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Mig-14 plays an important role in influencing gene expression of *Salmonella enterica* serovar Typhi, which contributes to cell invasion under hyperosmotic conditions

Xiumei Sheng, Hong Zhang, Qiufeng Xia, Shungao Xu, Huaxi Xu, Xinxiang Huang*

Department of Biochemistry and Molecular Biology, Jiangsu University School of Medical Technology, Zhenjiang, Jiangsu 212013, China

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Abstract

mig-14 is a horizontally acquired host-induced virulence gene in *Salmonella enterica* serovar Typhi. The molecular function of *mig-14* is still unknown; sequence analysis showed that *mig-14* shared homology with the helix-loop-helix motif of the AraC family of transcriptional regulatory proteins. In our previous microarray-based studies, *mig-14* was upregulated at the early stage of high osmotic stress, indicating a potential role under this condition. Therefore, we compared growth and the global transcriptional difference between wild-type and *mig-14* mutant strains to identify the role of Mig-14. The results showed that growth of *mig-14* mutant strain was clearly slower than that of the wild-type strain, and 148 genes showed significant differences in expression between these two strains under upshift high osmotic treatment for 30 min. In total, 77 genes and 71 genes in the *mig-14* mutant strain were upregulated and downregulated, respectively. Genes involved in invasion, virulence, flagellation, motility and chemotaxis of *Salmonella* were downregulated. Thus, cell invasion abilities of these two strains were further analyzed. The results confirmed that activities of *mig-14* were important for cell invasion.

Keywords: *Salmonella enterica* serovar Typhi; *mig-14*; Regulation of gene expression; Cell invasion

1. Introduction

Salmonella infections are a significant cause of morbidity in humans and animals. *Salmonella enterica* serovar Typhi (*S.* Typhi) is a Gram-negative human entero-invasive pathogen that causes typhoid fever, a major health problem in developing countries, especially those of Southeast Asia, and it kills an estimated 600,000 people annually (Mathur et al., 2012; Parry et al., 2002). After being ingested, *S.* Typhi traverses the stomach and invades the specialized intestinal epithelial M cells of the Peyer's patches. Following intestinal invasion, the pathogens migrate into the mesenteric lymph nodes and reach the liver, spleen, and bone marrow through blood and lymph systems and then replicate in these sites (Everest et al., 2001; House et al., 2001; Huang and DuPont, 2005; Whichard et al., 2010). The lack of animal models due to *S.* Typhi's strict human host specificity has hindered its study (Faucher et al., 2006). Recently, new breakthroughs have been achieved, and *tlr11*$^{-/-}$ mice and immunodeficient Rag2$^{-/-}$ γc$^{-/-}$ mice can be efficiently infected by *S.* Typhi and immunized against *S.* Typhi, which provides a small-animal model for further study on this important human pathogen (Song et al., 2010; Mathur et al., 2012).

mig-14 is a horizontally acquired host-induced virulence gene which is necessary for fatal infection in the mouse model of enteric fever (Bäumler, 1997; Valdivia and Falkow, 1997; Valdivia et al., 2000). It maps to centisome 61 in the *S. enterica* chromosome. This region (centisomes 59–61) contains several genetic markers and is responsible for some of *Salmonella*'s unique physiological and biochemical characteristics. Different segments in this region are present or absent

* Corresponding author.
E-mail addresses: shengxiumei@hotmail.com (X. Sheng), 564966815@qq.com (H. Zhang), xqf0688@sina.com (Q. Xia), xushungao@yahoo.com (S. Xu), xuhx56@yahoo.com.cn (H. Xu), huxinx@yahoo.com.hk (X. Huang).

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in various *Salmonella* serovars (Bäumler and Heffron, 1998; Valdivia et al., 2000). *mig-14* is one of the genes that was acquired by all *S. enterica* subspecies I serovars associated with infection of warm-blooded animals and deleted in *S. enterica* subspecies II and IV during the evolution of *S. enterica* (Valdivia et al., 2000).

Mig-14 is an inner-membrane-associated protein that promotes *S.* Typhimurium resistance to the cathelin-related antimicrobial peptide (CRAMP), polymyxin B (PB), protamine and the mammalian antimicrobial peptide protegrin-1, survival within activated macrophages and persistent infection, but resistance to antimicrobial peptides by *mig-14* was not correlated with direct modifications of the lipopolysaccharide structure (Brodsky et al., 2002; Brodsky et al., 2005). The molecular function of *mig-14* is still unclear. Sequence analysis showed that *mig-14* shared homology with the helix-loop-helix motif of the AraC family of transcriptional regulatory proteins (Gallegos et al., 1997), suggesting it can regulate gene expression. In our previous microarray-based studies, *mig-14* was upregulated at the early stage of high osmotic stress (Huang et al., 2007), which indicated that the Mig-14 protein had a potential regulatory function under this condition. To identify the function of Mig-14, we constructed a *mig-14* mutant (Δ*mig-14*) and compared the global transcriptional difference between the wild-type strain and the *mig-14* mutant strain by utilizing genomic DNA microarrays. Three plasmids harboring different segments of *mig-14* were introduced into Δ*mig-14* and the regulatory functions of these three segments were compared by quantitative real-time PCR. The three segments are the whole segment of *mig-14*, the segment of *mig-14* in the cytoplasm and the segment of *mig-14* in the periplasm, respectively. The different domain of Mig-14 is shown in Fig. 1.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The bacterial strains and plasmids used in this study are listed in Table 1. Bacteria were grown in an LB broth containing 50 mM NaCl (pH 7.0) at 37 °C overnight with shaking. A 300 μl overnight culture of bacteria strain was added to 30 ml LB broth containing 50 mM NaCl and incubated at 37 °C with shaking to an OD_{600} of 0.5. Then the log-phasic bacteria were incubated at upshift high osmolarity by increasing the sodium chloride concentration in the medium from 50 to 300 mM for 30 min.

VKIQEVKRILTRWQPSSFTLYREVFTQYGGSINMHPDIVDYFMKRHNWHFKFFH
YKEDDKIKGAYFICNDQNIGILTRRTFPLSSDEILIPMAPDLRCFLPDRTNRLSAL
HQPQIRNAIWKLARKKQNCLVKETFSSKFEKTRRNEYQRFLKKGGSVKSVADCS
SDELTHIFIELFRSRFGNTSSCYPADNLANFFSQL*HHLLFGHILYIEGIPCAF*DIVLKSE
SQMNVYFDVSNGAIKNECRPLSPGSILMWLNISRARHYCQERQKKLLFSIGILKPEWE
YKRMWSTPYFTGKSIC

Fig. 1. Amino acid sequence of the Mig-14 protein. The transmembrane domain is shown in italics, the periplasmic region is in bold, the cytoplasmic region is shaded and the underlined sequence shares homology with the helix–loop–helix motif of the AraC family of transcriptional regulatory proteins.

2.2. Construction of the mig-14 deletion mutant

Construction of the *mig-14* deletion mutant (Δ*mig-14*) was carried out as described in the previous method (Huang et al., 2004; Khan et al., 1998). Primer pairs F1A/F1B and F2A/F2B were used to amplify fragments F1 (459 bp) and F2 (857 bp) located upstream and downstream of *mig-14*, respectively. Primers used in this study are listed in Table 2. A *Bam*HI site was added to the 5′-termini of primers F1A and F2B and a *Bgl*II site was added to the 5′-termini of primers F1B and F2A. F1 and F2 amplified from the wild-type strain were digested with *Bgl*II and ligated with the DNA Ligation Kit Ver.2 (TaKaRa). This ligated product which lacked 390 bp of *mig-14* was then cloned into the *Bam*HI site of suicide plasmid pGMB151 and transferred into the wild-type strain by electroporation. After selective incubation on LB plates with ampicillin and streptomycin, the bacteria were selectively incubated on LB plates with 5% sucrose. The complete recombinant strain was selected as the candidate for the *mig-14* deletion mutant by PCR with primers F1A and F2B. Finally, the mutant without a polar mutation was confirmed by sequencing analysis and designated as Δ*mig-14*.

2.3. Measurement of bacterial growth under high osmotic treatment

Bacteria from a single colony were inoculated into 1 ml of LB broth containing 50 mM NaCl and incubated at 37 °C overnight with shaking (200 rpm). Each 300 μl of culture was transferred into 30 ml of pre-warmed (37 °C) fresh LB broth containing 300 mM NaCl. Growth was measured per h using a Bio Photometer (Eppendorf). The experiment was repeated three times.

2.4. RNA extraction and DNA microarray analysis

After cooling 30 ml of incubated medium on ice for 10 min, bacteria were harvested by a centrifuge (2500 × *g*, 10 min, 4 °C) and destroyed in 100 μl lysozyme–TE buffer (0.6 mg/ml, pH 8.0) within 5 min at room temperature. Total RNA of bacteria was extracted with an RNeasy kit (mini-column, Qiagen, Shanghai, China) according to the manufacturer's instructions. The quantity and quality of the extracted RNA were checked with agarose gel electrophoresis and an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). The extracted RNA was treated with 1 U RNase-free DNase I (TaKaRa, Japan) at 37 °C for 15 min to remove traces of mixed DNA and then incubated at 85 °C for 15 min to inactivate DNase I. Reverse transcription and fluorescence labeling of cDNA were performed in a 30 μl reaction volume containing 20 μg total RNA. Hybridization with the *S.* Typhi genomic DNA microarray, microarray scanning and