

The small non-coding RNA *rli106* contributes to the environmental adaptation and pathogenicity of *Listeria monocytogenes*

Yun Guo^{1*}, Chunhui Ji^{1*}, Lixia Wang^{1*}, Chengcheng Ning¹, Na Li¹,
 Zhiyuan Li¹, Yunxia Shang¹, Yaling Li¹, Yaoqiang Sun¹,
 Xiaoxing Huang¹, Jie Li¹, Xuepeng Cai², Qingling Meng¹, Jun Qiao^{1*}✉

¹College of Animal Science and Technology, Shihezi University, Shihezi, Xinjiang 832003, China

²State Key Lab of Veterinary Etiological Biology, Lanzhou Veterinary Research Institute, Chinese Academy of Agricultural Sciences, Lanzhou, Gansu 730046, China
 qj710625@shzu.edu.cn

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Abstract

Introduction: *Listeria monocytogenes* (LM) is an important food-borne pathogen, and the risk of its ingestion is a serious public health issue. The better its environmental adaptation mechanisms and pathogenicity are understood, the better the risk it poses can be countered. The regulatory role of the small non-coding RNA (sRNA) *rli106* in the environmental adaptation and pathogenicity of LM is still unclear and this study investigated that role through its biological function. **Material and Methods:** An LM-*Arli106* gene deletion strain and an LM-*Arli106/rli106* gene complementation strain were constructed using the homologous recombination technique. Then, the adaptation of these strains to temperature, alkalinity, acidity, salinity, ethanol and oxidative stressors, their biofilm-forming ability and their pathogenicity in mice were investigated to show the regulatory roles of sRNA *rli106* in LM. The target gene of *rli106* was also predicted, and the interaction between it and *rli106* was verified by a two-plasmid co-expressing system based on *E.coli* and Western blot analysis. **Results:** The adaptation of LM-*Arli106* to environmental stressors of pH 9, 5% NaCl and 8% NaCl, 3.8% ethanol and 5 mM H₂O₂ was significantly reduced when compared to the parental (LM EGD-e) and complementation strains. Also, the biofilm formation, cell adhesion, invasion, intracellular proliferation and pathogenicity of LM-*Arli106* in mice were significantly reduced. The results of two-plasmid co-expression and Western blot showed that *rli106* can interact with the mRNA of the predicted *DegU* target gene. **Conclusion:** The sRNA *rli106* may positively regulate the expression of the *DegU* gene in LM. This study sheds light on its regulatory roles in environmental adaptation and pathogenicity, providing new insights into the molecular mechanism of sRNA mediation in LM.

Keywords: *Listeria monocytogenes*, *rli106* sRNA, environmental adaptation, pathogenicity, *DegU* target gene.

Introduction

Listeria monocytogenes (LM), an important Gram-positive foodborne human-animal pathogen of the *Listeria* genus, is widely distributed in nature and can be transmitted by a variety of routes (2, 10). Infection with LM in humans and animals can manifest clinically as gastroenteritis, meningitis, abortion and sepsis (24, 28). Especially once neonates, pregnant females and immunocompromised individuals are infected, the pathogen can cause the death of the patient. The associated disease, listeriosis, has a mortality rate of 20% to 30% in these groups (13, 19, 21, 25). Usually, LM can contaminate meat, eggs and dairy products

during animal food production and processing, causing serious public health and safety problems (13, 22).

It has been revealed that small non-coding RNA (sRNA) sequences can bind to target messenger RNA (mRNA) and regulate expression at the post-transcriptional level of bacterial genes which play important roles in response to environmental changes and virulence regulation (1, 3, 12, 15). So far, more than 150 sRNA species have been identified in LM using bioinformatics and molecular biology techniques. However, the biological functions of most of them are still unclear (18, 26). One, *rli106*, is a newly identified sRNA species in the LM genome, which displays significant differences in intracellular and extracellular

expression following LM infection in host cells (5). To date, the regulatory role of *rli106* remains unknown.

The main purpose of this study was to investigate the biological function of the *rli106* sRNA in regard to the environmental adaptation and pathogenicity of LM. In this study, we constructed *rli106* deletion and complementation strains using a homologous recombination technique. By determining and comparing the differences in the environmental adaptability and pathogenicity of *rli106* deletion, complementation and parental strains, the regulatory roles of *rli106* in environmental adaptability and pathogenicity mechanisms were revealed. The target gene of *rli106* was also predicted and verified using a co-expression system based on *E. coli* and Western blot analysis, which provided new insights into the molecular mechanism of sRNA mediation of environmental adaptation and pathogenicity in *Listeria monocytogenes*.

Material and Methods

Primer design. The primers for the deletion and complementation strains were designed according to the genome sequence of the LM EGD-e parental strain registered in GenBank (accession number AL591824.1) using Primer 5.0 software (Premier Biosoft International, San Francisco, CA, USA). Enzyme-recognising sites

such as *Bam*H I, *Hind* III and *Kpn* I (TaKaRa Bio, Kusatsu, Japan) were introduced into the 5' end of these primers (Table 1).

Construction of deletion mutant and complementation strains. The LM EGD-e parental strain (kindly donated by Prof. W. Goebel of the University of Würzburg, Germany) was cultured in brain heart infusion broth (BHI) (Sangon, Shanghai, China). Briefly, LM genomic DNA was extracted through the steps specified in the Bacterial Genomic DNA Extraction Kit (Omega, Norcross, GA, USA), and the *rli106* gene was amplified using *rli106* forward (F) and *rli106* reverse (R) primers. The PCR product was recovered and ligated with a pMD19-T simple vector (TaKaRa Bio) to generate pMD19-T-*rli106* for sequencing. Next, the upstream and downstream homologous arms of the *rli106* gene were amplified with P1-P2 and P3-P4 primer pairs, respectively, and the deletion fragment Δ *rli106* was generated by splicing by overlap extension PCR. The Δ *rli106* fragment was cloned into the pMD19-T simple vector (TaKaRa Bio) to obtain the pMD19-T- Δ *rli106*. The recombinant shuttle pKSV7- Δ *rli106* plasmid was generated by inserting the Δ *rli106* fragment into the shuttle pKSV7 plasmid. After that, pKSV7- Δ *rli106* was electrotransformed into LM EGD-e competent cells at 2,500 V for 5.0 ms, and the positive clones were screened by PCR using the P5-P6 primer pair.

Table 1. Primers used in the study

Primer name	Primer sequences (5'→3')	Product size (bp)
<i>rli106</i> F	GCCTCTTGCAATCGAAA	225
<i>rli106</i> R	ACTACTATTACTTTAGACTAGTTTTCAATT	
P1	GGTACC AAATAGCCGACTGCTCCAGC	351
P2	AAATTCGTGTTATAATAAAATTGGTGAAATTAGATAAGTG	
P3	CACCTATCTAATTTACCAATTTTATTATAACACGAATTT	383
P4	CTGCAG GATCTCTAGCATTGGAAAGCCA	
P5	AAATAGCCGACTGCTCCAGC	722
P6	GATCTCTAGCATTGGAAAGCCA	
P7	CCGTGCTTGATTGCCGTTAC	1391/1166
P8	AACTAGGCCGCTGCACC	
CF	AAGCTTGCTCTTTGCAATCGAAA	239
CR	CGGGATCCACTACTATTACTTTAGACTAG	
<i>rli106</i> F1	GGATCCCAGTTGGCATATTAATATC	271
<i>rli106</i> R1	CGGGGTACCGGTTAAGCGGTTTTGC	
<i>Deg</i> UF	CAAGCTTAAAAGAGAAATGTTTAATT	721
<i>Deg</i> UR	GGTACCACTGTTTTTCTACTAATGA	
<i>16S rRNA</i> F	GATGCATAGCCGACCTGAGA	116
<i>16S rRNA</i> R	TGCTCCGTCAGACTTTCGTC	
<i>hem</i> CF	CGGATCCATGACGTTATCTAAAGGA	1122
<i>hem</i> CR	CCAAGCTTTTAAATTCGTTCTTCTACA	
<i>Deg</i> UF1	GGATCCATGGCACTCAAATCATGATTG	687
<i>Deg</i> UF2	CAAGCTTTTAGCGAATGTATACCCAGCC	

F – forward; R – reverse

The positive clones were cultured for 10 generations at 42°C in BHI medium containing 10 µg/mL chloramphenicol, and passaged for 20 generations in BHI liquid medium without chloramphenicol to generate an LM-*Arli106*-deletion mutant strain. To construct the *rli106* gene complementation strain, *rli106* was first cloned into a pHT304 vector to obtain the recombinant plasmid pHT304-*rli106*. In the next step, pHT304-*rli106* was electrotransformed into LM-*Arli106* competent cells and cultured on plates containing erythromycin-resistant BHI (final concentration 50 µg/mL) to generate the CLM-*Arli106* complementation strain.

Growth assay of LM. LM EGD-e, LM-*Arli106* and CLM-*Arli106* were inoculated into BHI liquid medium at the ratio of 1 : 100 and cultured at 30°C, 37°C and 42°C, respectively. The 600 nm optical density (OD_{600nm}) was measured at different times, and growth curves were plotted. At the same time, the bacterial broth was transferred to BHI liquid medium under different conditions of pH 4, pH 7, pH 9, 5% NaCl, 8% NaCl, 3.8% ethanol and 5mM H₂O₂. The growth curves were plotted to examine the effects of the *rli106* gene deletion on LM's response to environmental stressors under different conditions (18).

Assay of motility and biofilm formation of LM. Briefly, LM EGD-e, LM-*Arli106* and CLM-*Arli106* were inoculated into BHI solid medium and incubated overnight at 37°C. Then, the semi-solid BHI tube (containing 0.3% agar) was punctured with the inoculation needle containing the bacteria and cultured at 28°C for 36 h. In addition, 1 µL of the bacterial solution was spotted on a 0.3% semi-solid BHI plate and cultured at 28°C for 36 h, after which time the diameters of the bacterial colonies which formed on the plate were measured to access the motility of bacteria. Meanwhile, LM EGD-e, LM-*Arli106* and CLM-*Arli106* were inoculated into 96-well microplates and cultured at 37°C for 12 h, 24 h and 48 h, respectively. The plates were stained with a 1% crystalline violet solution according to Zetzmann *et al.* (28) and decolourised by adding 95% ethanol, and biofilm size was measured by reading at OD_{570nm} using an enzyme-linked detector (BioTek Instruments, Winooski, VT, USA). Before decolourisation, the microtitre plate was placed under an inverted microscope (Leica Microsystems, Jena, Germany) for observation and photographic recording.

Determination of adhesion, invasion and intracellular survival of LM. In brief, RAW264.7 mouse macrophages were cultured in six-well microplates, and the cells were inoculated with LM EGD-e, LM-*Arli106* and CLM-*Arli106* at a 10:1 ratio of multiples of infection. One hour after infection, the cells were washed three times with phosphate-buffered saline (PBS), and 500 µL of 0.1% Triton X-100 was added to lyse the cells for 10 min. The lysate was collected for bacterial counting, and the adhesion rate was calculated according to the reference described by Peng (18). Bacteria were allowed to infect RAW264.7 cells for 1 h, and then replaced with fresh Dulbecco's modified Eagle's medium containing 100 µg/mL gentamicin for

another 1 h. The cells were washed with PBS three times and lysed, their bacterial contents were counted and then the invasion rate was calculated. Additionally, 100 µg/mL gentamicin was used to kill the bacteria on the surface of infected RAW264.7 cells. After 2, 4, 6, 8, 10 and 12 h of cell culturing, the cells were lysed and the bacteria in them were counted to determine the intracellular viability of LM EGD-e, LM-*Arli106* and CLM-*Arli106*.

Determination of pathogenicity of LM. One hundred and twenty 6–8-week-old Kunming mice were randomly divided into four groups. The use of these mice was approved by the Research and Ethical Committee of Shihezi University. Each group was further divided into five subgroups of six mice, each of which was injected intraperitoneally either with 500 µL of LM EGD-e, LM-*Arli106* and CLM-*Arli106* bacterial solutions at one of the dilution gradients 10⁵, 10⁶, 10⁷, 10⁸ or 10⁹ CFU/mL, or with sterile PBS (pH 7.2). After observation for seven consecutive days, the mice deaths were counted every day, the survival curves were plotted and the lethal dose, 50% (LD₅₀) was calculated using Karber's method. In parallel, twenty-four 6–8-week-old Kunming mice were randomly divided into four groups. Each group was intraperitoneally injected with one of a sublethal dose of LM EGD-e, a sublethal dose of LM-*Arli106*, a sublethal dose of CLM-*Arli106* or sterile PBS (pH 7.2). After infection, these mice were humanely sacrificed and their spleens and livers were collected aseptically every day to determine bacterial loads in the organs. These organs were fixed with formalin and tissue sections were prepared for histopathological observation.

Predictive analysis of target genes of *rli106*. Potential target genes were predicted by the online TargetRNA2 software (<http://cs.wellesley.edu/TargetRNA2>). The possible regulatory mode of *rli106* on target genes was analysed.

Analysis and verification of interaction between *rli106* and target gene mRNA. Briefly, *rli106* sRNA and the target gene were amplified and cloned into the pUT18C and pMR-LacZ vectors to generate the recombinant pUT18C-*rli106* and pMR-LacZ-*target* plasmids. The pUT18C vector and pUT18C-*rli106* plasmid were electrotransformed into the BTH101 *E. coli* competent cells, and then the pMR-LacZ-*target* was electrotransformed into BTH101 containing the pUT18C or pUT18C-*rli106* plasmid. The bacteria were cultured in Luria-Bertani (LB) liquid medium containing ampicillin (Amp, 100 µg/mL) and kanamycin (Kan, 100 µg/mL) for 4 h at 37°C, and then cultivated in LB plates containing 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-gal), Amp (100 µg/mL) and Kan (100 µg/mL). Then the colour and OD_{420nm} of the colonies of different strains were observed and recorded.

Quantitative RT-PCR. The mRNA level of the *DegU* gene relative to the internal reference *16S rRNA* gene was measured using a quantitative RT-PCR (RT-qPCR). In brief, the cultures of LM EGD-e, LM-*Arli106* and CLM-*Arli106* were diluted 100-fold with BHI medium and cultured to OD_{600nm} ≈ 0.5. Total RNA was

extracted using Trizol (Invitrogen, Carlsbad, CA, USA) and reverse transcribed into cDNA *via* the AMV reverse transcriptase kit (TaKaRa). The RT-qPCR was performed using the SYBR *Premix Ex Taq* kit (TaKaRa) on a LightCycler 480 PCR device (Roche, Basel, Switzerland) according to the manufacturers' instructions. Each sample was assayed in three replicates and the mRNA level of the target gene was calculated using the $2^{-\Delta\Delta CT}$ method (15, 18).

Western blot analysis. Briefly, LM EGD-e, LM- $\Delta rli106$ and CLM- $\Delta rli106$ were inoculated into BHI liquid medium and incubated overnight at 37°C. Protein extracts were prepared by ultrasonic lysis for sodium dodecyl sulphate polyacrylamide gel electrophoresis. After these proteins were transferred, the membrane was blocked by a 5% BSA buffer at 4°C overnight. Western

blot analysis was then performed using the mouse anti-target protein antibody as the primary antibody, and HRP-labelled rabbit anti-mouse IgG as the secondary antibody. The bands of Western blot analysis were scanned using ImageJ 1.8.0 software (23), and the effect of *rli106* on the protein level of the target mRNA was quantitatively analysed.

Statistical analysis of data. GraphPad Prism 5.0 software (GraphPad Software, San Diego, CA, USA) was used to analyse the experimental data. Statistical analysis was performed on continuous variables using one-way analysis of variance to compare whether there were significant differences between the groups. Variable data were analysed using the chi-squared test. Differences were considered significant when $P < 0.05$, and extremely significant when $P < 0.01$.

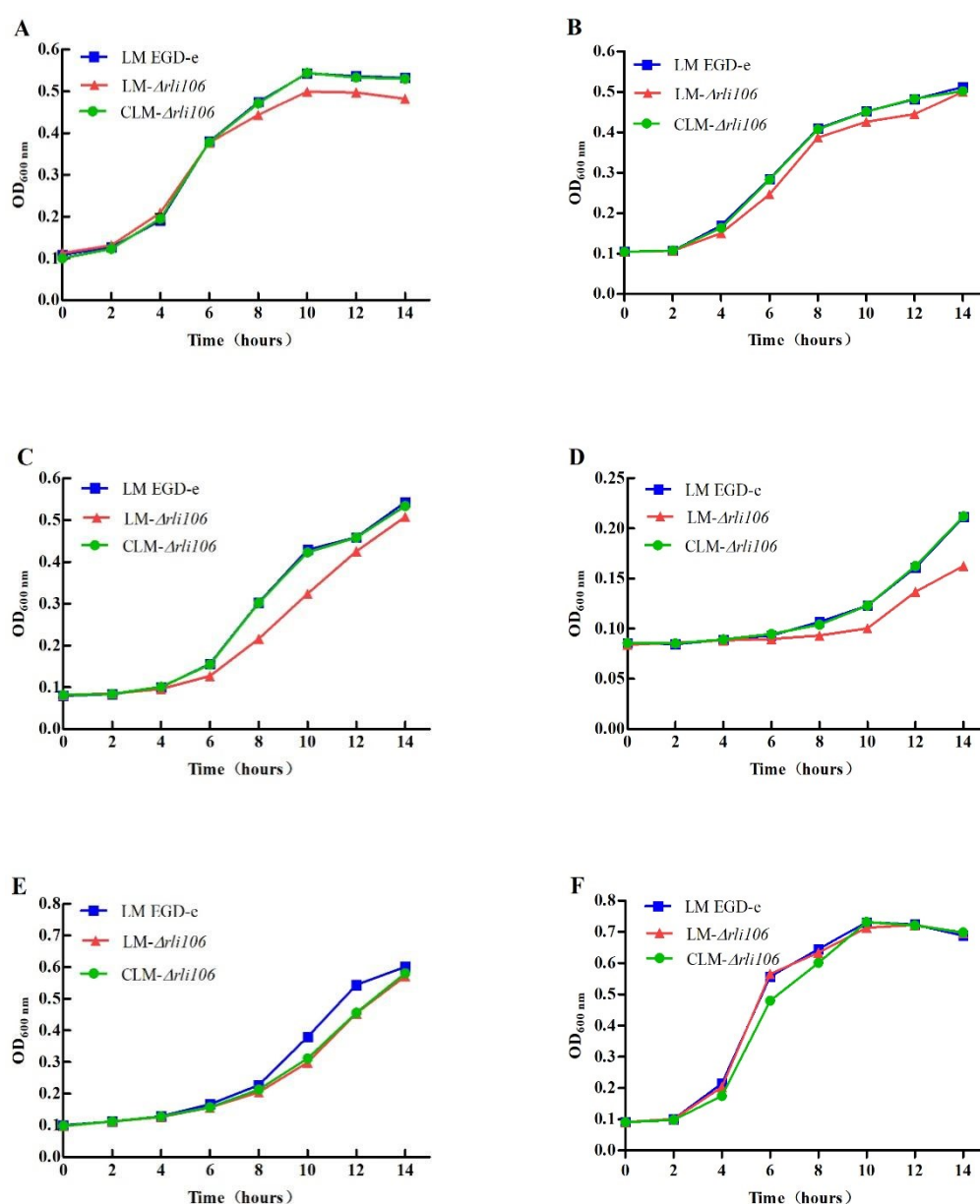


Fig. 1. Growth curves of LM strains under different environmental stresses. LM EGD-e – parental strain of *Listeria monocytogenes*; LM- $\Delta rli106$ – *rli106* deletion strain of *Listeria monocytogenes*; CLM- $\Delta rli106$ – *rli106* complementation strain of *Listeria monocytogenes*
A–F – Growth curves of LM EGD-e, LM- $\Delta rli106$ and CLM- $\Delta rli106$ under the conditions of 42°C, pH 9, 5% NaCl and 8% NaCl, 3.8% ethanol and 5 mM H₂O₂. Bars indicate the standard error of the mean

Results

The deletion mutant strain LM-*Arli106* and the complementation strain CLM-*Arli106* were successfully generated, which was confirmed by PCR and sequencing (Supplementary Figs S1, S2, S3 and S4).

The growth of the three strains did not differ significantly ($P > 0.05$) under the conditions of 30°C and 37°C. However, at 42°C, the growth of LM-*Arli106* slowed after 8 h when compared with that of LM EGD-e and CLM-*Arli106*, indicating that LM-*Arli106* was less adaptable in high-temperature environments (Fig. 1A). Moreover, under the conditions of pH 9, 5% NaCl and 8% NaCl, 3.8% ethanol and 5 mM H₂O₂, the growth of LM-*Arli106* was less than that of LM EGD-e and CLM-*Arli106* (Fig. 1B–1F).

As shown in Fig. 2, there were no significant differences ($P > 0.05$) in the motility of LM-*Arli106* when compared to that of LM EGD-e and CLM-*Arli106* in the semi-solid BHI test tube (Fig. 2A) and on the BHI plates (Fig. 2B and 2C), indicating that *rli106* gene deletion did not affect the motility of LM.

All strains were able to form biofilms over 12–24 h. However, the biofilm formation by LM-*Arli106* was significantly weaker than that by LM EGD-e and CLM-*Arli106* ($P < 0.01$) at 48 h (Fig. 3A and 3B).

Compared with LM EGD-e and CLM-*Arli106*, the adhesion and invasion capacity of LM-*Arli106* were

notably decreased, with a highly significant difference ($P < 0.01$) (Fig. 4A and 4B). Bacterial loads in RAW264.7 cells were significantly lower in LM-*Arli106* ($P < 0.01$) when compared with those of LM EGD-e and CLM-*Arli106* (Fig. 4C).

The LD₅₀ of LM-*Arli106* was significantly decreased when compared with those of LM EGD-e and CLM-*Arli106* ($P < 0.05$) (Supplementary Table 1). Survival curves were significantly longer in mice inoculated with LM-*Arli106* when compared with the curves of mice in the LM EGD-e and CLM-*Arli106* groups ($P < 0.05$) (Fig. 5A). On days 1, 2 and 5 post infection, the bacterial loads in the livers and spleens of mice infected with LM-*Arli106* were significantly lower than those of mice infected with LM EGD-e and CLM-*Arli106* ($P < 0.01$) (Fig. 5B and 5C).

Compared with the control mice (PBS-injected group), the livers, spleens and kidneys of mice of the LM EGD-e, LM-*Arli106* and CLM-*Arli106* groups displayed different degrees of pathological changes. Cell degeneration and necrosis were present in the livers of the LM EGD-e and CLM-*Arli106* group mice. Pathological changes in the spleens were haemorrhage and lymphocyte infiltration. Also, granular degeneration and hyperplasia in the kidney were observed. In contrast, the pathological changes in LM-*Arli106*-infected mice were significantly weaker (Fig. 6), indicating that deletion of the *rli106* gene can weaken the pathogenicity of LM.

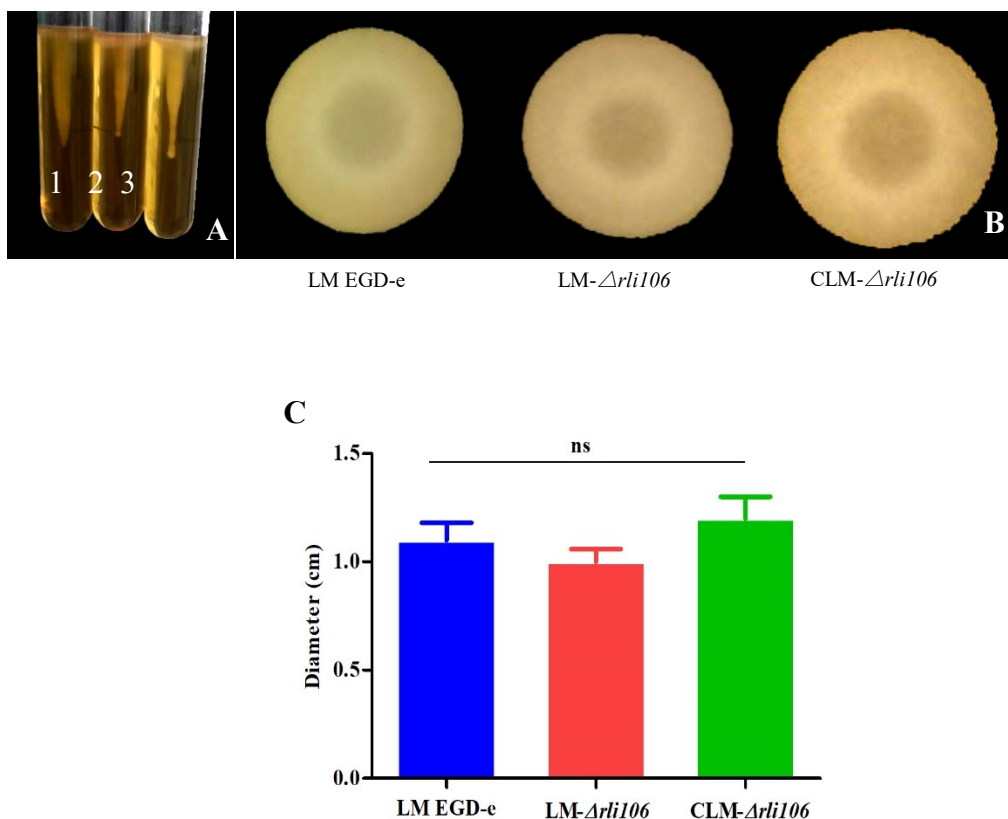


Fig. 2. Determination and comparisons of the motility of different strains of LM. LM EGD-e – parental strain of *Listeria monocytogenes*; LM-*Arli106* – *rli106* deletion strain of *Listeria monocytogenes*; CLM-*Arli106* – *rli106* complementation strain of *Listeria monocytogenes*

A – Kinetics of LM EGD-e (1), LM-*Arli106* (2) and CLM-*Arli106* (3) in semi-solid medium; B – Kinetics in tablets; C – Diameter size of the bacterial colony on the plate; ns – not significant, $P > 0.05$. Bars indicate the standard error of the mean

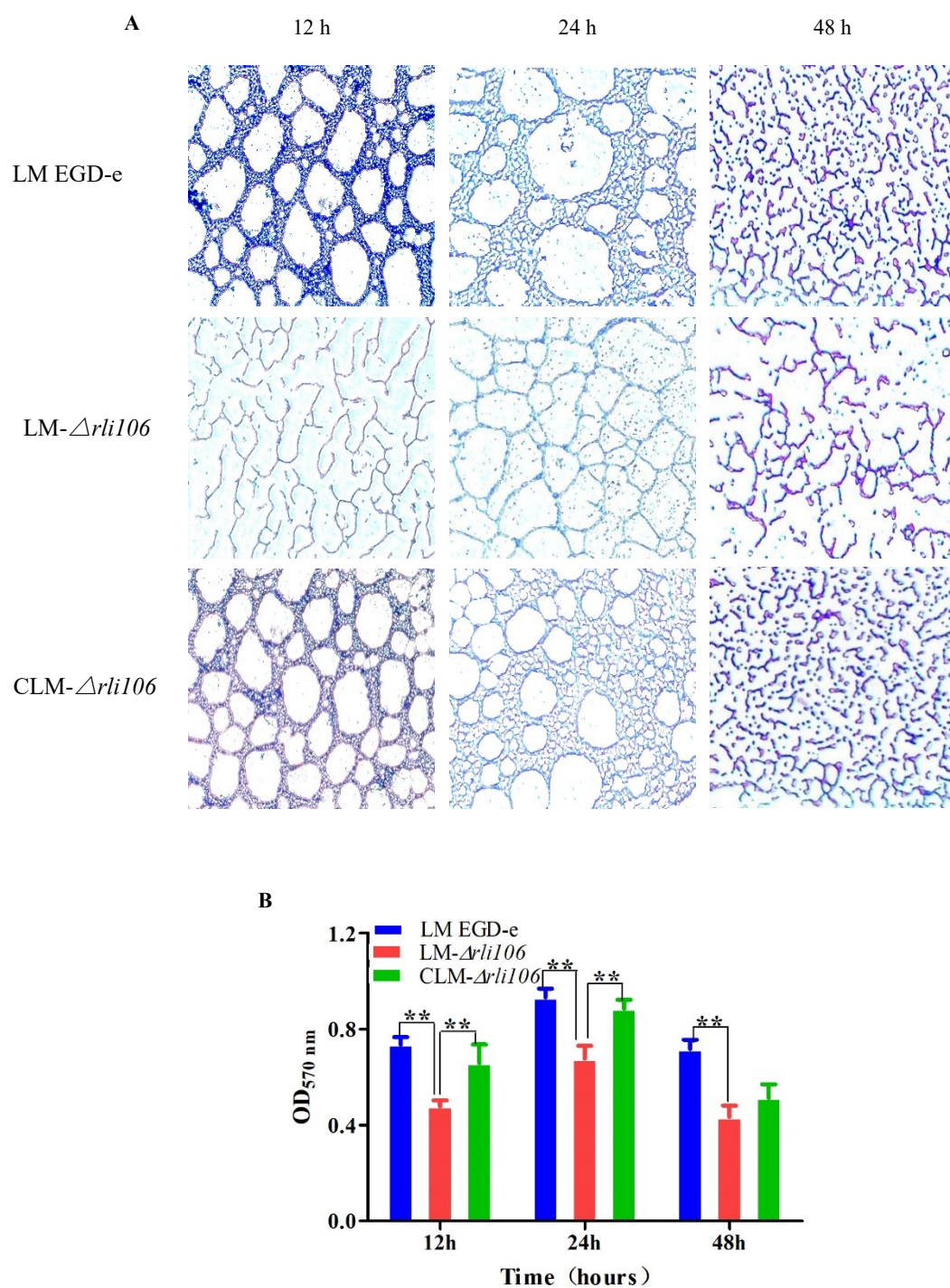


Fig. 3. Effects of *rli106* gene deletion on biofilm formation of LM

A – Formation of biofilm by the parental strain of *Listeria monocytogenes* (LM EGD-e), *rli106* deletion strain of *Listeria monocytogenes* (LM- $\Delta rli106$) and *rli106* complementation strain of *Listeria monocytogenes* (CLM- $\Delta rli106$) at 12 h, 24 h and 48 h; B – Comparison of biofilm formation at 12 h, 24 h and 48 h. Bars indicate the standard error of the mean (SE); ** – $P < 0.01$

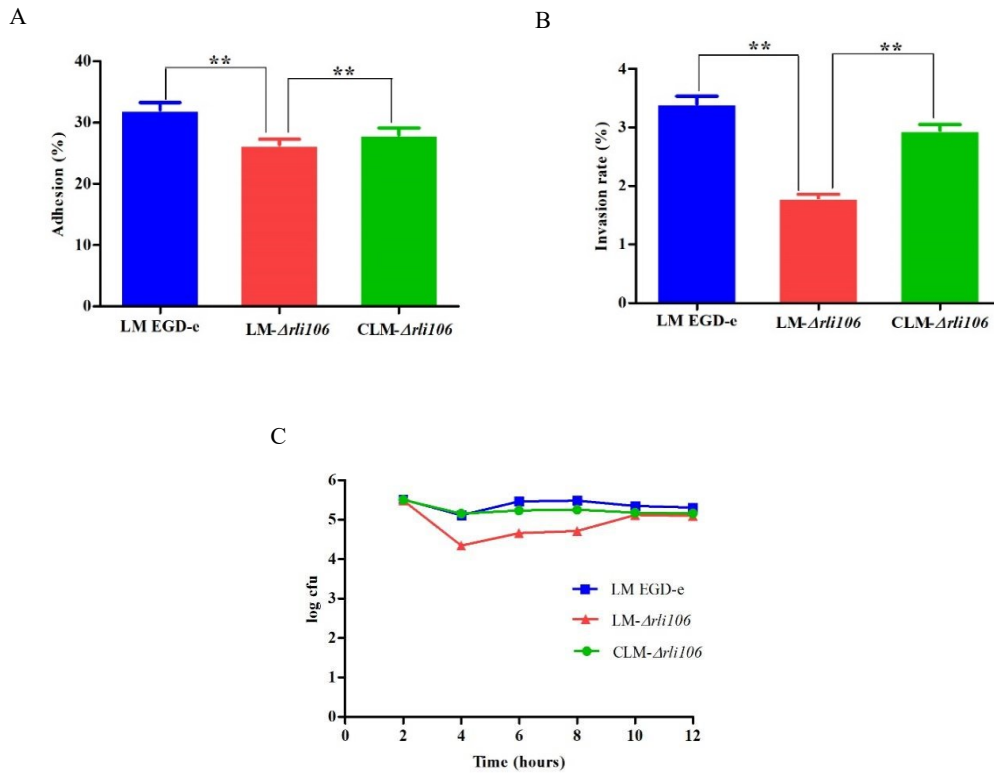


Fig. 4. Determination of adhesion, invasion and intracellular viability in RAW264.7 macrophages for the parental strain of *Listeria monocytogenes* (LM EGD-e), *rli106* deletion strain of *Listeria monocytogenes* (LM- $\Delta rli106$) and *rli106* complementation strain of *Listeria monocytogenes* (CLM- $\Delta rli106$). A – Adhesion rate of LM EGD-e, LM- $\Delta rli106$ and CLM- $\Delta rli106$ in RAW264.7 cells; B – Invasion rate of LM EGD-e, LM- $\Delta rli106$ and CLM- $\Delta rli106$ in RAW264.7 cells; C – Determination of intracellular viability in macrophages. Bars indicate the standard error of the mean (SE); ** – $P < 0.01$

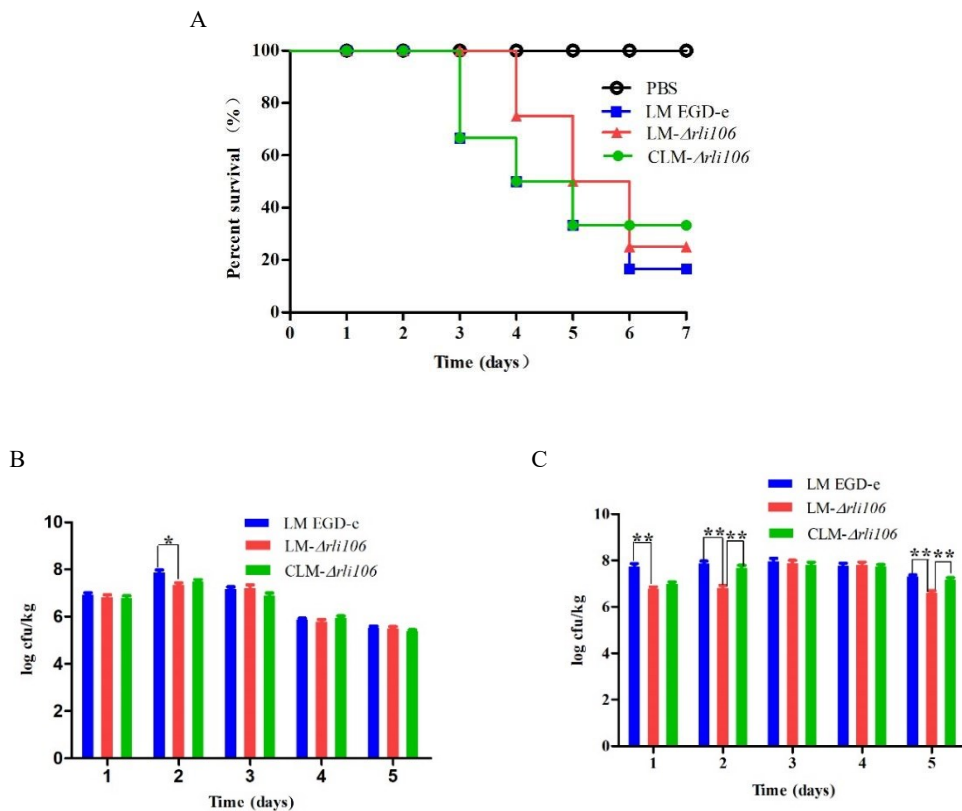


Fig. 5. Effects of *rli106* gene deletion on virulence of LM. A – Survival curves of mice infected with the parental strain of *Listeria monocytogenes* (LM EGD-e), *rli106* deletion strain of *Listeria monocytogenes* (LM- $\Delta rli106$) and *rli106* complementation strain of *Listeria monocytogenes* (CLM- $\Delta rli106$); B and C – Bacterial loads in the livers and spleens of mice infected by LM EGD-e, LM- $\Delta rli106$ and CLM- $\Delta rli106$; bars indicate the standard error of the mean (SE); * – $P < 0.05$; ** – $P < 0.01$ (LM EGD-e)

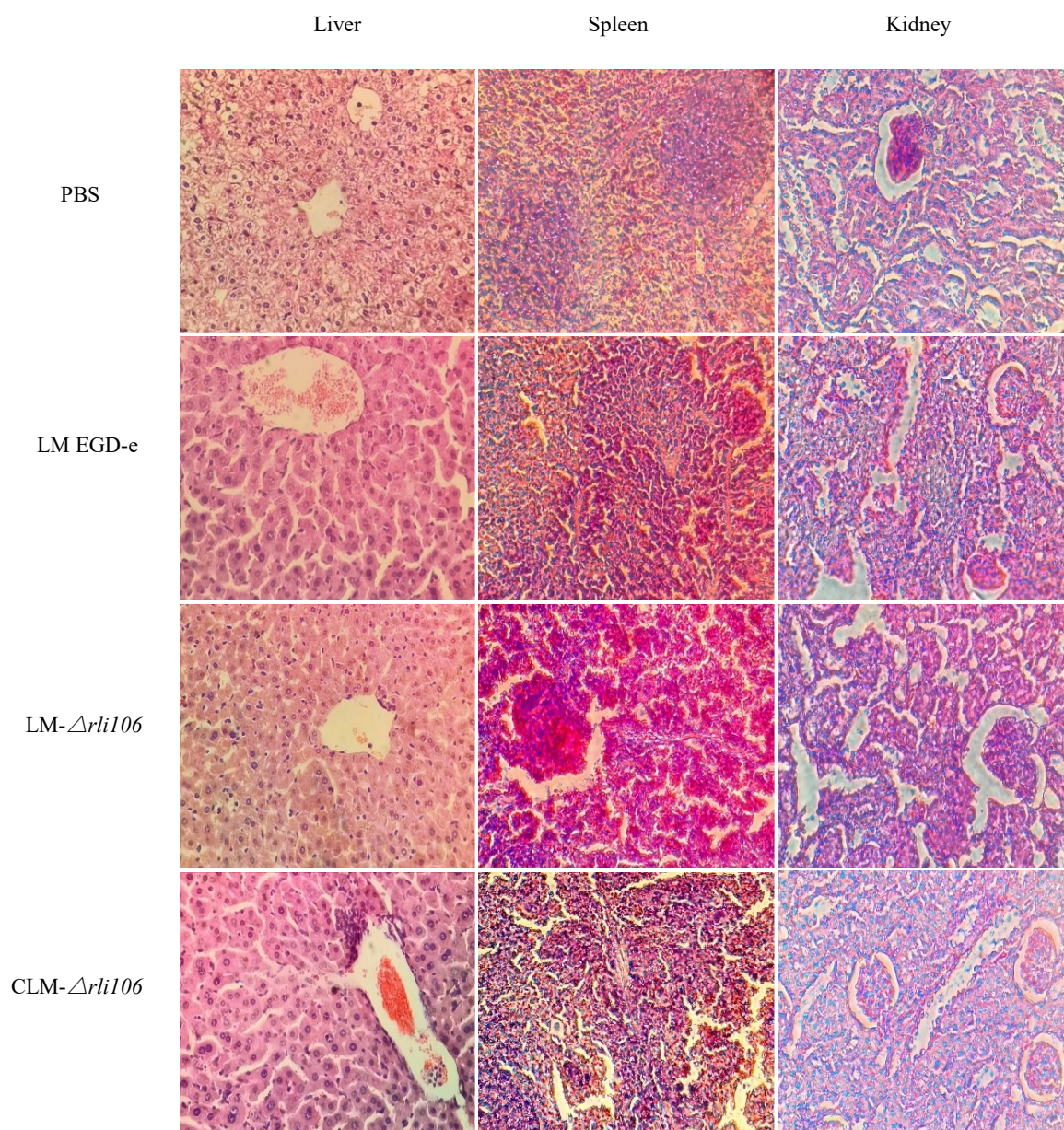


Fig. 6. Histopathological examination of liver, spleen and kidney of mice infected by LM strains (haematoxylin and eosin staining, $\times 400$). LM EGD-e – parental strain of *Listeria monocytogenes* *rli106*; LM- $\Delta rli106$ – *rli106* deletion strain of *Listeria monocytogenes*; CLM- $\Delta rli106$ – *rli106* complementation strain of *Listeria monocytogenes*

The bases 12–32 of *rli106* can complementarily pair with the bases 49–69 of *DegU* 5' untranslated region (UTR), suggesting that *rli106* may interact with *DegU* mRNA. This interaction possibly plays a biological role in regulating *DegU* mRNA (Fig. 7A). The results of the expression experiment in the two plasmid co-expression system showed that the lawns of *E. coli* co-transformed with pUT18C-*rli106* and pMR-LacZ-*DegU* turned a deeper blue on X-gal plates than lawns co-transformed by the pUT18C and pMR-LacZ-*DegU* plasmids (Fig. 7B, 7C and 7D), which suggests that *rli106* can positively regulate the expression of the *DegU* gene through the *rli106*–*DegU* mRNA interaction.

Quantitative RT-PCR results revealed that the mRNA level of the *DegU* gene was significantly downregulated in the deletion strain compared to LM EGD-e and CLM- $\Delta rli106$ ($P < 0.01$), indicating that *rli106* can modify the mRNA level of the *DegU* gene via base pairing (Fig. 7E).

Compared with the level in the LM EGD-e and CLM- $\Delta rli106$ strains, Western blot analysis showed that the protein level of *DegU* in the LM- $\Delta rli106$ strain was decreased ($P < 0.01$) (Fig. 7F and 7G), which suggests that *rli106* can modulate the expression of the *DegU* gene at the post-transcriptional level via a positive regulatory mechanism.

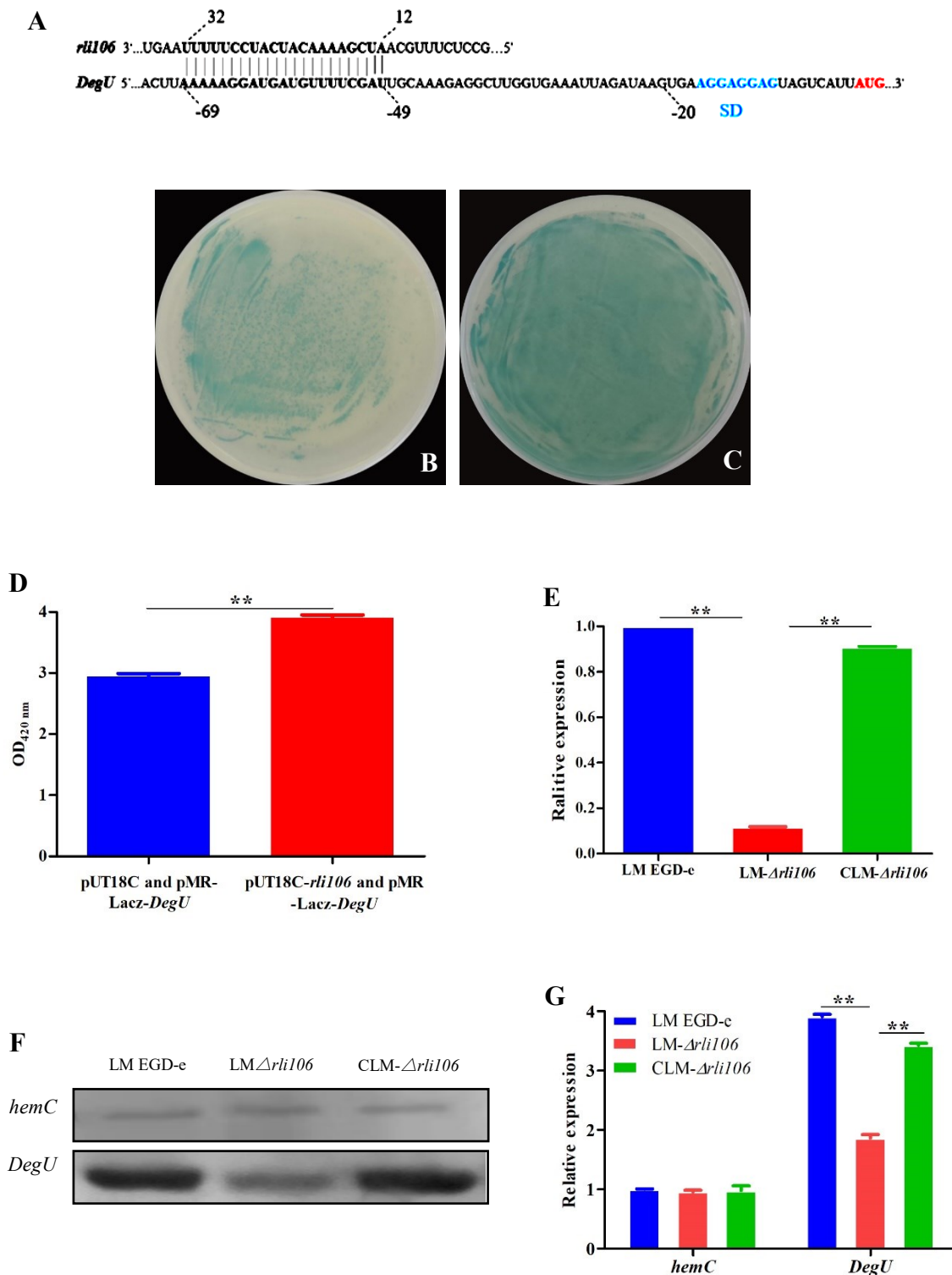


Fig. 7. Verification of interaction between *rli106* sRNA and mRNA of target gene *DegU*. A – Complementary pairing position of *DegU* target gene and *rli106*; B and C – Bacterial lawns of BTH101 co-transformed by pUT18C and pMR-LacZ-*DegU*, and BTH101 co-transformed by pUT18C-*rli106* and pMR-LacZ-*DegU*; D – OD_{420 nm} of bacterial lawns; E – mRNA level of *DegU* target gene in the parental strain of *Listeria monocytogenes* (LM EGD-e), *rli106* deletion strain of *Listeria monocytogenes* (LM- Δ *rli106*) and *rli106* complementation strain of *Listeria monocytogenes* (CLM- Δ *rli106*); F and G – protein level of *DegU* in LM EGD-e, LM- Δ *rli106* and CLM- Δ *rli106*

Discussion

Current studies have shown that sRNA species can bind to target mRNA to regulate gene expression at the post-transcriptional level and are considered to be key negative or positive regulators of gene expression (8, 9, 16, 17). Therefore, they may participate in the modulation

of physiological processes in bacteria, including stress responses, population sensing, toxin-antitoxin systems or pathogenicity (5, 7, 14). Several sRNA species in LM, such as *rliB*, *rli31*, *rli33-1*, *rli27*, *rli47* and *rli50*, have recently been shown to be involved in the regulation of bacterial growth and virulence. Among them, *rli27* can regulate the expression of wall proteins by binding to the

5'-UTR of the *lmo0514* gene (20), while *rli47* may repress isoleucine biosynthesis through a direct interaction with the *ilvA* transcript in LM (14). In addition, existing studies have verified that sRNA species, as well as positive regulatory factor A and alternative general stress sigma factor σ B, formed a highly complex regulatory network in LM, which enabled the bacteria to survive and propagate *in vitro* and *in vivo* (9, 18).

Listeria monocytogenes can adapt to various stressful environments and survive in food processing, storage and transportation. It has been proved that sRNA species play important regulatory roles in bacteria during environmental changes such as variations in temperature, nutrition, oxidative stress and pH. Some sRNA species were involved in transmitting the signal and regulating stress responses through two-component signal transduction systems. In this investigation, we demonstrated that deletion of the *rli106* gene can suppress the adaptation and biofilm formation of LM under environmental stresses. It was shown that *rli106* played important regulatory roles in LM's resistance to environmental stress. Notably, a binding site of the *cpxR* transcription factor was predicted in the 5'-UTR sequence of the *rli106* gene (Supplementary Fig. S5). It has been proved that *cpxR* is a regulatory response protein of two-component systems in bacteria, which contributes to membrane stability and virulence by regulating the expression of target genes (6, 27). Thus, it was reasonably hypothesised that *cpxR* might regulate *rli106* to enhance the environmental adaptation of LM.

In the present study, the effects of *rli106* on the virulence of LM were also analysed by macrophage infection and mouse infection assays. The results showed that in interaction with macrophages, LM-*Arli106* had significantly lower adhesion, invasion and intracellular proliferation ability, and displayed significantly lower virulence compared to LM EGD-e and CLM-*Arli106*. These results indicated that *rli106* plays an important role in the regulation of LM pathogenicity.

Using TargetRNA2 software, the target genes of sRNA *rli106* were successfully predicted. To verify the regulatory relationship between sRNA *rli106* and *DegU* gene mRNA, two plasmid co-expressing systems based on *E. coli* and Western blot analysis were employed to confirm the interaction between sRNA *rli106* and the *DegU* gene. The results showed that sRNA *rli106* could positively regulate the expression of the *DegU* target gene. We deduced that sRNA *rli106* can protect *DegU* mRNA from degradation by binding to the sequence of the *DegU* target gene mRNA. This regulatory mode is similar to the mode described previously in several publications (6, 16, 17, 25, 27). Given that the *DegU* protein is a pleiotropic regulator involved in the regulation of virulence genes (4, 11), it can be inferred that sRNA *rli106* can modulate environmental adaptation and pathogenicity through the *DegU* protein in LM.

In conclusion, this study demonstrated for the first time that *rli106* sRNA contributed to the modulation of environmental adaptation and pathogenicity through regulating the expression of *DegU* mRNA, which provides new insights into the regulatory roles and mechanisms of sRNA mediation in LM.

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

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Animal Rights Statement: The authors declare that the experiments on animals were approved by the Research and Ethical Committee of Shihezi University (No. A2019216).

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*These authors contributed equally.

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