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BACTERIAL AND FUNGAL PATHOGENESIS - RESEARCH PAPER

Salmonella Typhimurium O-antigen and VisP play an important role in swarming and osmotic stress response during intracellular conditions

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Abstract

Salmonella Typhimurium is a pathogen of clinical relevance and a model of study in host–pathogen interactions. The virulence and stress-related periplasmic protein VisP is important during *S*. Typhimurium pathogenesis. It supports bacteria invading host cells, surviving inside macrophages, swimming, and succeeding in murine colitis model, O-antigen assembly, and responding to cationic antimicrobial peptides. This study aimed to investigate the role of the O-antigen molecular ruler WzzST and the periplasmic protein VisP in swarming motility and osmotic stress response. Lambda red mutagenesis was performed to generate single and double mutants, followed by swarming motility, qRT-PCR, Western blot, and growth curves. Here we demonstrate that the deletion of *visP* affects swarming under osmotic stress and changes the expression levels of genes responsible for chemotaxis, flagella assembly, and general stress response. The deletion of the gene encoding for the O-antigen co-polymerase *wzzST* increases swarming motility but not under osmotic stress. A second mutation in O-antigen co-polymerase *wzzST* in a *ΔvisP* background affected gene expression levels. The *ΔvisP* growth was affected by sodium and magnesium levels on N-minimum media. These data indicate that WzzST has a role in swarming the motility of *S*. Typhimurium, as the VisP is involved in chemotaxis and osmotic stress, specifically in response to $MgCl_2$ and NaCl.

Keywords VisP, O-antigen · Chemotaxis · Osmolarity · Motility

Introduction

Salmonella enterica is a major foodborne pathogen that comprises more than 2500 typhoidal and non-typhoidal serovars [1]. *S*. Typhimurium and *S*. Enteritidis are serovars of clinical interest, causing 10 million cases of salmonellosis and thousands of deaths every year [2]. Moreover, *S*. Typhimurium is a great model for host–pathogen interactions since it invades the gut epithelium and promotes inflammatory diarrhea [1, 3].

The bacterial membrane has a crucial role in the pathogenic process inside the host and protects the cell against different environmental conditions. It harbors several proteins, including fimbriae, flagella, and porins, which allow bacteria to detect and respond to the environment [3]. *Salmonella* has the lipopolysaccharide (LPS) layer composed of the lipid-A, or the endotoxin portion; the oligosaccharide core; and the O-antigen (O-Ag), distributed in a tri-modal manner [4]. The immune system recognizes the lipid-A, and the O-Ag is detected by the Toll-like receptors 4, resulting in the secretion of TNF-α and IL-1β [5].

The periplasmic space connects the outer membrane to the inner membrane and has several proteins of importance for bacteria. The virulence and stress-related periplasmic protein (VisP) is conserved among Enterobacteria and predicted to have an oligonucleotide/oligosaccharide-binding fold [6]. A transcriptome released with environmental *Escherichia coli* revealed that *visP* (former *ygiW*) was one of the most upregulated genes during stress response [7]. Our group previously described that VisP is essential during *S*. Typhimurium pathogenesis. It helps bacteria survive inside macrophages, perform swimming motility, succeed in the murine colitis model, and respond to CAMPs by interacting

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with the lipid-A enzyme LpxO [6]. VisP is known to regulate the stress response to cadmium chloride, hydrochloric acid, and hydrogen peroxide [6]. We also have found that VisP participates in O-Ag assembly, affects the transcription of SPI2-related effectors, and impairs the intramacrophage survival and replication process [8].

The flagellum is a macromolecular structure assembled via its secretion system [9]. Flagella are essential for virulence and allow bacteria to explore new niches through their movement in liquid and semi-solid media (swimming) and surfaces (swarming) [10]. Swarming motility works with chemotaxis, in which bacteria sense the environment via methyl-accepting chemotaxis proteins (MCP), followed by phosphorylation of kinase proteins like CheA and CheY, which are responsible for controlling flagella direction (clockwise and counterclockwise) [11, 12]. Swarming helps in community establishment and survival and contributes to bacterial virulence [11].

A correct setup and fine-tuning of motility, chemotaxis, and O-Ag assembly systems depend on membrane integrity and the interaction in its surroundings. During the infection, *Salmonella* faces high osmolarity in the intestinal lumen, reducing as bacteria reach the epithelium [13]. It is essential that *S*. Typhimurium gain the epithelium and start the invasion process since the bacteria that stay in the lumen are killed by neutrophils and cannot colonize the host [14]. Herein, we aimed to investigate the role of VisP and the O-Ag co-polymerase WzzST in swarming motility and their participation in osmotic stress response.

Material and methods

Bacterial strains and cell cultures

All bacterial strains are listed in Table 1 and the plasmids used are listed in Table 2. The bacterial strains were grown in Luria–Bertani (LB) base broth (Invitrogen), containing 10 g/l NaCl. The 2% NaCl media were made by adding NaCl to the same broth, totalizing 20 g/l NaCl, as indicated.

Table 2 Plasmids used in this study

Plasmids	Description	Reference or source
pBadMycHisA	Cloning vector	[16]
pKD3	λ red template plasmid	[16]
pKD46	λ red plasmid (recombinase)	[16]
pCP20	λ resolvase plasmid	[17]

Recombinant DNA and molecular biology techniques were performed as previously described [18]. All oligonucleotides used in this study are cited in Table 3.

Construction of isogenic mutants

Construction of isogenic nonpolar *S*. Typhimurium SL1344 mutants *ΔvisP* and *ΔvisP/wzzST* was achieved by lambda red recombination [16]. The pKD46 plasmid with Lambda recombinant system was inserted in WT strain to construct single mutants and in *ΔvisP* background to construct the *ΔvisP/wzzST* strain. The PCR containing the chloramphenicol cassette was amplified using primers with homology sequences in the target genes, under the following conditions: 94 °C for 5 s, 94 °C for 1 min, 55 °C for 30 s, 68 °C for 1 min (high-fidelity DNA polymerase), 68 °C for 10 min, and 4 °C hold. The PCR products were electroporated in the strain harboring the pKD46 plasmid. Mutants were selected for Cm^R (20 µg/ml) in LB agar plates and confirmed by sequencing [16]. All strains were complemented with respective genes cloned into the vector pBADMycHisA (KpnI and EcoRI) [6] to generate strains *visP*+, *wzzST*+, and *ΔvisP/wzzST/visP*+. The single mutant strain *ΔvisP* was resolved via electroporation of the pCP20 plasmid, followed by incubation at 30 °C for 3 h, and plated in LB ampicillin (Amp) agar. Single colonies were chosen and grown in LB at 42 °C overnight and then plated on LB streptomycin (Sm) at 37 °C. Single colonies were patched in Amp, Sm, and Cm and incubated at 37 °C. The resolved clones were chosen based on Sm^R (100 µg/ml), Amp^S (100 µg/ml), and Cm^S (20 µg/ml) [16]. All mutants were previously constructed at

Table 1 Strains used in this study

Strain	Relevant genotype or description	Reference or source
SL1344	*Salmonella enterica* serovar Typhimurium prototype	[15]
ΔvisP	SL1344 *visP* mutant	[6]
visP+	SL1344 *visP* complemented strain	[6]
ΔwzzST	SL1344 *wzzST* mutant	[8]
wzzST+	SL1344 *wzzST* complemented strain	This study
ΔvisP/wzzST	SL1344 *visP* and *wzzST* double mutant	[8]
ΔvisP/wzzST /visP+	SL1344 *visP* and *wzzST* double mutant, *visP* complemented	[8]

Table 3 Oligonucleotides used in this study

Primer	Forward sequence	Reverse sequence	Reference or source
wzzST_lambda_red	ATGACAGTGGATAGTAATACGTCTTCCGGG CGTGGGAACGATCCGGAACAGTGTAGG CTGGAGCTGCTTC	TTACAAGGCTTTTGGCTTATAGCTACGTAG CGCATTGCGTCCCAGCACAATCCATAT GAATATCCTCCTTA	[8]
visP_lambda_red	AAGGGAAAAGTAATCATGAAAAAATTA GCTGCAATCGTTGCGTGTAGGCTGGGA GCTGCTTC	TTACGGATTCACTTTACGAATCTGTTTTAC GTCGATTTCGACATATGAATATCCTCC TTA	[6]
wzzST_external	CGTAAGCGTCATCAATAAGC	CTATCCACTGTCATAGATA	[8]
visP_external	GAATAAGCCGCGCTGATCAG	GCAACGCGAGTTACCGCAAG	[6]
cheA	TGGTAATGAGATCGCCGTGG	AAACCTGGTCGGCGAGTTAG	This study
cheZ	ACATCCCTGAGAAACTGGCG	ATCCGATCGAGCTTTCCGAC	This study
envZ	GGTCATCGGCCAATTGCTTC	GACCCGGGCGTTTAACCATA	This study
rpoS	TGTCCAGCAACGCTTTTTCG	TCAGCCGTATGCTTCGTCTC	This study
flgM	TAACGTTAAGCGACGCGCA	ATGCTTCGACGCGTTCCATA	This study

the UT Southwestern Medical Center in the USA under NIH Biosafety regulations [6, 8].

Motility assays

The swarming motility assay was performed in LB agar 0.5% as previously described [19], containing 1% or 2% NaCl. A total inoculum of 1×10^6 CFU in a volume of 5 µl bacteria was added to the surface of agar plates. The plates were incubated at 37 °C, and the motility halos were measured after 12, 24, and 48 h of incubation at 37 °C. Two independent experiments of five replicates were performed.

qRT-PCR

Quantitative real-time reverse transcription-PCR (qRT-PCR) was performed as previously described [20]. Cells were harvested to $O.D._{600}$ of 1.0, and RNA was extracted using the RiboPure® Bacteria Purification kit (Thermo Fisher). Each 20 µl reaction contained the SYBR® Master Mix, the Multiscribe® Reverse Transcriptase, and an RNAse Inhibitor (Thermo Fisher). The reactions were normalized by the *rpoA* endogenous control, and RNA was used to a final concentration of 100 ng. All reactions were performed in QuantStudio 3 (Thermo Fisher), and data were analyzed via comparative critical threshold (ΔΔCt).

Western blot assay

Culture supernatants were obtained from strains grown statically in LB medium at 37 °C overnight. The medium was removed by centrifugation, and the cells were resuspended in 10 ml of phosphate-buffered saline (PBS) plus 100 µl of phenylmethylsulfonyl fluoride (100 mM). Bacteria were lysed by sonication, pelleted by centrifugation ($4200 \times g$, 10 min, 4 °C), and analyzed by SDS-PAGE. The total proteins were quantified by the Bradford method, and 6 µg of protein was loaded into each lane. The proteins were transferred to nitrocellulose membranes using a semi-dry transfer cell (Bio-Rad) for 1 h. The immunoblots were made using monoclonal antibodies anti-FliC (InvivoGen) at 1:1000 and anti-RpoA (Santa Cruz Biotechnology) at 1:5000. The antibodies were detected with the horseradish peroxidase–conjugated secondary antibody anti-mouse IgG (Promega) at 1:2500, followed by chemiluminescence detection (ECL Western blotting) in the ChemiDoc MP imaging system (Bio-Rad).

Growth rates

Bacteria were grown in a 125-ml Erlenmeyer containing 15 ml of LB 2% NaCl, N-minimum media (100 µM $MgCl_2$), N-minimum low $MgCl_2$ (10 µM), or N-minimum plus 2% of NaCl, on a shaker at 250 rpm and 37 °C. Optical densities ($O.D._{600}$) were acquired after 30 min, 1 h, 2 h, 3 h, 4 h, 5 h, 7 h, 8 h, and 18 h of incubation.

Statistical analysis

Data were analyzed for statistical significance using GraphPad Prism 8. Results were compared by two-way ANOVA with Bonferroni post hoc test (P-value < 0.05).

Results

VisP and WzzST are needed for swarming on high osmolarity

To determine whether the flagellar system was affected in the mutant strains, we assessed the motility swarming phenotype of SL1344 and the isogenic mutants in LB and LB 2% NaCl conditions. In the swarming assays performed in

LB (1% NaCl), *ΔvisP* presented a similar phenotype to WT, whereas Δ*visP/wzzST* and Δ*visP/wzzST/visP* + had their motility reduced after 48 h of incubation, with 37% and 35% of reduction, respectively (Fig. 1A). The single mutant *ΔwzzST* showed an increase of 36% in swarming motility compared to WT (Fig. 1A). The complemented strains *visP* + and *wzzST* + had an increase in swarming compared to WT: 122% and 154%, respectively. However, when the swarming is performed under osmotic stress (2% NaCl), all strains presented smaller motility halos when compared to WT (Fig. 1B), indicating that disruption of membrane changes the stress response on surface movement. Moreover, the *visP* + and *wzzST* + strains did not restore the WT phenotype (Fig. 1B). The *ΔwzzST* mutant, which had swarming increased in LB, presented less motility than WT in 2% NaCl, around 63% less at the end of 48 h (Fig. 1B). On the other hand, the WT motility increased 55% in osmotic stress, indicating that flagella are working in response to high osmolarity.

Flagella assembly follows a complex cascade of genes encoded by several operons. Here we have evaluated the expression of the anti-sigma factor *flgM*. The *ΔvisP* strain presented higher expression levels in both conditions, whereas Δ*wzzST* and Δ*visP/wzzST* presented high levels of *flgM* gene expression only under stress conditions—around ninefold compared to WT levels (Fig. 1C). The deletion of *wzzST* in *ΔvisP* background restored the expression to WT levels in LB 1% NaCl (Fig. 1C). The complemented strains *visP* + and *wzzST* + could restore the expression to the WT levels, different from what we have seen for phenotype on Fig. 1A and B.

VisP and WzzST are required for chemotaxis

Swarming requires flagella and chemotaxis system expression. Thus, we assessed the expression of *cheA* (kinase sensor) and *cheZ* (phosphatase) of two-component systems (TCS) involved in this process. The WT levels were higher under osmotic stress for both genes, around 6- and tenfold, respectively (Fig. 2A and B), which corroborates the increase in swarming in LB 2% NaCl. The Δ*visP* presented increased *cheA* expression in LB 1% NaCl, whereas Δ*wzzST* and Δ*visP/wzzST* strains showed reduced *cheA* expression of 2- and 0.4-fold, respectively, compared to WT in 1% NaCl

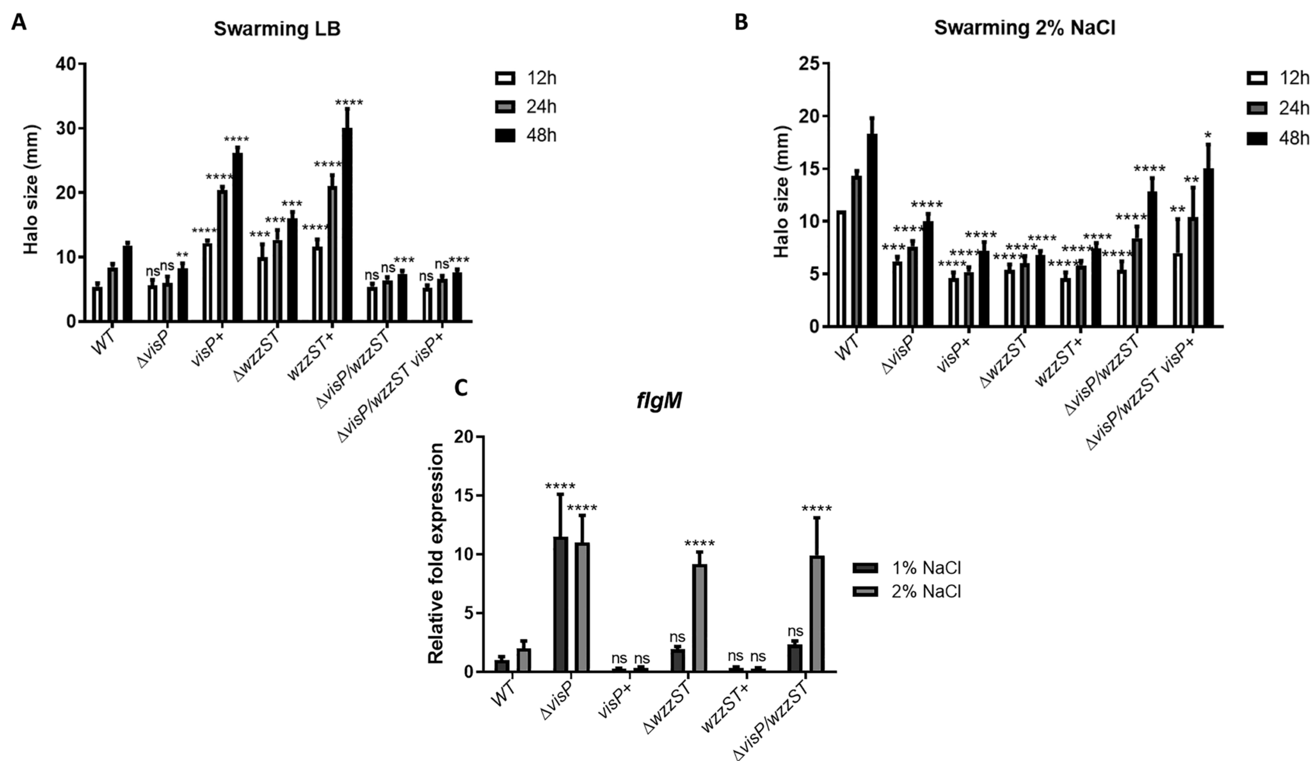

Fig. 1 Swarming motility of *Salmonella* WT and isogenic mutants. **A** Swarming motility performed in LB 0.5% agar after 12, 24, and 48 h of incubation. Motility halos of WT, *ΔvisP*, *visP*+, *ΔwzzST*, *wzzST*+, *ΔvisP/wzzST*, and *ΔvisP/wzzST visP*+. ns, non-significant; $*p<0.05$; $**p<0.01$; $***p<0.001$; $****p<0.0001$. **B** Swarming motility perfomed in LB 0.5% agar plus 2% NaCl after 12, 24, and 48 h of incubation. Motility halos of WT, *ΔvisP*, *visP*+, *ΔwzzST*, *wzzST*+, *ΔvisP/wzzST*, and *ΔvisP/wzzST/visP*+. ns, non-significant; $*p<0.05$; $**p<0.01$; $****p<0.0001$. **C** qRT-PCR of *flgM* in LB 1% and 2% NaCl. Relative fold expression of *flgM* in WT, *ΔvisP*, *visP*+, *ΔwzzST*, *wzzST*+, and *ΔvisP/wzzST* strains. ns, non-significant; $****p<0.0001$

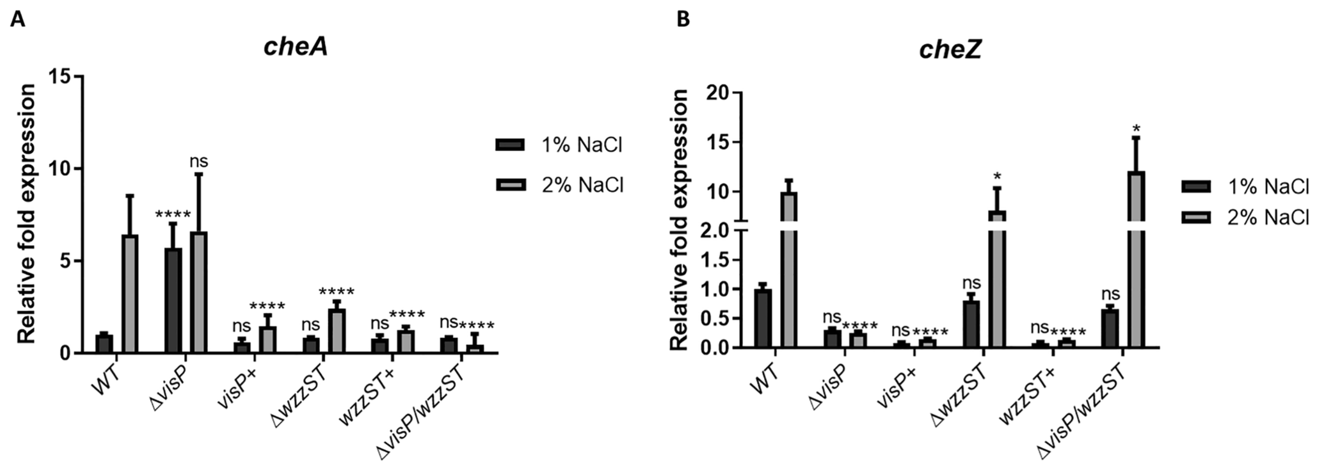

Fig. 2 Expression levels of chemotaxis genes in *Salmonella* WT and isogenic mutants. **A** qRT-PCR of *cheA* in LB 1% and 2% NaCl. Relative fold expression of *cheA* in WT, *ΔvisP*, *visP+*, *ΔwzzST*, *wzzST+*, and *ΔvisP/wzzST* strains. ns, non-significant; ****$p < 0.0001$. **B** qRT-PCR of *cheZ* in LB 1% and 2% of NaCl. Relative fold expression of *cheZ* in WT, *ΔvisP*, *visP+*, *ΔwzzST*, *wzzST+*, and *ΔvisP/wzzST* strains. ns, non-significant; *$p < 0.05$; ****$p < 0.0001$

(Fig. 2A). These expression levels may indicate that the deletion of *wzzST* and/or *visP* compromises the fine-tuning of CheAB TCS. Furthermore, deletion of *wzzST* in Δ*visP* background restored *cheA* expression to WT levels in 1% but not in 2% NaCl condition (Fig. 2A). The complementation with *visP+* and *wzzST+* genes restored expression in 1% NaCl but not in 2% NaCl, with a significant reduction ($p < 0.0001$). The expression of the phosphatase *cheZ* was similar to WT in Δ*wzzST* and Δ*visP/wzzST* strains, and Δ*visP* presented low levels mainly in 2% NaCl (Fig. 2B). The *visP+* and *wzzST+* strains did not restore the WT expression levels in 2% NaCl (Fig. 2B).

WzzST impacts on *envZ*-dependent porin expression

The regulation of porins is essential during the osmotic stress response. We checked *envZ* expression levels, and except for Δ*visP*, all strains presented reduced levels compared to WT, mainly in 1% NaCl (Fig. 3A). The complementation with *visP+* did not restore the *envZ* expression to WT levels (Fig. 3A). The *wzzST+* complemented strain did not change the expression compared to Δ*wzzST* and did not reach the WT expression levels (Fig. 3A). The double mutant Δ*visP/wzzST* reduced *envZ* expression compared to WT at 1% NaCl, which might indicate that *envZ* expression

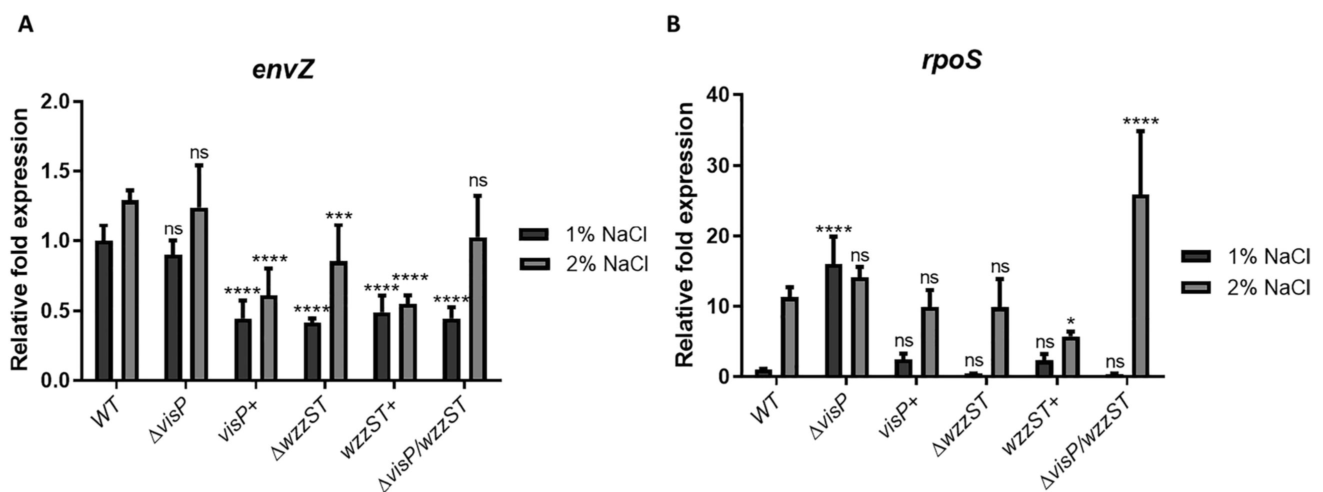

Fig. 3 Expression levels of *envZ* and *rpoS* genes in *Salmonella* WT and isogenic mutants. **A** qRT-PCR of *envZ* in LB 1% and 2% NaCl. Relative fold expression of *envZ* in WT, *ΔvisP*, *visP+*, *ΔwzzST*, *wzzST+*, and *ΔvisP/wzzST* strains. ns, non-significant; ***$p < 0.001$; ****$p < 0.0001$. **B** qRT-PCR of *rpoS* in LB 1% and 2% NaCl. Relative fold expression of rpoS in WT, *ΔvisP*, *visP+*, *ΔwzzST*, *wzzST+*, and *ΔvisP/wzzST* strains. ns, non-significant; ****$p < 0.0001$

is WzzST-dependent. Furthermore, the expression levels of the global stress regulator *rpoS* were checked, and, except for Δ*visP*, all strains presented similar expression levels in LB 1% NaCl media (Fig. 3B). Under osmotic stress, WT expression increased tenfold, also observed for Δ*wzzST* and *visP*+. The double mutant Δ*visP/wzzST* expression increased 25-fold in 2% NaCl compared to the LB condition (Fig. 3B). In this case, the deletion of *wzzST* in a Δ*visP* background changes the stress response and increases *rpoS* transcription. The complementation with *wzzST*+ did not restore to the WT expression levels in 2% NaCl condition (Fig. 3B).

VisP absence affects growth on minimal media.

Because of the differences observed in gene expression levels in 1% and 2% NaCl conditions, we performed Western blot targeting the flagellin FliC protein expression. For that, the monoclonal α-FliC antibody and the control α-RpoA were employed. The FliC expression in Δ*visP* was lower than WT in both LB 1% NaCl and LB 2% NaCl (Additional File 1), and the complemented strain *visP*+ could not restore WT flagellin levels. Furthermore, no difference was observed in FliC expression between 1 and 2% NaCl (Additional File 1). Lastly, we performed growth curves in N-minimum media to evaluate differences in stress response, such as the influence of magnesium and sodium on growth. Employing regular N-minimum conditions (100 μM $MgCl_2$), Δ*visP* growth was defected at 50% less than WT growth, peaking $O.D._{600}$ of 0.5 (Fig. 4A). Under $MgCl_2$ starvation (10 μM), both WT and Δ*visP* struggled to grow, as the deletion of *visP* caused a slow growth when the two curves were compared (Fig. 4B). The same pattern was observed after adding 2% NaCl in regular N-minimum, in which the Δ*visP* strain presented half of the $O.D._{600}$ of WT strain after 18 h (Fig. 4C). We did not observe this difference in LB 2% NaCl, where the Δ*visP* strain grows similar to WT levels (Additional File 2). These data indicate that VisP protein is involved in the osmotic response, but mainly in poor media conditions, where *Salmonella* must change the metabolic routes to survive.

Discussion

Swarming requires one or more flagella, surfactant synthesis, and a grouped movement [10]. *S*. Typhimurium promotes cell elongation and increases torque, but flagella amounts in cells do not change, while some bacteria as *Vibrio parahaemolyticus* and *Aeromonas* spp. may increase flagellar synthesis [10]. When compared Δ*visP* to the WT during swarming on LB, both have similar motility phenotypes

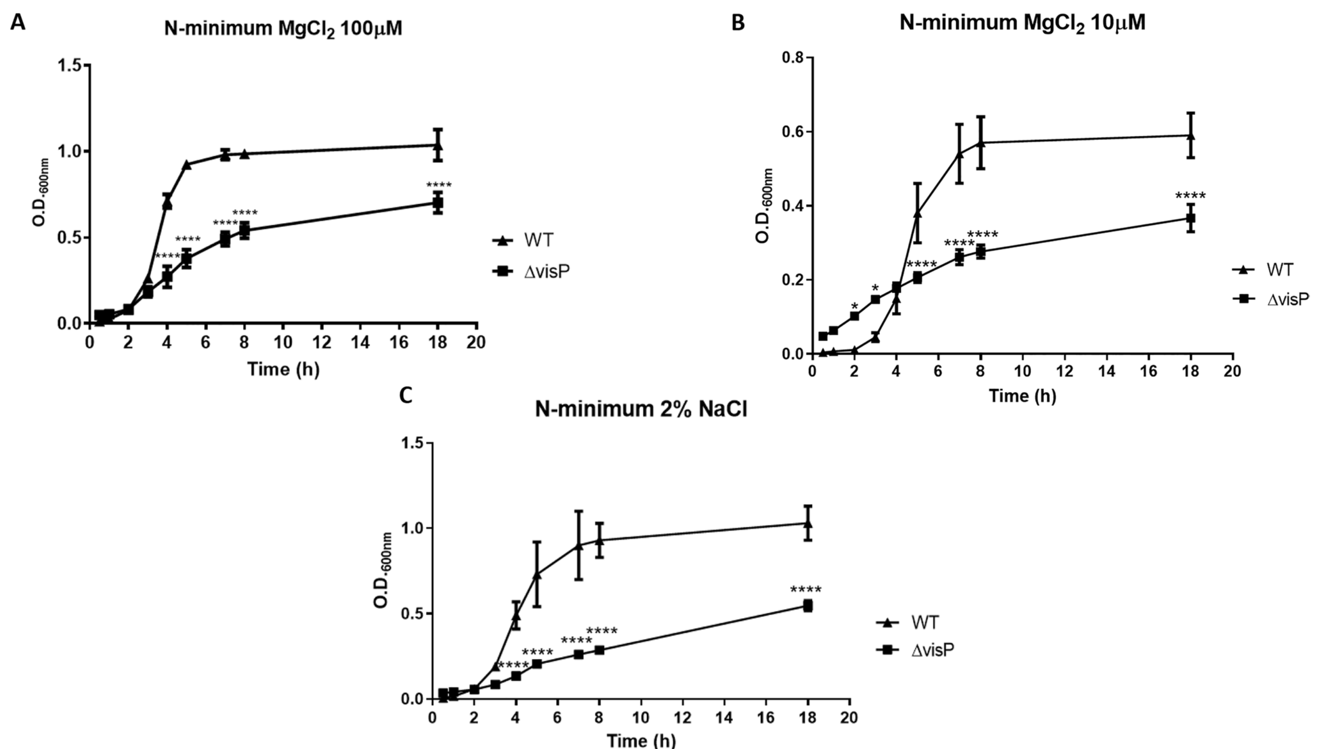

Fig. 4 Growth curves performed in N-minimum media. **A** N-minimum 100 μM $MgCl_2$. **B** N-minimum 10 μM $MgCl_2$. **C** N-minimum media 2% NaCl. Optical density ($O.D._{600}$) of WT and Δ*visP* at different time points after 18 h of incubation. *$p < 0.05$; ****$p < 0.0001$

(Fig. 1A). Our previous data showed that swimming motility on LB is reduced in *ΔvisP*, and flagellar genes were downregulated [8]. All strains had swarming reduced in 2% NaCl compared to WT phenotype (Fig. 1B). The single mutant Δ*wzzST* had an increase compared to WT, and the complementation with *wzzST*+ increased the motility even more (Fig. 1A). The WzzST protein encodes for the long O-Ag assembly forms (L), while the WzzfepE (which is the remaining co-polymerase in Δ*wzzST*) encodes for very long O-Ag forms (VL) [21]. Even in the absence of WzzST, the swarming is still facilitated by O-Ag VL forms. After deletion of *wzzST* in a *ΔvisP* background, the swarming motility had decreased, and the complemented strain *ΔvisP/wzzST visP*+ did not restore the Δ*wzzST* phenotype (Fig. 1A). After deletion of *wzzST* in a *ΔvisP* background, the swarming motility had decreased, and the complemented strain *ΔvisP/wzzST visP*+ did not restore the Δ*wzzST* phenotype (Fig. 1A).

The qRT-PCR of *flgM* was performed and exhibited increased expression levels under osmotic stress, except for *ΔvisP*, which presented overexpression in both conditions tested (Fig. 1C). The reduced swimming motility in LB previously observed in *ΔvisP* is related to the low levels of expression in flagellar genes [8]. The overexpression of the *flgM* can affect the substrate shift from class 2 to class 3 genes since FlgM is secreted via flagellar T3SS. Then, FliA initiates transcription of the FliC or FljB strand [9]. The complemented strains *visP*+ and *wzzST*+ presented a slight decrease compared to WT (Fig. 1C). These data corroborate previous studies and indicate the importance of VisP for membrane homeostasis.

The expression of chemotaxis genes *cheA* and *cheZ* exhibited different levels in *ΔvisP* compared to WT (Fig. 2A and B). The CheA kinase sensor phosphorylates CheY and starts the flagella rotation, while CheZ is responsible for resetting the switch and changing direction [19]. The *cheA* expression on LB was increased fivefold in *ΔvisP* when compared to WT levels. However, the *cheZ* expression in the *ΔvisP* mutant was reduced in both conditions. In the WT strain, the *cheZ* expression is higher under osmotic conditions. Because VisP has a role in flagella expression and the chemotaxis genes are part of the flagella cascade [8], the chemotactic system might not work properly during osmotic stress. The *cheA* expression for both *ΔwzzST and ΔvisP/wzzST* strains was similar to WT levels in 1% NaCl, but it was reduced compared to WT in 2% NaCl (Fig. 2A). When comparing *cheZ* expression, the mutant strains presented similar levels to WT, except for *ΔvisP* (Fig. 2B). Despite these differences observed in all mutant strains, none of them had the same swarming phenotype under osmotic stress as observed in WT (Fig. 1B). For both *cheA* and *cheZ* expressions, the complemented strains *visP*+ and *wzzST*+ could not restore the WT levels (Fig. 2A and B). The results have shown that the importance of a functional chemotactic system is reflected in the stress response phenotype.

The regulation of porins in osmotic gradient depends on a critical bacterial TCS: the EnvZ/OmpR system. EnvZ is a kinase sensor and phosphorylates the response regulator OmpR in the presence of high osmolarity [22]. In *S*. Typhi, EnvZ binds to *tviA* and activates transcription of the capsule; in contrast, in *S*. Typhimurium, it regulates SPI-1 and SPI-2 through the SsrAB system [22]. The expression of *envZ* had decreased in all mutant strains compared to WT in LB, except for *ΔvisP* (Fig. 3A). OmpR tends to inhibit *flhDC* expression in *Escherichia coli* [23]; however, this regulation needs to be more explored in *Salmonella*. The complementation with *visP*+ and *wzzST*+ could not restore the WT expression levels (Fig. 3A). The *ΔvisP* strain did not show a significant difference in expression levels; on the other hand, WzzST showed to be essential for *envZ* expression (Fig. 3A). RpoS is on the top of the stress response cascade and responds to osmotic and oxidative stress, low pH, UV radiation, temperature, ethanol, microaerophilic growth, among others [17, 24, 25]. The *rpoS* expression in *ΔvisP* presented an increase compared to WT in LB, but not in LB 2% NaCl (Fig. 3B). The VisP protein is involved in stress response and membrane homeostasis, and its absence could impact *rpoS* expression. The complementation with *visP*+ restored the expression to WT levels. The *ΔwzzST* strain presented the same *rpoS* levels as WT, indicating that the absence of the O-antigen PCP protein did not impact *rpoS* transcription (Fig. 3B). However, the double mutation *ΔvisP/wzzST* had a significant increase in expression levels under osmotic stress, about 25-fold (Fig. 3B).

The FliC expression in *ΔvisP* showed to be lower than WT levels but did not change in 2% NaCl (Additional File 1). The genomic localization of the *visP* gene suggested that it is part of the *qseBC* regulon, encoded in an adjacent operon and the opposite direction [6]. Our previous data demonstrated that the *ΔvisP/wzzST* strain overexpressed FliC protein [8] and presented overexpression of the *qseC* gene (Manieri, data not shown). The deletion of *visP* also abrogates bacterial resistance to acid, heavy metals, and oxidative stress [6]. Thus, the growth curves exhibited that *ΔvisP* grows slow in N-minimum and even slower than WT in $MgCl_2$ starvation and 2% NaCl (Fig. 4A–C). It corroborates with previous data about VisP and includes the osmotic stress response as another stressor agent related to VisP function.

Conclusion

Our findings highlight the direct importance of VisP protein and indirect via WzzST for *S*. Typhimurium in motility and stress response. VisP and WzzST have a more specific role in

swarming, chemotaxis, and osmotic stress response; WzzST is also required for *envZ* expression, and, consequently, porin regulation. VisP is required for growth in minimal media, specifically in response to $MgCl_2$ and NaCl. These data open new perspectives about swarming and mechanisms involved in flagella-independent motility.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42770-022-00701-9.

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Author contribution FZM and CGM designed the study. FZM performed the research. FZM and CGM performed the data analysis. FZM wrote the manuscript. CGM supervised the research.

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Data availability All data and material are available under request.

Code availability Not applicable.

Declarations

Ethics approval Not applicable.

Consent to participate Not applicable.

Consent for publication All authors consent for publication.

Competing interests The authors declare no competing interests.

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