Characterization of a Novel Two-Component Regulatory System, 
HptRS, the Regulator for the Hexose Phosphate Transport System in 
Staphylococcus aureus

Joo Youn Park, Jong Wan Kim, Bo Youn Moon, Juyeun Lee, Ye Ji Fortin, Frank W. Austin, Soo-Jin Yang, Keun Seok Seo

Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, Mississippi State, Mississippi, USA; Animal Diseases Diagnostic Division, Quarantine and Inspection Agency, Anyang, Gyeonggi, Republic of Korea; Department of Microbiology, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea; Division of Infectious Diseases, Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center, Torrance, California, USA; The David Geffen School of Medicine at UCLA, Los Angeles, California, USA

Hexose phosphate is an important carbon source within the cytoplasm of host cells. Bacterial pathogens that invade, survive, and multiply within various host epithelial cells exploit hexose phosphates from the host cytoplasm through the hexose phosphate transport (HPT) system to gain energy and synthesize cellular components. In Escherichia coli, the HPT system consists of a two-component regulatory system (UhpAB) and a phosphate sensor protein (UhpC) that tightly regulate the expression of a hexose phosphate transporter (UhpT). Although growing evidence suggests that Staphylococcus aureus also can invade, survive, and multiply within various host epithelial cells, the genetic elements involved in the HPT system in S. aureus have not been characterized yet. In this study, we identified and characterized the HPT system in S. aureus that includes the hptRS (a novel two-component regulatory system), the hpta (a putative phosphate sensor), and the uhpt (a hexose phosphate transporter) genes. The hpta, hptrs, and uhpt markerless deletion mutants were generated by an allelic replacement method using a modified pMAD-CM-GFPuv vector system. We demonstrated that both hpta and hptrs are required to positively regulate transcription of uhpt in response to extracellular phosphates, such as glycerol-3-phosphate (G3P), glucose-6-phosphate (G6P), and fosfomycin. Mutational studies revealed that disruption of the hpta, hptrs, or uhpt gene impaired the growth of bacteria when the available carbon source was limited to G6P, impaired survival/multiplication within various types of host cells, and increased resistance to fosfomycin. The results of this study suggest that the HPT system plays an important role in adaptation of S. aureus within the host cells and could be an important target for developing novel antistaphylococcal therapies.

Staphylococcus aureus is a major human pathogen that causes a diverse array of diseases in nosocomial and community-acquired settings, ranging from superficial skin infections to severe diseases, such as endocarditis, septicemia, and necrotizing pneumonia (1). S. aureus adapts extremely well to various environmental conditions and hosts by regulating expression of colonization factors, such as microbial surface components recognizing adhesive matrix molecules (MSCRAMM), and virulence factors, such as cytotoxins, proteases, and superantigens. Expression of these factors is regulated by global transcriptional regulatory systems, such as sigma factors (2), Staphylococcus aureus regulator (ArlRS) and LytSR, which regulate autolysis (7, 8), WalKR, GraSR, and VraRS, which regulate cell wall synthesis (9–11), HssRS, which regulates iron acquisition (12), NreBC, which regulates nitrogen sensing (13), SrrAB and AirRS, which regulate oxygen sensing (14, 15), and BraRS, which regulates bacitracin resistance (16). However, relatively little is known about the role of TCSs on nutritional challenges.

Sugar uptake is an essential process for microorganisms to gain energy and synthesize cellular compartments. The transport of sugar into bacterial cells is mediated by the phosphoenolpyruvate phosphotransferase system (PTS) (17) and by non-PTS mechanisms, such as the hexose phosphate transport (HPT) system.

Bacterial pathogens can survive and multiply within various host epithelial cells by exploiting nutrients available within the host cytoplasm, such as hexose phosphates, through the HPT system (18, 19). The HPT system has been well characterized in Esch...
erichia coli, where it is composed of a two-component regulatory system (UhpAB) (18), a phosphate sensor protein (UhpC) (20), and a hexose phosphate transporter (UhpT) (21). UhpC is required to sense extracellular hexose phosphates, resulting in autophosphorylation of a conserved histidine residue in the cytoplasmic domain of UhpB. Subsequently, a phosphoryl group is transferred to a conserved aspartic acid residue in the response regulator domain of UhpA, which activates transcription of uhpT (22). UhpT is a hexose phosphate transporter that exchanges intracellular phosphorylated hexose phosphate for extracellular hexose phosphate and fosfomycin (23).

Although there is growing evidence that S. aureus can survive and multiply within various types of host epithelial cells (24), little is known about the mechanism by which S. aureus adapts to intracellular conditions that are nutritionally limited to host cell metabolites. In this study, we characterized the HPT system in S. aureus, encoded by the SAUSA300_0216, SAUSA300_0217, SAUSA300_0218, and SAUSA300_0219 genes. We demonstrated that transcription of a hexose phosphate transporter, UhpT, encoded by SAUSA300_0216, is regulated by a novel TCS, HptRS, encoded by SAUSA300_0217 and SAUSA300_0218, and by a phosphate-sensing protein, HptA, encoded by SAUSA300_0219. We demonstrated that the HPT system is important to support intracellular survival/multiplication of S. aureus, where the carbon source is limited to hexose phosphate and affects the sensitivity to fosfomycin.

**MATERIALS AND METHODS**

**Bacterial strains, plasmids, and growth conditions.** All strains used in this study are listed in Table 1. All S. aureus strains were cultured in Mueller-Hinton II (MH2) broth or agar (BBL Becton Dickinson) supplemented with chloramphenicol (100 μg/ml) when necessary. All E. coli DH5α strains were cultured in Luria-Bertani (LB) broth or agar (BBL Becton Dickinson) supplemented with ampicillin (100 μg/ml) when necessary.

**Construction of S. aureus deletion mutants.** The gene deletion mutants were generated by allelic replacement using a modified PMAD-CM temperature-sensitive shuttle vector system. To enhance the screening processes without an antibiotic selection marker, to thereby generate a markerless deletion mutant, the promoter region of the lactate dehydrogenase gene from Listeria monocytogenes was joined to the coding region of the GFPuv gene (Clontech) by overlapping PCR using primers listed in Table 2 and cloned between BglII and Stul sites in PMAD-CM (25), which resulted in PMAD-CM-GFPuv. To generate deletion mutants, the upstream and downstream flanking regions of hpta, hprtS, or uhpT were amplified from S. aureus LAC chromosomal DNA using primers listed in Table 2 and cloned between the BamHI/Sall and EcoRI/BglII sites, respectively, in PMAD-CM-GFPuv. The plasmids were constructed in E. coli DH5α and then transferred to S. aureus RN4220 prior to electroporation into S. aureus LAC by using electroporation, as described previously (25). Constructed strains were cultured on MH2 plates containing chloramphenicol at a nonpermissive plasmid replication temperature of 43°C overnight to promote the first recombination event. A single colony was transferred to MH2 broth and cultured at 37°C overnight to promote the second recombination event. The mutant candidate, showing the GFPuv-negative and chloramphenicol-sensitive phenotypes, was screened by PCR using the primers listed in Table 2 and DNA sequencing analysis. To complement the gene deletions, gene fragments from 500 bp upstream of the start codon containing the putative promoter regions along with each open reading frame were amplified by PCR using primers listed in Table 2 and cloned into the shuttle plasmid, pMK4 vector (26).

**Growth analysis.** Chemically defined medium (CDM) was prepared as described previously (27). Overnight cultures from MH2 broth were washed three times with MH2 broth or CDM supplemented with a single carbon source (glucose, glycerol-3-phosphate [G3P], or glucose-6-phosphate [G6P]; 0.5%, wt/vol) and adjusted to an optical density at 600 nm (OD600) of 0.01. Cells were cultured at 37°C with shaking at 200 rpm, and bacterial growth was monitored by measuring OD600 using a Genesys 20 spectrophotometer (Thermo Electron).

**Quantitative real-time PCR (qRT-PCR).** To measure transcriptional changes in the uhpT, glpT, and glcU genes in response to various nutritional conditions, strains were cultured in various nutritional conditions as described above. To measure transcriptional changes in the genes related to fosfomycin resistance, overnight cultures from MH2 broth were washed three times and adjusted to an OD600 of 0.5 with MH2 broth or CDM supplemented with a single carbon source as described above in the presence of fosfomycin (128 μg/ml) for 6 h. Bacterial cells were harvested by centrifugation, and total RNA was isolated from the cell pellets using

### Table 1: Strains and plasmids

<table>
<thead>
<tr>
<th>Strain or plasmid</th>
<th>Description</th>
<th>Reference or source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RN4220</td>
<td>Cloning intermediate derived from 8325-4</td>
<td>45</td>
</tr>
<tr>
<td>USA300 LAC</td>
<td>USA300 wild-type reference strain</td>
<td></td>
</tr>
<tr>
<td>ΔhptA mutant</td>
<td>SAUSA300_0219, hptA deletion mutant</td>
<td>This study</td>
</tr>
<tr>
<td>ΔhprtS mutant</td>
<td>SAUSA300_0217 and 0218, hprtS double deletion mutant</td>
<td>This study</td>
</tr>
<tr>
<td>ΔUhpT mutant</td>
<td>SAUSA300_0216, uhpT deletion mutant</td>
<td>This study</td>
</tr>
<tr>
<td>Δhpta-Comp mutant</td>
<td>LAC ΔhptA mutant complemented with pMK4-HptA</td>
<td>This study</td>
</tr>
<tr>
<td>ΔhptRS-Comp mutant</td>
<td>LAC ΔhptRS mutant complemented with pMK4-HptRS</td>
<td>This study</td>
</tr>
<tr>
<td>ΔUhpT-Comp mutant</td>
<td>LAC ΔhptA mutant complemented with pMK4-UhpT</td>
<td>This study</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pMK4</td>
<td>E. coli-S. aureus shuttle vector</td>
<td>This study</td>
</tr>
<tr>
<td>pMK4-HptA</td>
<td>pMK4 harboring complementation of the hptA deletion</td>
<td>This study</td>
</tr>
<tr>
<td>pMK4-HptRS</td>
<td>pMK4 harboring complementation of the hptR and hptS deletions</td>
<td>This study</td>
</tr>
<tr>
<td>pMK4-UhpT</td>
<td>pMK4 harboring complementation of the uhpT deletion</td>
<td>This study</td>
</tr>
</tbody>
</table>

April 2015 Volume 83 Number 4 *Infection and Immunity* iai.asm.org 1621

Downloaded from http://iai.asm.org on October 24, 2017 by guest
FastPrep (Thermo Fisher Scientific) and RNeasy kits (Qiagen), according to the manufacturers’ recommendations.

For qRT-PCR, cDNA samples were generated from 1 μg of total RNA using SuperScriptase III (Invitrogen) according to the manufacturer’s instructions. The qRT-PCR was performed using primers listed in Table 2, Power SYBR green master mix, and the ABI 7500 real-time PCR system (Applied Biosystems) according to the manufacturer’s instructions. Real-time PCR data were analyzed using sequence detector system software (Applied Biosystems). The data were normalized by calculating the threshold cycle ($C_T$) of the target minus the $C_T$ of the internal control (16S RNA and gyrB) ($\Delta C_T$). Relative quantification of the target gene was determined by the comparative $C_T$ method ($2^{- \Delta \Delta C_T}$).

### Intraglomerular survival and multiplication assay.
Human monocytic cells (THP-1) and alveolar epithelial cells (A549) were obtained from ATCC. Murine bone marrow-derived macrophages (BMDM) were prepared as previously described (28). Cell monolayers (~80% confluence in 24-well tissue culture plates) were infected with bacteria at a multiplicity of infection (MOI) of 5 (5 bacteria per cell). After 30 min of infection, cells were washed and treated with cell culture medium containing gentamicin (100 μg/ml) to remove extracellular bacteria. After 30 min of gentamicin treatment, cells were washed and lysed, and the numbers of intracellular bacteria were determined by plate counting ($n_0$). Parallel cultures were further maintained and harvested at the indicated time points ($t = 3 h$ and $t = 6 h$). The percentage of surviving bacteria was calculated by the following formula: (the number of bacterial cells at $t = 3 h$ or $t = 6 h$ / the number of bacteria at $t = 0$) × 100.

### Antibiotic sensitivity assays.
The MIC of fosfomycin was determined using a fosfomycin epsilometer test (Etest; bioMérieux) according to the manufacturer’s instructions.

### Statistical analysis.
Statistical significance was analyzed with the Student $t$ test using GraphPad Prism software (GraphPad).

## RESULTS

### Identification of a putative hexose phosphate transport system in *S. aureus*.
Genome sequence analysis showed that genes encoding a hexose phosphate transporter, UhpT (SAUSA300_216), a putative histidine kinase (SAUSA300_217), a response regulator protein (SAUSA300_218), and a putative iron compound binding protein (SAUSA300_0219) are highly conserved in all sequenced *S. aureus* strains, but these genes have not been characterized yet. **In silico** analysis showed that the SAUSA300_216 gene encodes a putative 458-amino-acid transmembrane protein with a major facilitator superfamily 1 (MFS_1) domain showing 51%
The HPT system is required for growth under nutritional conditions specifically limited to G6P. To determine the function of HptA, HptRS, and UhpT, we generated deletion mutants of HptA (ΔHptA mutant), HptRS (ΔHptRS mutant), and UhpT (ΔUhpT mutant) by an allelic replacement method using a modified temperature-sensitive pMAD-CM-GFPuv vector system, an efficient approach for generating and screening a markerless deleted temperature-sensitive pMAD-CM-GFPuv vector system, an (UhpT mutant) by an allelic replacement method using a mod/H9004 mutant, and the corresponding complemented mutant, and UhpT (ΔUhpT mutant) and the corresponding complemented strains, pMK4-HptA, pMK4-HptRS, and pMK4-UhpT, respectively.

FIG 1 Identification of a putative hexose phosphate transport system in S. aureus. (A) A schematic map of the genomic organization of the hexose phosphate transport system in S. aureus LAC and its mutant derivatives; (B) a PCR confirmation of a deletion mutant of HptA (ΔHptA mutant), HptRS (ΔHptRS mutant), and UhpT (ΔUhpT mutant) and the corresponding complemented strains, pMK4-HptA, pMK4-HptRS, and pMK4-UhpT, respectively.

identities and 67% positive matching with the UhpT in E. coli. The SAUSA300_0217 gene encodes a putative 252-amino-acid protein with a response regulator receiver domain at the N terminus and a helix-turn-helix domain at the C terminus, which are typically associated with a sensor protein in TCSs (29). The SAUSA300_0218 gene encodes a putative 518-amino-acid protein with a histidine kinase domain and a histidine kinase-type ATPase catalytic (HATPase_c) domain at the C terminus, which are typically associated with a response regulator in TCSs (29). The SAUSA300_0219 gene encodes a putative 323-amino-acid protein with a bacterial extracellular solute binding protein 2 (SBP II) domain, which potentially can interact with a phosphate (30). These results suggested that the SAUSA300_216, 217, 218, and 219 genes may represent the HPT system in S. aureus, similar to uhpatBC in E. coli. Based on this assumption, we refer here to this putative TCS (SAUSA300_0218 and 0217) as the hexose phosphate transporter regulation system (HptRS), a putative iron compound binding protein (SAUSA300_0219) as HptA, and a hexose phosphate transporter as UhpT (SAUSA300_216).

The HPT system is required for growth under nutritional conditions specifically limited to G6P. To determine the function of HptA, HptRS, and UhpT, we generated deletion mutants of HptA (ΔHptA mutant), HptRS (ΔHptRS mutant), and UhpT (ΔUhpT mutant) by an allelic replacement method using a modified temperature-sensitive pMAD-CM-GFPuv vector system, an efficient approach for generating and screening a markerless deletion mutant, and a corresponding complement strain using pMK4, in the S. aureus LAC strain background (Fig. 1B). To analyze the function of HptA, HptRS, and UhpT in carbohydrate metabolism, the growth of the wild-type, mutants, and complemented strains in various nutritional conditions was measured. When cultured in MH2 broth, the growth of wild-type and mutant strains was not affected (Fig. 2A). In contrast, when cultured in CDM supplemented with glucose-6-phosphate (G6P) as a sole carbon source, the growth of the ΔHptA, ΔHptRS, and ΔUhpT strains was significantly inhibited compared to that of the wild type (Fig. 2B), and the complemented mutant strains restored the growth (see Fig. S2 in the supplemental material). When cultured in CDM supplemented with glucose or glycerol-3-phosphate (G3P), the growth of the wild type and mutants was not affected (Fig. 2C and D). These results indicated that the hpta, hptrs, and uhpt genes are required for growth under nutrition-limited conditions, especially when G6P is the sole carbon source.

The hpta and hptrs genes are required for transcriptional activation of uhpt in response to extracellular G6P or G3P as the sole carbon source. To determine whether the hpta and hptrs genes regulate transcription of the uhpt gene, as in E. coli, we determined the transcriptional profiles of the uhpt gene in various nutritional conditions by using qRT-PCR. When cultured in MH2 broth, transcription of the uhpt gene was not significantly different in the wild-type, mutant, and complemented strains (Fig. 3A; see also Fig. S1A in the supplemental material). In contrast, when cultured in CDM supplemented with G6P as the sole carbon source, transcription of the uhpt gene in the wild-type and complemented strains significantly increased (approximately 18-fold), but not in the ΔHptA or ΔHptRS strains, compared to the result obtained from the MH2 culture (Fig. 3B; see also Fig. S1B). Interestingly, when cultured in CDM supplemented with G3P, transcription of the uhpt gene in the wild-type strain also significantly increased (Fig. 3C; see also Fig. S1C). When cultured in CDM supplemented with glucose as the sole carbon source, no significant changes were observed (Fig. 3D; see also Fig. S1D). To further analyze the role of HptA and HptRS on other carbohydrate translocases, such as GlcU and GlpT, translocases for glucose and G3P, respectively, transcription of these genes was determined but showed no differences (Fig. 3A to D). Combined with the in silico analysis and the growth results, these results suggest that HptA is a phosphate sensor protein that activates HptRS TCS, resulting in transcriptional activation of the uhpt gene; thus, HptA and HptRS represent the HPT system in S. aureus.

The hptrs gene is required for intracellular survival and multiplication of S. aureus. Since hexose phosphates are important carbon sources within the host cell cytosol, we hypothesized that any defect in the HPT system would negatively affect intracellular survival and multiplication of S. aureus. As shown in Fig. 4, the intracellular survival and multiplication of the ΔHptRS mutant in murine bone marrow-derived macrophages (BMDM), human monocyte cells (THP-1), and human alveolar epithelial cells (A549) were clearly impaired compared to those of the wild-type and complemented strains. These results suggest that uptake of hexose phosphate by the HPT system is important for intracellular survival and multiplication of S. aureus.

The hptrs deletion mutant exhibits fosfomycin resistance in S. aureus. Fosfomycin is a bactericidal agent that acts by inhibiting bacterial cell wall biosynthesis by inactivating the enzyme UDP-N-acetylglucosamine-3'-enolpyruvyltransferase, also known as MurA (31). Since fosfomycin requires UhpT gene expression to
enter bacterial cells (32), the fosfomycin MIC of the wild-type, ΔHptA, ΔHptRS, and ΔUhpT strains was determined using an Etest. As shown in Fig. 5, the fosfomycin MIC of the wild-type LAC strain was found to be 0.5 to 1.5 μg/ml. In contrast, the ΔHptRS and ΔUhpT strains were highly resistant to fosfomycin (>1,024 μg/ml). Both complemented mutant strains exhibited restored sensitivity to fosfomycin (1.0 to 2.5 μg/ml).

Resistance to fosfomycin could be induced by (i) the impaired uptake of fosfomycin through UhpT and GlpT (33), (ii) overexpression or mutation in MurA (33), (iii) biochemical modifications of fosfomycin (bacillithiol conjugation) by FosB (34), and (iv) impaired bacillithiol synthesis by downregulation of the bacillithiol synthesis enzyme, BshA (35). It is noteworthy that the wild-type LAC strain is highly sensitive to the fosfomycin, although it harbors both the fosB and bshA genes. To understand the effect of HptRS on the genes related to fosfomycin resistance

FIG 2 Role of the HPT system in growth under nutrition specifically limited to glucose-6-phosphate. Results shown are the growth kinetics of the wild-type, ΔHptA, ΔHptRS, and ΔUhpT strains in MH2 (A) or in chemically defined medium (CDM) supplemented with G6P (B), G3P (C), or glucose (Glu) (D) as the sole carbon source. Results shown are combined from triplicate measurements from three independent experiments. The asterisk indicates statistical difference from the wild type at P values of <0.001.

FIG 3 Role of HptA and HptRS in transcriptional regulation of the UhpT in response to extracellular G3P and G6P. Transcription of UhpT in the wild-type, ΔHptA, and ΔHptRS strains, cultured in MH2 broth (A) or in CDM supplemented with G6P (B), G3P (C), or glucose (Glu) (D) as the sole carbon source, was measured using qRT-PCR. Results shown are combined from triplicate measurements from two independent experiments.
(glpT, uhpT, murA, fosB, and bshA) and how the wild-type LAC strain is sensitive to fosfomycin, transcription of these genes in response to an exposure to fosfomycin was measured using qRT-PCR. When cultured in MH2 broth, transcription of the glpT, uhpT, murA, fosB, and bshA genes was not different among the wild-type, ΔHptRS, and complemented strains (Fig. 6A). However, when exposed to a high level of fosfomycin (128 µg/ml) for 6 h, transcription of the glpT, uhpT, murA, and fosB genes increased (2- to 4-fold) but that of the bshA gene was not changed in the wild-type and complemented strains, compared to the data obtained from the wild-type strain cultured in MH2 broth without fosfomycin (Fig. 6B). On the contrary, transcription of the glpT, uhpT, murA, fosB, and bshA genes decreased in the ΔHptRS strain (Fig. 6B). These results suggested that, in the wild-type strain, a transcriptional increase of the glpT and uhpT genes without a transcriptional increase of the bshA gene resulted in an increased fosfomycin uptake but not a sufficient supply of bacillithiol, thereby making the bacteria sensitive to fosfomycin. In contrast, in the ΔHptRS strain, a transcriptional decrease of the glpT and uhpT genes resulted in a decreased fosfomycin uptake, thereby causing resistance to fosfomycin.

**DISCUSSION**

For successful survival/multiplication within host cells, invading pathogens must adapt to the available nutrients and other physiological conditions, such as pH, temperature, and oxygen, existing in these cells. Within host cells, glucose and related metabolic derivatives, such as G3P, G6P, pyruvate, lactate, and glycerol, are the most important carbon sources (36). The transport and use of nutrients by invading pathogens are mediated by multiple specific transport systems and metabolic pathways controlled by both global and specific transcriptional regulators (37). Differential gene expression profile (DGE) analysis of *Listeria monocytogenes* and *Shigella flexneri* grown in host epithelial cells or macrophages, compared with laboratorially-defined medium (38–40), showed that upregulation of genes involved in uptake, such as glpT and uhpT, in catabolism of G3P and G6P, such as glpF, glpK, glpD, and dhaK, and in downregulation of glycolysis genes suggested the importance of G3P and G6P for intracellular growth.

In this study, we identified and characterized the HPT system in *S. aureus*. We demonstrated that the disruption of the hptA, hptRS, and uhpT genes impaired the growth under nutrition-limited conditions when the available carbon source was limited to G6P but not to glucose or MH2 broth. Similarly, transcription of the uhpT gene in the wild-type strain was increased when phosphate substrates, such as G3P, G6P, and fosfomycin, were present. The disruption of the hptA or hptRS gene ablated transcriptional activation of the uhpT gene. These results demonstrate that UhpT is the only transporter for G6P in *S. aureus* and that transcriptional activation of uhpT by the HPT is required for G6P or fosfomycin uptake. In combination with the *in silico* analysis data, these results strongly

---

**FIG 4** Role of HptRS in intracellular survival/multiplication of *S. aureus*. A cell monolayer of murine bone marrow-derived macrophages (BMDM), human monocyteic cells (THP-1), and human alveolar epithelial cells (A549) was infected with the wild-type or ΔHptRS strains at an MOI of 5. After 30 min of infection, extracellular bacteria were removed by gentamicin treatment, and survival/multiplication of *S. aureus* within the host cells were measured at the indicated time points. Results shown are combined from triplicate measurements from three independent experiments. The asterisk indicates statistical difference from the wild type at *P* values of <0.05.

**FIG 5** Role of HptRS and UhpT in fosfomycin sensitivity. The fosfomycin MIC of the wild-type, ΔHptRS, and ΔUhpT strains was determined using the Etest by following the manufacturer’s instructions.
UDP-GlcNAc, which is the first step in the peptidoglycan biosynthetic use against both Gram-positive and -negative bacteria (43). It and the HPT system plays an important role in this process.

-phosphoenolpyruvate, a peptidoglycan precursor, from N-acetylgalactosamine-3-phosphate substrates and triggering an adaptive response to take inositol pentakisphosphate (InsP5) (51). The HPT system is conserved across all bacteria (22, 23) but is not required for growth in the absence of stress. In addition, the HPT system appears to be a critical component for the regulation of multiple virulence factors in S. aureus.

We also demonstrated that the disruption of the hptRS genes impaired survival/multiplication of S. aureus within various types of host cells, possibly due to the lack of transcriptional activation of the uhpT gene, similar to the HPT system described in E. coli (22).

We thank Stephen Pruett for revising the manuscript.

REFERENCES


6. Ibarra JA, Perez-Rueda E, Carroll RK, Shaw LN. 2013. Global analysis of


35. Miro JM, Entenza JM, Del Rio A, Velasco M, Castaneda X, Garcia de la
