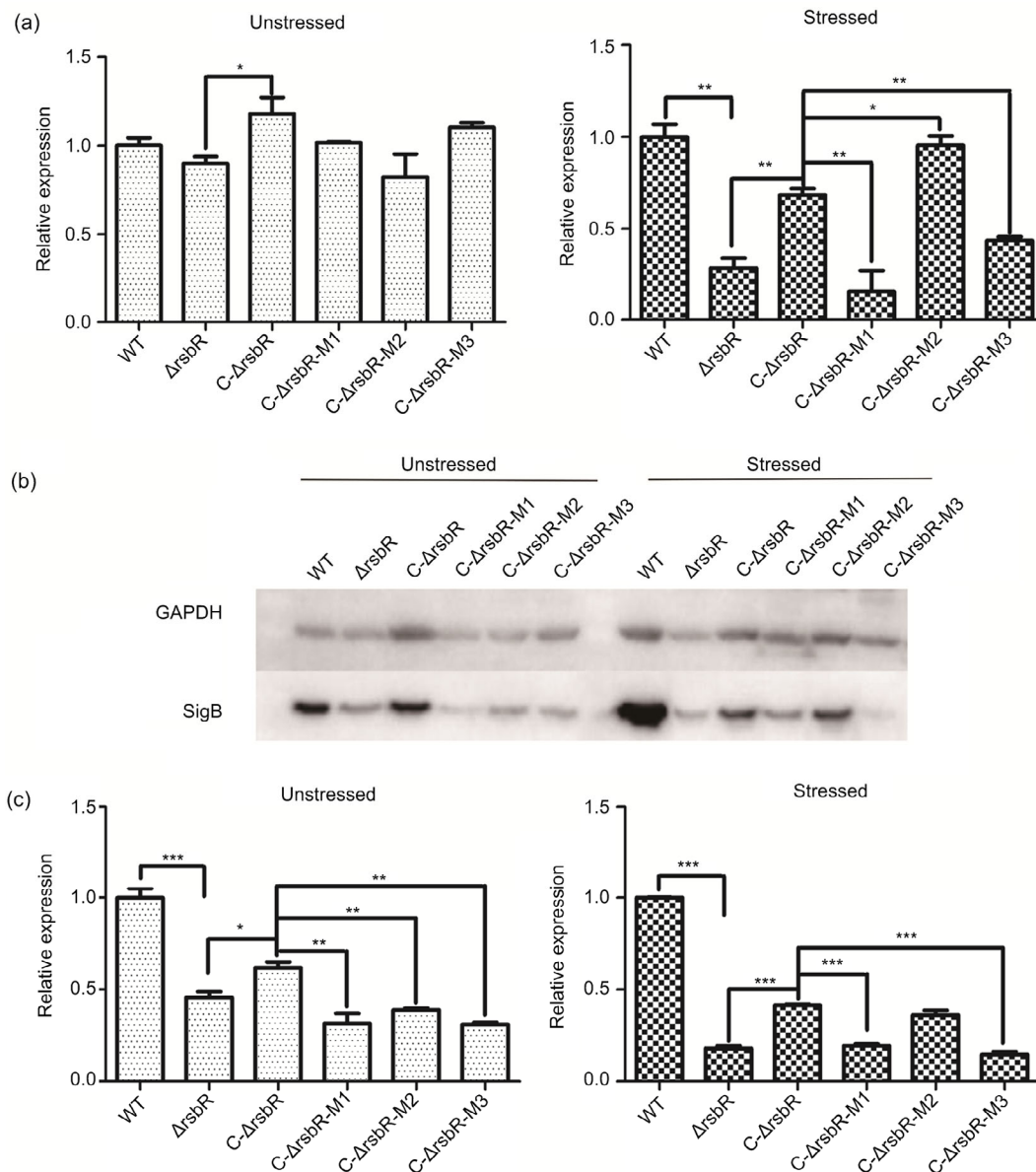


Fig. 4 Analysis of SigB expression in *Listeria monocytogenes* wild-type (WT) strain and related mutants in BHI at pH 7.4 (unstressed) or pH 5.0 (stressed) by qRT-PCR (a), western blotting (b), and densitometric quantification of SigB of panels B (c)

Data are expressed as mean±SD from three independent experiments. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

4 Discussion

RsbR was identified as a potential receptor to environmental stresses. In *B. subtilis*, two phosphorylation sites in RsbR behave differently in unstressful and stressful conditions (Kim et al., 2014a, 2004b; Eymann et al., 2011). Until now, there has been no study of the function of this protein in *L. monocytogenes*. Here we clearly show that RsbR is involved in

the growth and survival of *L. monocytogenes* under acidic stress, and that RsbR-T175 is required to trigger induction of SigB expression in response to acidic stress for improved survival.

It has been reported that deletion of *rsbT*, *rsbV*, or *rsbU* of *L. monocytogenes* reduces SigB expression under environmental stress (Chaturongakul and Boor, 2004; Shin et al., 2010; Zhang et al., 2013). All these genes are considered essential in the SigB pathway.

rsbR, the first gene in the SigB operon, is considered an initial sensory input from environmental stresses. Although the RsbR ortholog in *L. monocytogenes* has only 49.6% identity to the RsbR in *B. subtilis* at the amino acid level, the putative key sites for phosphorylation are conserved (T171 and T205 in the RsbR of *B. subtilis* and T175 and T209 in the RsbR of *L. monocytogenes*). Therefore, we supposed that LM-RsbR could play a role similar to that of the RsbR in *B. subtilis*. By genetic deletion, complementation, and site-directed mutagenesis, we proved that RsbR and its residue T175 are involved in listerial survival under acidic stress, most likely via increased expression of the general stress regulator SigB.

In *B. subtilis*, some of the proteins in the SigB operon (RsbRA, RsbRB, RsbRC, RsbRD, RsbT, and RsbS) form a large molecular mass structure called a stressome and function via a partner switch mechanism in response to environmental stress. The homologous RsbRs that contain the N- and C-terminal sulfate transporter and anti-sigma factor antagonist (STAS) domains and a conserved 13-residue α -helical linker (Marles-Wright et al., 2008) are the major players in this functionality. Each RsbR has two conserved threonine residues in its STAS domain (Pané-Farré et al., 2005). The STAS domain forms the complex core, and receives sensory input from the N-terminal domain (Gaidenko et al., 2012). Chen et al. (2004) suggested that RsbR proteins might serve as redundant co-antagonists necessary for RsbS, as shown by the phenotypes of triple mutants with only one or another RsbR family member. Although the *L. monocytogenes* genome contains a number of RsbR paralogues, the threonine and serine residues are absent (Pané-Farré et al., 2005). It is also less likely that the RsbR-RsbS-RsbT proteins would form stressomes in *L. monocytogenes* because these would be physically beyond the holding capacity of the listerial cells. Thus, we speculate that RsbR is the only receptor of environment stress in the *L. monocytogenes* SigB operon.

RsbRA T171 phosphorylation is thought to be an important prerequisite, but is not the trigger for signaling in *B. subtilis*. This is supported by the phenotypes of T171A and T171D substitutions and the analysis of the phosphorylation states of the RsbR family in vivo (Kim et al., 2004b). RsbRA T205 is found phosphorylated under strong stresses, and this

phosphorylation is considered a feedback mechanism to inhibit signaling under extreme conditions (Eymann et al., 2011). We showed that removal of the phosphorylation residue RsbR-T175A (C- Δ rsbR-M1) could disable the complementation, suggesting that this residue is required to initiate SigB signaling for improved listerial growth or survival. However, dephosphorylation on RsbR-T209A (C- Δ rsbR-M2) seemed to enhance SigB expression and listerial survival, which might indicate a negative regulatory role of T209, similar to that of RsbRA T205 in *B. subtilis* (Eymann et al., 2011).

In conclusion, this study has provided clear evidence that RsbR T175 is important in triggering increased expression of SigB and listerial survival under acidic stress. It remains to be determined how RsbR residues T175 and T205 coordinate in response to acidic stress, but T175 appeared to function as the major player in this strain with double mutations (C- Δ rsbR-M3).

Contributors

Ke HE and Wei-huan FANG designed the experimentation and data analysis, wrote and edited the manuscript. Ke HE, Yong-ping XIN, Ying SHAN, and Xian ZHANG performed the experimental research and data analysis. Hou-hui SONG contributed to editing of the manuscript. All authors read and approved the final manuscript and, therefore, had full access to all the data in the study and take responsibility for the integrity and security of the data.

Compliance with ethics guidelines

Ke HE, Yong-ping XIN, Ying SHAN, Xian ZHANG, Hou-hui SONG, and Wei-huan FANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

References

- Chaturongakul S, Boor KJ, 2004. RsbT and RsbV contribute to σ^B -dependent survival under environmental, energy, and intracellular stress conditions in *Listeria monocytogenes*. *Appl Environ Microbiol*, 70(9):5349-5356. <https://doi.org/10.1128/AEM.70.9.5349-5356.2004>
- Chen CC, Yudkin MD, Delumeau O, 2004. Phosphorylation and RsbX-dependent dephosphorylation of RsbR in the RsbR-RsBs complex of *Bacillus subtilis*. *J Bacteriol*, 186(20):6830-6836. <https://doi.org/10.1128/JB.186.20.6830-6836.2004>
- Chen JS, Cheng CY, Xia Y, et al., 2011. Lmo0036, an ornithine and putrescine carbamoyltransferase in *Listeria monocytogenes*, participates in arginine deiminase and

- agmatine deiminase pathways and mediates acid tolerance. *Microbiology*, 157(11):3150-3161.
<https://doi.org/10.1099/mic.0.049619-0>
- Cotter PD, Gahan CGM, Hill C, 2000. Analysis of the role of the *Listeria monocytogenes* F₀F₁-ATPase operon in the acid tolerance response. *Int J Food Microbiol*, 60(2-3): 137-146.
[https://doi.org/10.1016/S0168-1605\(00\)00305-6](https://doi.org/10.1016/S0168-1605(00)00305-6)
- Delumeau O, Chen CC, Murray JW, et al., 2006. High-molecular-weight complexes of RsbR and paralogues in the environmental signaling pathway of *Bacillus subtilis*. *J Bacteriol*, 188(22):7885-7892.
<https://doi.org/10.1128/JB.00892-06>
- Eymann C, Schulz S, Gronau K, et al., 2011. *In vivo* phosphorylation patterns of key stressosome proteins define a second feedback loop that limits activation of *Bacillus subtilis* σ^B . *Mol Microbiol*, 80(3):798-810.
<https://doi.org/10.1111/j.1365-2958.2011.07609.x>
- Gaidenko TA, Bie XM, Baldwin EP, et al., 2012. Two surfaces of a conserved interdomain linker differentially affect output from the RST sensing module of the *Bacillus subtilis* stressosome. *J Bacteriol*, 194(15):3913-3921.
<https://doi.org/10.1128/JB.00583-12>
- Hecker M, Pané-Farré J, Völker U, 2007. SigB-dependent general stress response in *Bacillus subtilis* and related Gram-positive bacteria. *Annu Rev Microbiol*, 61:215-236.
<https://doi.org/10.1146/annurev.micro.61.080706.093445>
- Hecker M, Reder A, Fuchs S, et al., 2009. Physiological proteomics and stress/starvation responses in *Bacillus subtilis* and *Staphylococcus aureus*. *Res Microbiol*, 160(4): 245-258.
<https://doi.org/10.1016/j.resmic.2009.03.008>
- Kim TJ, Gaidenko TA, Price CW, 2004a. A multicomponent protein complex mediates environmental stress signaling in *Bacillus subtilis*. *J Mol Biol*, 341(1):135-150.
<https://doi.org/10.1016/j.jmb.2004.05.043>
- Kim TJ, Gaidenko TA, Price CW, 2004b. *In vivo* phosphorylation of partner switching regulators correlates with stress transmission in the environmental signaling pathway of *Bacillus subtilis*. *J Bacteriol*, 186(18):6124-6132.
<https://doi.org/10.1128/JB.186.18.6124-6132.2004>
- Liebal UW, Thomas M, Jon MW, et al., 2013. Simulations of stressosome activation emphasize allosteric interactions between RsbR and RsbT. *BMC Syst Biol*, 7:3.
<https://doi.org/10.1186/1752-0509-7-3>
- Livak KJ, Schmittgen TD, 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2^{- $\Delta\Delta C_T$} . *Methods*, 25(4):402-408.
<https://doi.org/10.1006/meth.2001.1262>
- Marles-Wright J, Grant T, Delumeau O, et al., 2008. Molecular architecture of the "stressosome," a signal integration and transduction hub. *Science*, 322(5898):92-96.
<https://doi.org/10.1126/science.1159572>
- Martinez L, Reeves A, Haldenwang W, 2010. Stressosomes formed in *Bacillus subtilis* from the RsbR protein of *Listeria monocytogenes* allow σ^B activation following exposure to either physical or nutritional stress. *J Bacteriol*, 192(23):6279-6286.
<https://doi.org/10.1128/JB.00467-10>
- NicAogáin K, O'Byrne CP, 2016. The role of stress and stress adaptations in determining the fate of the bacterial pathogen *Listeria monocytogenes* in the food chain. *Front Microbiol*, 7:1865.
<https://doi.org/10.3389/fmicb.2016.01865>
- O'Byrne CP, Karatzas KAG, 2008. Chapter 5: the role of sigma B (σ^B) in the stress adaptations of *Listeria monocytogenes*: overlaps between stress adaptation and virulence. *Adv Appl Microbiol*, 65:115-140.
[https://doi.org/10.1016/S0065-2164\(08\)00605-9](https://doi.org/10.1016/S0065-2164(08)00605-9)
- Okpo E, Leith J, Smith-Palmer A, et al., 2015. An outbreak of an unusual strain of *Listeria monocytogenes* infection in North-East Scotland. *J Infect Public Health*, 8(6):612-618.
<https://doi.org/10.1016/j.jiph.2015.05.009>
- Pané-Farré J, Lewis RJ, Stülke J, 2005. The RsbRST stress module in bacteria: a signalling system that may interact with different output modules. *J Mol Microbiol Biotechnol*, 9:65-76.
<https://doi.org/10.1159/000088837>
- Price CW, 2011. General stress response in *Bacillus subtilis* and related Gram-positive bacteria. In: Storz G, Hengge R (Eds.), *Bacterial Stress Responses*, 2nd Ed. American Society of Microbiology (ASM) Press, Washington, DC, p.301-318.
<https://doi.org/10.1128/9781555816841.ch17>
- Shan Y, Liu ZQ, Li GW, et al., 2018. Nucleocapsid protein from porcine epidemic diarrhea virus isolates can antagonize interferon- λ production by blocking the nuclear factor- κ B nuclear translocation. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 19(8):570-580.
<https://doi.org/10.1631/jzus.B1700283>
- Shin JH, Brody MS, Price CW, 2010. Physical and antibiotic stresses require activation of the RsbU phosphatase to induce the general stress response in *Listeria monocytogenes*. *Microbiology*, 156:2660-2669.
<https://doi.org/10.1099/mic.0.041202-0>
- Varma JK, Samuel MC, Marcus R, et al., 2007. *Listeria monocytogenes* infection from foods prepared in a commercial establishment: a case-control study of potential sources of sporadic illness in the United States. *Clin Infect Dis*, 44(4):521-528.
<https://doi.org/10.1086/509920>
- Xia Y, Xin YP, Li XL, et al., 2016. To modulate survival under secondary stress conditions, *Listeria monocytogenes* 10403S employs RsbX to downregulate σ^B activity in the poststress recovery stage or stationary phase. *Appl Environ Microbiol*, 82(4):1126-1135.
<https://doi.org/10.1128/AEM.03218-15>
- Zhang ZC, Meng QL, Qiao J, et al., 2013. RsbV of *Listeria monocytogenes* contributes to regulation of environmental stress and virulence. *Arch Microbiol*, 195(2):113-120.
<https://doi.org/10.1007/s00203-012-0855-5>

Zhou YS, Gu YX, Qi BZ, et al., 2017. Porcine circovirus type 2 capsid protein induces unfolded protein response with subsequent activation of apoptosis. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*, 18(4):316-323.
<https://doi.org/10.1631/jzus.B1600208>

中文概要

题目: 单核细胞增多性李斯特菌 RsbR 抗酸应激机制

目的: 探明单增李斯特菌 RsbR 的两个保守磷酸化位点是否影响 SigB (sigma B) 的表达。

创新点: 解析了 RsbR 在单增李斯特菌中 SigB 网络调控中的作用。

方法: 通过基因缺失、定点突变和回补技术构建 *rsbR* 基因缺失株、回补株和回补突变菌株, 进行酸应激条件下的生长能力和强酸条件下的存活能力试验, 并检测在酸应激下菌株中的 SigB 基因和蛋白表达的改变情况。

结论: RsbR 缺失能够显著降低单增李斯特菌的抗酸应激能力和下调 SigB 表达, RsbR-175 位点是 SigB 调控途径中 RsbT 释放的先决条件。

关键词: 单增李斯特菌; RsbR; SigB; 抗酸应激