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The *Staphylococcus aureus* KdpDE Two-Component System Couples Extracellular K^+ Sensing and Agr Signaling to Infection Programming▽†‡

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The Kdp system is widely distributed among bacteria. In *Escherichia coli*, the Kdp-ATPase is a high-affinity K^+ uptake system and its expression is activated by the KdpDE two-component system in response to K^+ limitation or salt stress. However, information about the role of this system in many bacteria still remains obscure. Here we demonstrate that KdpFABC in *Staphylococcus aureus* is not a major K^+ transporter and that the main function of KdpDE is not associated with K^+ transport but that instead it regulates transcription for a series of virulence factors through sensing external K^+ concentrations, indicating that this bacterium might modulate its infectious status through sensing specific external K^+ stimuli in different environments. Our results further reveal that *S. aureus* KdpDE is upregulated by the Agr/RNAIII system, which suggests that KdpDE may be an important virulence regulator coordinating the external K^+ sensing and Agr signaling during pathogenesis in this bacterium.

Staphylococcus aureus is a significant human pathogen that causes a wide range of infections. Its capacity to cause diseases arises from its production of a diverse array of virulence factors during different stages of infection, including secreted proteins, such as serine protease (Ssp), nuclease, hemolysins, enterotoxins, lipase, and coagulase, and proteins exposed on the cell surface, such as protein A (Spa) and fibrinogen-, fibronectin-, and collagen-binding proteins (9, 14). Expression of these factors is regulated by a range of global regulators that mainly comprise two families: two-component regulatory systems, which are sensitive to environmental signals and consist of a sensor histidine kinase and a response regulator protein, and the Sar homologs, a family of DNA-binding proteins homologous to SarA (9, 14, 17, 31). Genomic scans have revealed that there are 16 two-component systems in the genome of *S. aureus* (28). Among these, several have been revealed to have specific physiological roles, including Agr (accessory gene regulator) and ArlRS, SaeRS, SrrAB, LytRS, VraRS, HssRS, and GraRS, some of which are known as virulence-associated sensor-regulator systems involved in the induced production of toxins and exoproteins and the regulation of biofilm formation (12, 20, 24, 28, 30, 33, 39, 49, 55). The Agr system is also the major quorum sensing system in which *agrD* encodes the autoinducing peptide pheromone (AIP), which activates the two-component AgrC-AgrA system; the latter two function as sensor and response regulator proteins, respectively (31, 33, 39). As the most important quorum sensing system, Agr controls the expression of many virulence factors and primarily regulates alterations in the gene expression pattern when cells enter the post-exponential phase. In contrast, the physiological functions of several *S. aureus* two-component systems still remain to be explored. The orthologues of these systems are widely found in Gram-positive bacteria, suggesting that they play important roles in cell physiology (9).

The KdpDE two-component system was first characterized in *Escherichia coli*, in which proteins KdpD and KdpE regulate the production of the high-affinity K^+ transporter Kdp-ATPase (6, 19, 38, 40). In *E. coli*, Kdp-ATPase is an efficient K^+-scavenging system that is expressed when cells are subjected to extreme K^+ limitation or osmotic upshock and other low-affinity K^+ transporters cannot meet the cellular requirements for K^+ (2, 4, 18, 26, 27, 29). The *E. coli* Kdp system consists of four proteins encoded by a single operon, *kdpFABC*, and its regulatory element, *kdpDE*, which is situated downstream of the *kdpC* gene (2, 19). Under K^+ limitation or high osmolarity imposed by a salt, the histidine kinase KdpD autophosphorylates and transfers the phosphoryl group to the response regulator KdpE (51). Phosphorylated KdpE exhibits increased affinity for a 23-bp sequence upstream of the canonical −35 and −10 regions of the *kdpFABC* promoter and thereby triggers *kdpFABC* transcription (47). A BLAST search of Kdp protein sequences shows that the Kdp-ATPase system is widely distributed among Gram-negative bacteria (e.g., *E. coli*, *Salmonella enterica* serovar Typhimurium LT2, and *Clostridium acetobutylicum*) and Gram-positive bacteria (e.g., *Bacillus cereus* E33L, *Alicyclobacillus acidocaldarius*, and *Mycobacterium tuberculosis*). In distantly related bacteria, the ordering of the *kdpA*, *kdpB*, and *kdpC* genes is relatively fixed, but the *kdpDE* genes show different arrangements (6, 44, 52). In *S. aureus*, the organization of the *kdpFABC* operon is similar to that of *E. coli*, but the *kdpDE* genes are arranged in a reverse orientation upstream of the *kdpA* gene. Although ex-

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perimental evidence has shown that the Kdp-ATPase system in several bacteria also functions as a high-affinity K^+ transporter (1, 5, 21, 22, 50), the role of the Kdp system in pathogenic bacteria has not been investigated in detail.

Recently, several lines of evidence have shown that the two-component system KdpD-KdpE is involved in virulence in some bacteria. In *Mycobacterium tuberculosis*, deletion of *kdpDE* resulted in increased virulence. Mice infected with the *M. tuberculosis kdpDE* mutant died more rapidly than did those infected with wild-type bacteria (36). In *S. aureus*, the function of the Kdp system has not yet been clarified, although several reports have shown that the transcript level of *kdpDE* changes under certain environmental stresses (exposure to neutrophil microbicides or growth under biofilm conditions) (7, 35). Our previous work showed that the transcript level of *kdpDE* in the *luxS* mutant increased compared with that in the wild type and the addition of exogenous autoinducer 2 (AI-2) restored the parental phenotype; besides this, the inactivation of *kdpDE* resulted in a decreased transcript level of *cap*, indicating that the LuxS/AI-2 signaling system regulates capsular polysaccharide synthetase gene expression via KdpDE (56). All these data suggest that KdpDE might be a functional two-component system in *S. aureus*; however, detailed information about its physiological role and how it functions needs further exploration.

In the present study, we identified the function of KdpDE in *S. aureus*. In *S. aureus* NCTC8325, KdpDE displays a repression effect on the transcription of *kdpFABC* under all of the different K^+ conditions that were tested and KdpFABC is not a major K^+ transporter. However, inactivation of *kdpDE* results in alterations of transcription for a range of virulence genes, including *spa*, *cap*, *hla*, *aur*, *geh*, and *hlgB*. In addition, our electrophoretic mobility shift assay (EMSA) data showed that KdpE can directly bind to the promoter regions of most of these genes so as to regulate their transcription. Besides this, we also revealed that the transcript level of *kdpDE* was influenced by the external K^+ concentration, indicating that this bacterium might modulate its infectious status by sensing specific external K^+ stimuli in different environments. Finally, we found that Agr/RNAIII strongly activated the transcript level of *kdpDE* in the post-exponential phase of the cells, and we confirmed that this regulatory effect was via Rot.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains and plasmids used in this study are described in Table 1. *Staphylococcus aureus* and *E. coli* were grown in Luria-Bertani (LB) medium or tryptic soy broth (TSB; soybean-casein digest medium USP; Oxoid) medium with the appropriate antibiotics for plasmid selection and maintenance. The mutants were constructed using a method previously described (11). All primers used in this study are listed in Table S1 in the supplemental material.

Development of the CDM. To study the effect of external K^+ on *S. aureus* strains under an increasing gradient of K^+ conditions, a formulation based on the one by Onoue and Mori was used (34). Initially, all potassium-containing salts of this medium were replaced with their sodium equivalents. The detailed composition of the formulation is described in Table S2 in the supplemental material. Three groups of wild-type bacteria were initially cultivated in chemically defined medium (CDM) with 0.2 mM K^+ to an optical density at 600 nm (OD_{600}) of 0.3, and then K^+ was added to two groups of these until final concentrations of 4 mM and 100 mM were reached. Each group was divided into three parts on average, and the three parts of the cells were harvested after cultivation for 10 min, 40 min, and a longer time (about 3 h to reach an OD_{600} of 0.5). The RNA from each group was subsequently extracted for real-time reverse transcription-PCR (RT-PCR) assays.

Measurement of the internal potassium concentration of *S. aureus*. The cells were cultivated in CDMs with different K^+ concentrations to an OD_{600} of 0.6, harvested by centrifugation, and then washed with deionized water six times to eliminate any residual K^+ in the medium. The cleaned cells were dried and lysed with sulfuric acid (metal-oxide-semiconductor grade), and the internal potassium concentration was assessed using an AAnalyst 800 atomic absorption spectrometer (Perkin-Elmer Corporation).

Total RNA isolation, cDNA generation, real-time RT-PCR, and microarray processing. For the microarray assays, overnight cultures of *S. aureus* were diluted 1:100 in LB medium and grown to the late exponential phase (OD_{600} = 2.0). Cells were collected by centrifugation and resuspended in Tris-EDTA (TE) buffer (pH 8.0) containing 10 g/liter lysozyme and 40 mg/liter lysostaphin. After incubation at 37°C for 5 min, *S. aureus* cells were prepared for total RNA extraction using the Trizol method (Invitrogen), and any residual DNA was removed with DNase (RNase free; TaKaRa). The cDNAs were synthesized and labeled according to the manufacturer's recommendations for *S. aureus* antisense genome arrays (Affymetrix Inc., Santa Clara, CA). Further preparation, hybridization, and scanning were conducted by the Biochip Company of Shanghai, China. Microarray data were analyzed with the Affymetrix Microarray Suite software 5.1 (Affymetrix Inc.) and a four-comparison survival method. Real-time RT-PCR was performed with the PrimeScript 1st Strand cDNA synthesis kit and the SYBR Premix Ex *Taq* (TaKaRa) using the StepOne real-time PCR system (Applied Biosystems). The quantity of cDNA measured by real-time PCR was normalized to the abundance of 16S cDNA. All the real-time PCR assays were repeated at least four times.

Purification of KdpE and Rot. The same purification methods were used for KdpE and Rot. Plasmid was transformed into *E. coli* BL21(DE3). The transformant was grown in 1 liter of LB at 37°C to an OD_{600} of 0.3 and induced with 0.5 mM IPTG (isopropyl-β-D-thiogalactopyranoside) for 3 h. Cells were harvested by centrifugation and washed with cell washing buffer (20 mM Tris-HCl, pH 8.0, and 0.5 M NaCl). The cells were resuspended in 50 ml of lysis buffer (20 mM Tris-HCl, pH 8.0, and 0.5 M NaCl) and were then lysed by sonication and centrifuged at 12,500 rpm for 30 min at 4°C. The supernatant was mixed with 2 ml of Ni-NTA agarose solution (Invitrogen), and the suspension was loaded onto a column at 4°C. After the column was washed with 5 ml of washing buffer I (5 mM imidazole, 20 mM Tris-HCl, pH 8.0, 0.5 M NaCl) and then with 100 ml of washing buffer II (20 mM imidazole, 20 mM Tris-HCl, pH 8.0, 0.5 M NaCl) and 10 ml of washing buffer III (100 mM imidazole, 20 mM Tris-HCl, pH 8.0, 0.5 M NaCl), the Rot protein was eluted with 5 ml of elution buffer (250 mM imidazole, 20 mM Tris-HCl, pH 8.0, 0.5 M NaCl, 10% glycerol). The imidazole in the eluant was removed by using a Centrifuge Biomax-5 column (Millipore), and then the protein solution was stored at −80°C until use. The purity of the protein was analyzed by SDS-PAGE, and the protein concentration was measured using the Bradford assay with bovine serum albumin (BSA) as a standard.

Electrophoretic mobility shift assay. The DNA fragments containing the promoters were amplified from the *S. aureus* NCTC8325 chromosome. The PCR products were labeled using the digoxigenin (DIG) gel shift kit (Roche) according to the manufacturer's instructions. The labeled fragment was incubated at 25°C for 15 min with various amounts of purified proteins in 10 μl of incubation buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 3 mM magnesium acetate, 0.1 mM EDTA, 0.1 mM dithiothreitol). After incubation, the mixtures were electrophoresed in a 4% native polyacrylamide gel in an 0.5× Tris-borate-EDTA (TBE) buffer. The band shifts were detected and analyzed according to the manufacturer's instructions.

DNase I footprinting assay. The forward primer was synthesized and subsequently 5′ labeled with 6-carboxyfluorescein (6-FAM), resulting in the labeled primer p-kdp-f-FAM. The labeled DNA fragments were prepared by PCR using *S. aureus* NCTC8325 genomic DNA as the template. The labeled DNA fragments were purified by PAGE. The DNase I footprinting assays were performed with a 3730XL DNA analyzer (Applied Biosystems) using a modified method based on previous studies (54).

***S. aureus* survival in human blood and in U937 monocytic cells.** Heparinized venous blood samples were collected from healthy donors who provided written informed consent to participate in the study. The bacterial strains were harvested from TSB plates after being cultured at 37°C for 16 h, washed twice in phosphate-buffered saline (PBS), and suspended to an OD_{600} of 0.8. The heparinized human blood (1 ml) was inoculated with 1×10^6 CFU of *S. aureus* and incubated at 37°C with shaking (250 rpm). A total of 5×10^6 U937 monocytic cells were mixed with 2×10^6 CFU of *S. aureus* opsonized with 10% normal human serum and incubated at 37°C under an atmosphere of 5% CO_2 with intermittent shaking. The bacteria were diluted to the appropriate concentration for testing at the

TABLE 1. Strain and plasmid list

Strain or plasmid	Relevant genotype	Source or reference
Strains		
S. aureus		
WT[b]	NCTC8325, wild type	NARSA[a]
RN4220	8325-4, r^-	NARSA
RN6911	*agr* locus in 8325-4 replaced by *tetM*	NARSA
SX8	8325 *kdpDE*::*ermB*	L. Zhao
SX9	8325 *kdpDE*::*ermB* pLIkdpDE	L. Zhao
SX10	8325 *kdpE*::*ermB*	L. Zhao
SX11	8325 *kdpE*::*ermB* pLIkdpE	L. Zhao
SX13	8325 *kdpFABC*::*ermB*	This study
SX14	8325 *kdpFABC*::*ermB* pLIkdpFABC	This study
SX15	8325 *agr*::*ermB*	This study
SX16	8325 *agr*::*ermB* pLIagr	This study
SX17	8325 RNAIII::*ermB*	This study
SX18	8325 RNAIII::*ermB* pLIRNAIII	This study
SX19	8325 *rot*::*ermB*	This study
SX20	8325 *rot*::*ermB* pLIrot	This study
SX21	RN6911 *rot*::*ermB*, *agr rot* double mutant	This study
E. coli		
DH5α	Clone host strain, *supE44* Δ*lacU169*(ϕ80 *lacZ*ΔM15) *hsdR17 recA1 endA1 gyrA96 thi-1 relA1*	Invitrogen
BL21	Expression strain, F^- *ompT hsdS*(r_B^- m_B^-) *gal dcm* (DE3)	Invitrogen
Plasmids		
pEASY-Blunt	Clone vector, Kan^r Ap^r	Transgen
pET28a(+)	Expression vector	Novagen
pGkdpE	pET28a(+) with *kdpE* gene	This study
pGrot	pET28a(+) with *rot* gene	This study
pEC1	pBluescript derivative; source of *ermB* gene; Ap^r	R. Bruckner
pBT2	Shuttle vector, temperature sensitive, Ap^r Cm^r	R. Bruckner
pBTkdpFABC	pBT2 derivative, for *kdpFABC* mutagenesis; Ap^r Cm^r Em^r	This study
pBTkdpDE	pBT2 derivative, for *kdpDE* mutagenesis; Ap^r Cm^r Em^r	L. Zhao
pBTkdpE	pBT2 derivative, for *kdpE* mutagenesis; Ap^r Cm^r Em^r	L. Zhao
pBTagr	pBT2 derivative, for *agr* mutagenesis; Ap^r Cm^r Em^r	This study
pBTRNAIII	pBT2 derivative, for RNAIII mutagenesis; Ap^r Cm^r Em^r	This study
pBTrot	pBT2 derivative, for *rot* mutagenesis; Ap^r Cm^r Em^r	This study
pLI50	Shuttle cloning vector, Ap^r Cm^r	Addgene
pLIkdpFABC	pLI50 with *kdpFABC* and its promoter, Ap^r Cm^r	This study
pLIkdpDE	pLI50 with *kdpDE* and its promoter, Ap^r Cm^r	L. Zhao
pLIkdpE	pLI50 with *kdpE* and the promoter of *kdp* operon, Ap^r Cm^r	L. Zhao
pLIagr	pLI50 with *agr* operon and its promoter, Ap^r Cm^r	This study
pLIRNAIII	pLI50 with RNAIII and its promoter, Ap^r Cm^r	This study
pLIrot	pLI50 with *rot* and its promoter, Ap^r Cm^r	This study

[a] NARSA, Network on Antimicrobial Resistance in *Staphylococcus aureus*.
[b] WT, wild type.

required intervals, and CFU were calculated by plate counts performed in duplicate on TSB agar. The percentage of *S. aureus* CFU that survived was determined by comparing the bacterial burden in each sample after the indicated time with the bacterial burden at the start of the assay (0 h).

Microarray data accession number. The microarray data and detailed protocols have been deposited in the CIBEX database (http://cibex.nig.ac.jp) with accession number CBX136.

RESULTS

KdpE can bind to the promoters of *kdpF* and *kdpD* and always represses transcription of the *kdpFABC* operon. The available genomic information shows that the organization of the *S. aureus* Kdp system is different from that in *E. coli*. In *E. coli*, the *kdpDE* operon is located downstream of *kdpC*, while in *S. aureus*, *kdpDE* is arranged in a reverse orientation upstream of *kdpA* (Fig. 1A). Since the *E. coli kdpDE* and other investigated *kdpDE* operons all activated the transcription of *kdpFABC*, it was reasonable for us to first investigate whether *S. aureus kdpDE* also has the same regulatory effect on the transcription of *kdpFABC*. The transcript levels of *kdpFABC* in the wild type, the *kdpDE* mutant, and the *kdpE* mutant were measured by real-time reverse transcription-PCR (RT-PCR) analysis. Unexpectedly, the transcript levels of *kdpFABC* in both the *kdpDE* mutant and the *kdpE* mutant displayed an increase compared with that in the wild type, whether the cells were grown in LB medium to an optical density at 600 nm (OD_{600}) of 1, 2, or 3 (Fig. 1B), suggesting that, in contrast to all of the other investigated *kdpDE* operons, the *S. aureus kdpDE* represses transcription of *kdpFABC* throughout the growth phase. Since KdpE is the response regulator containing a helix-turn-helix DNA-binding domain, we supposed that its regulatory effect on the transcription of *kdpFABC* might be through direct binding to the promoter regions of the *kdpFABC* operon. In order to determine this, we carried out EMSAs. The intergenic region between *kdpF* and *kdpD* was divided into two

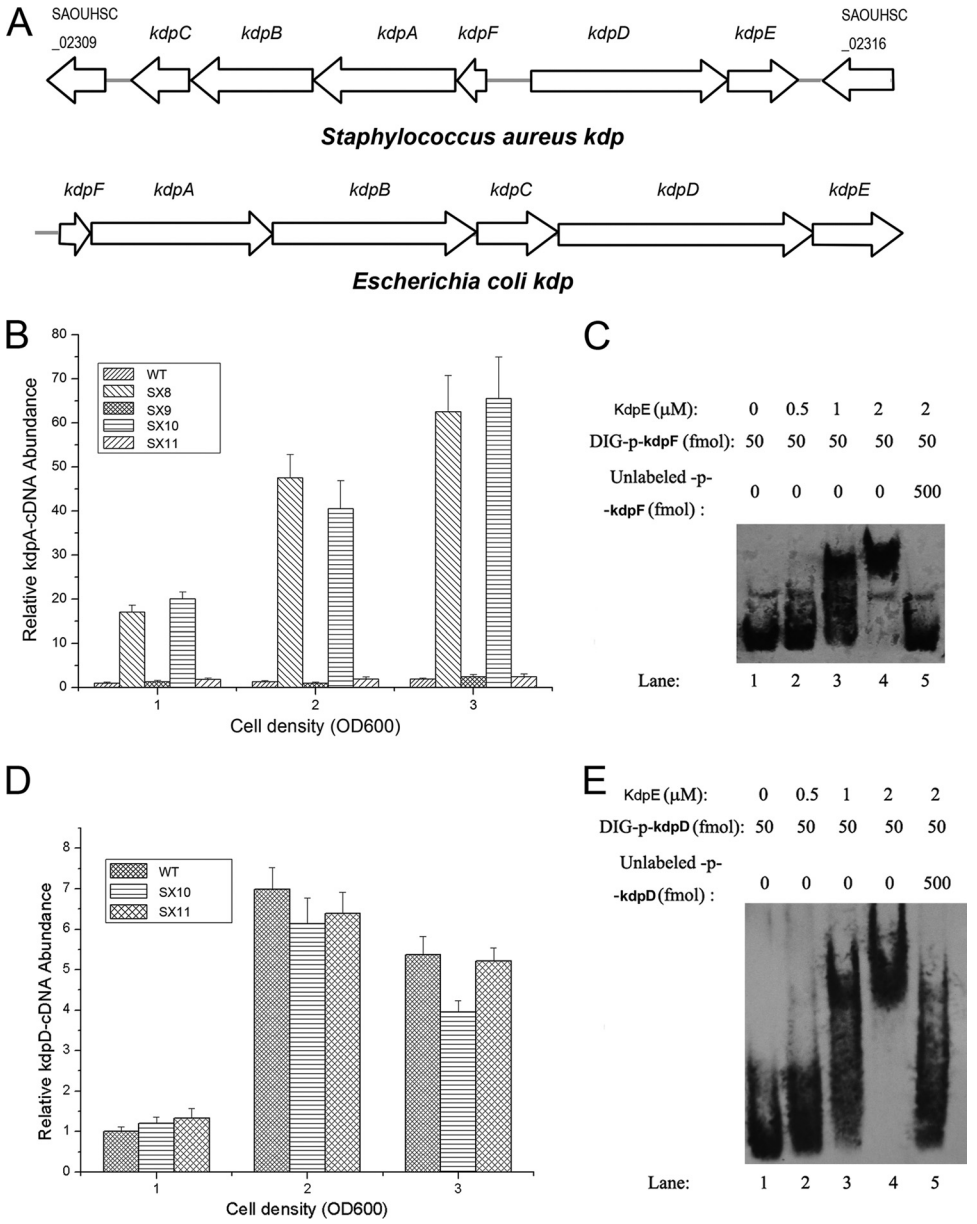

FIG. 1. Regulatory effect of KdpE on the *kdp* operon and *kdpDE* transcription. (A) Organization of the *kdp* operons in *S. aureus* and *E. coli*. The arrows indicate the directions of translation as determined from the nucleotide sequence. (B) The regulatory effect of KdpDE on the transcription of *kdpFABC* in cells grown in LB medium. The transcript levels of *kdpFABC* were compared using real-time RT-PCR in wild type (WT; *S. aureus* NCTC8325), SX8 (*kdpDE* mutant), SX9 (*kdpDE* mutant with a plasmid encoding KdpDE), SX10 (*kdpE* mutant), and SX11 (*kdpE* mutant with a plasmid encoding KdpE). The strains were grown in LB medium to OD_{600}s of 1, 2, and 3. (C) The ability of KdpE to bind to the *kdpFABC* promoter as determined by EMSAs. (D) The regulatory effect of KdpE on the transcription of *kdpD.* The transcript levels of *kdpD* were compared between WT, SX10 (*kdpE* mutant), and SX11 (*kdpE* mutant with a plasmid encoding KdpE). (E) The ability of KdpE to bind to the *kdpDE* promoter as determined by EMSAs. All the real-time PCR assays were repeated five times with similar results. Error bars indicate standard deviations.

parts, which were used as p-kdpF and p-kdpD, respectively. As expected, KdpE appeared to have a strong ability to bind to the promoter regions of *kdpFABC in vitro* (Fig. 1C).

Most of the response regulators of the two-component system can bind to the promoter region of their own operons and regulate transcription. Therefore, it was appropriate to investigate whether *S. aureus* KdpE also has this common feature. We performed real-time RT-PCR experiments and EMSAs for this purpose. Interestingly, our results showed that although KdpE can directly bind to the promoter region of *kdpDE* (Fig. 1E), it displayed no apparent influence on the transcription of this operon when the cells were grown in LB medium to different growth phases (Fig. 1D).

KdpFABC is not a major K^+ transporter in *S. aureus*. In *E. coli*, KdpFABC is a highly efficient K^+ transporter and inactivation of *kdpFABC* will result in notable growth inhibition of the cells when the external K^+ concentration is low. However, according to the above data, the regulatory effect of *S. aureus*

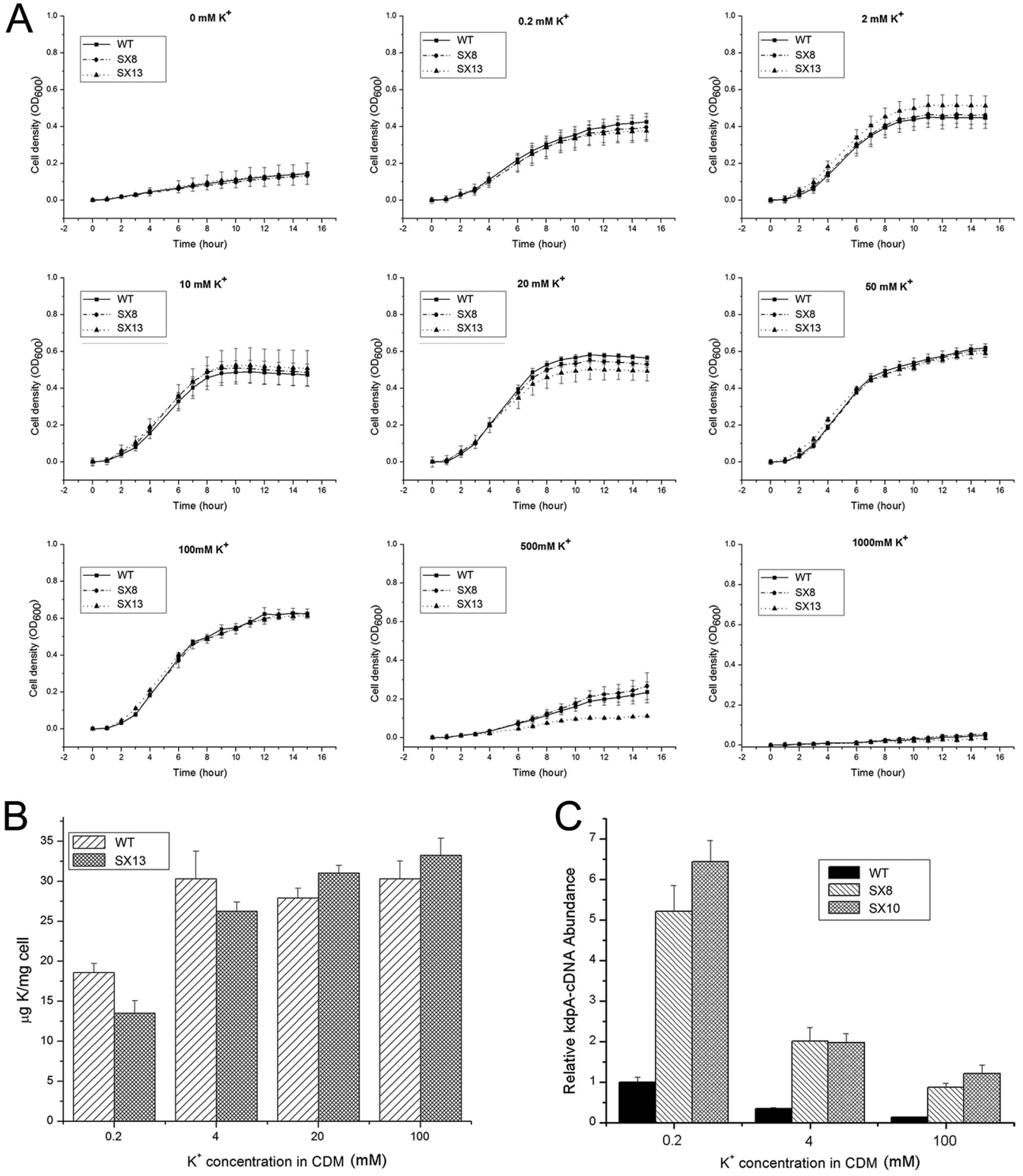

FIG. 2. KdpFABC is not a major K^+ transporter in *S. aureus*. (A) Comparison of growth rates of WT, SX8 (*kdpDE* mutant), and SX13 (*kdpFABC* mutant) in CDM with different K^+ concentrations. (B) Measurement of the internal K contents of the WT and SX13 (*kdpFABC* mutant) at different K^+ concentrations of 0.2 mM, 4 mM, 20 mM, and 100 mM. (C) The regulatory effect of KdpDE on the transcription of *kdpFABC* in cells grown under different external K^+ conditions. The transcript levels of *kdpFABC* were compared between WT, SX8 (*kdpDE* mutant), and SX10 (*kdpE* mutant) cells at different external K^+ concentrations of 0.2 mM, 4 mM, and 100 mM. The real-time PCR assay was repeated four times with similar results. Error bars indicate standard deviations.

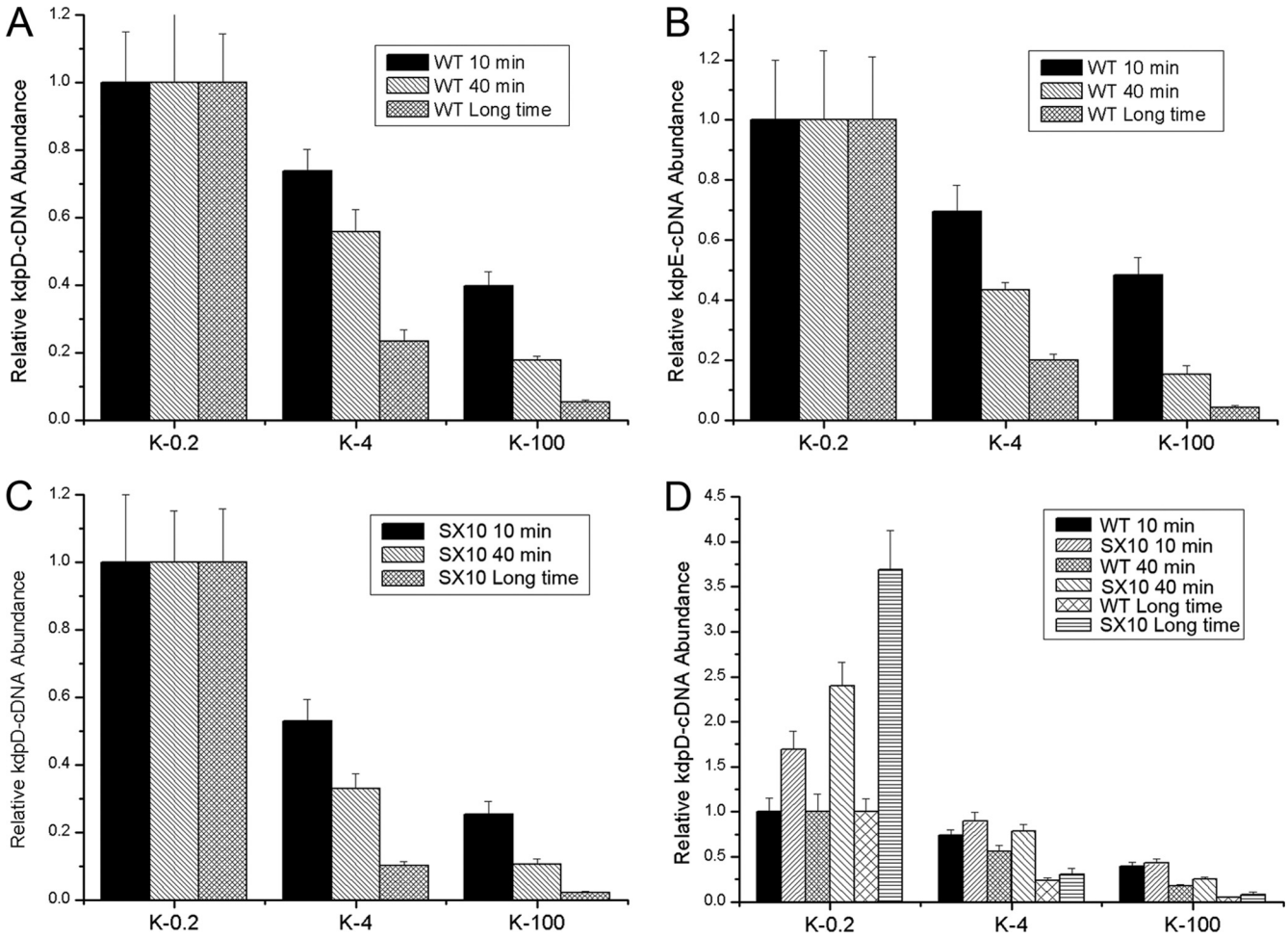

FIG. 3. Influence of external K^+ on *kdpDE* transcription. (A and B) Influence of K^+ stimuli on the transcription of *kdpDE*. The transcript levels of *kdpD* and *kdpDE* in the WT were tested when the cells were grown under different K conditions for different times. Three groups of wild-type bacteria were initially cultivated in CDM with 0.2 mM K^+ to an OD_{600} of 0.3, and then K^+ was added to two groups of these until final concentrations of 4 mM and 100 mM were reached. Each group was divided into three parts on average, and the three parts of the cells were harvested after cultivation for 10 min, 40 min, and a longer time (about 3 h to reach an OD_{600} of 0.5). (C) Experiments to explore whether or not the K^+ stimuli in the environment influenced the transcription of *kdpDE* through KdpE. The transcript levels of *kdpD* in SX10 (*kdpE* mutant) were tested with cells grown under different K^+ conditions for different times. (D) Comparison of the transcript levels of *kdpD* between WT and SX10 (*kdpE* mutant) under different K^+ conditions. All the real-time PCR assays were repeated four times with similar results. Error bars indicate standard deviations.

KdpDE on the transcription of *kdpFABC* appears to be contrary to that of its homolog in *E. coli*, which led us to suspect that the KdpFABC in *S. aureus* may not function like that in *E. coli*. We designed a series of experiments in order to explore whether the *S. aureus* KdpFABC is associated with K^+ transport. We first compared the growth rates of the wild type, the *kdpDE* mutant, and the *kdpFABC* mutant when they were grown in chemically defined medium (CDM) with different K^+ concentrations. The results showed that no remarkable difference was observed between the growth rates of the three strains, whether the external K^+ concentration was low or high (Fig. 2A). After this, we assessed the internal K contents of the wild type and the *kdpFABC* mutant strain when they were grown under different external K^+ conditions. As shown in Fig. 2B, the internal K contents of the *kdpFABC* mutants were quite high no matter which external K^+ condition they were grown under. When the external K^+ concentration was 0.2 mM or 4 mM, the internal K content of the *kdpFABC* mutant was a little lower than that of the wild type. Under the other external K^+ conditions, the two strains showed no remarkable difference in the internal K content. We further investigated the regulatory effect of KdpDE on *kdpFABC* transcription when the cells were grown under different external K^+ conditions. Our results still confirmed that transcription of *kdpFABC* is repressed by KdpDE (Fig. 2C). Compared with the activation of *kdpFABC* transcription by KdpDE in *E. coli*, the sustained repression effect of KdpDE on *kdpFABC* in *S. aureus* strongly suggests that KdpFABC is not a major K^+ transporter.

Transcription of *kdpDE* is influenced by external K^+ concentration. In *E. coli*, the transcript level of *kdpDE* changes with fluctuations in external K^+ concentrations. Therefore, we performed experiments to determine whether or not the transcription of *S. aureus kdpDE* can also be influenced by external K^+ concentrations. We tested the effects of three different K^+ concentrations: 0.2 mM, which is close to the usual K^+ concentration in the natural environment; 4 mM, which is almost equal to the K^+ concentration in human blood and serum; and

TABLE 2. Main genes affected by KdpDE

Gene function and identifier	Gene product	Fold change, mutant vs WT[c]
Metabolism genes		
SAOUHSC_01389	Thioredoxin reductase	2
SAOUHSC_00113	AdhE product = alcohol-acetaldehyde dehydrogenase	2
SAOUHSC_01619	Probable exodeoxyribonuclease VII small subunit	2.14
SAOUHSC_01825	Aminotransferase, class V	2
SAOUHSC_02281	IlvD product = dihydroxy-acid dehydratase	2.63
SAOUHSC_02282	IlvB product = acetolactate synthase large subunit	2.14
SAOUHSC_02283	Similar to acetolactate synthase small subunit	2.63
SAOUHSC_02671	NarK product = nitrite extrusion protein	2
SAOUHSC_02679	Similar to nitrate reductase delta chain	3.73
SAOUHSC_02680	NarH product = nitrate reductase beta chain	3.03
SAOUHSC_02682	NasF product = uroporphyrin III *C*-methyltransferase	2.29
SAOUHSC_02684	NasD product = nitrite reductase	2
SAOUHSC_02849	Pyruvate oxidase	2.14
SAOUHSC_02945	Precorrin-2 dehydrogenase	2
SAOUHSC_02969	ArcA product = arginine deiminase	2.46
SAOUHSC_03011	HisB product = imidazole glycerolphosphate dehydratase	2.14
SAOUHSC_03012	Hypothetical protein	2.29
SAOUHSC_03013	Histidinol dehydrogenase	2
SAOUHSC_01030	Putative NrdH-redoxin	2
SAOUHSC_00465	Veg product = VEG protein homolog	2.29
SAOUHSC_00898	ArgH product = argininosuccinate lyase	2.63
SAOUHSC_00899	ArgG product = argininosuccinate synthase	2.46
SAOUHSC_02118	Glutamyl-tRNA^{Gln} amidotransferase subunit C	2.29
SAOUHSC_01191	RpmB product = 50S ribosomal protein L28	0.47
SAOUHSC_01216	SucC product = succinyl-CoA[a] synthetase subunit beta	0.5
SAOUHSC_01218	SucD product = succinyl-CoA synthetase alpha subunit	0.5
SAOUHSC_00195	Acetyl-CoA acetyltransferase homolog	0.5
SAOUHSC_00196	Putative 3-hydroxyacyl-CoA dehydrogenase FadB	0.47
SAOUHSC_00197	Putative acyl-CoA dehydrogenase FadD	0.5
SAOUHSC_00198	Putative acyl-CoA synthetase FadE	0.44
SAOUHSC_00199	Putative acetyl-CoA/acetoacetyl-CoA transferase	0.47
SAOUHSC_00206	LctE product = L-lactate dehydrogenase	0.44
SAOUHSC_00365	AhpC product = alkyl hydroperoxide reductase subunit C	0.5
SAOUHSC_01002	Quinol oxidase polypeptide II QoxA	0.5
SAOUHSC_02366	FbaA product = fructose-bisphosphate aldolase	0.5
Cell surface protein genes		
SAOUHSC_01385	PstB product = phosphate ABC transporter	3.24
SAOUHSC_01386	Similar to phosphate ABC transporter	2.63
SAOUHSC_02311	KdpB product = probable potassium-transporting ATPase B	2
SAOUHSC_02310	KdpC product = probable potassium-transporting ATPase C	2.14
SAOUHSC_01990	Glutamate ABC transporter ATP-binding protein	4
SAOUHSC_00636	Similar to ABC transporter, permease protein	2.14
SAOUHSC_00637	Similar to ABC transporter ATP-binding protein	2.14
SAOUHSC_02661	ScrA product = PTS[b] system, sucrose-specific IIBC component	0.43
Regulator genes		
SAOUHSC_01384	Similar to negative regulator PhoU	2.46
SAOUHSC_01490	Hu product = DNA-binding protein II	0.44
SAOUHSC_02314	KdpD product = sensor protein	0.0059
SAOUHSC_02261	Accessory gene regulator protein B	0.5
SAOUHSC_02264	Accessory gene regulator protein C	0.5
SAOUHSC_02262	Accessory gene regulator protein D	0.41
SAOUHSC_02810	Transcriptional regulator, MerR family	0.44
SAOUHSC_00794	GapR product = glycolytic operon regulator	0.35
Virulence genes		
SAOUHSC_02629	Similar to multidrug resistance protein	2
SAOUHSC_02851	LrgA family protein	2
SAOUHSC_02855	Similar to secretory antigen precursor SsaA	2.63
SAOUHSC_02971	Aur product = zinc metalloproteinase aureolysin	2.29
SAOUHSC_00069	Spa product = immunoglobulin G binding protein A precursor	0.31
SAOUHSC_00114	CapA	0.38
SAOUHSC_00115	CapB	0.35
SAOUHSC_00116	CapC	0.35
SAOUHSC_00117	CapD	0.38
SAOUHSC_00118	CapE	0.44

Continued on following page

TABLE 2—*Continued*

Gene function and identifier	Gene product	Fold change, mutant vs WT[c]
SAOUHSC_00119	CapF	0.41
SAOUHSC_00120	CapG	0.44
SAOUHSC_00121	CapH	0.35
SAOUHSC_00122	CapI	0.43
SAOUHSC_00123	CapJ	0.43
SAOUHSC_00124	CapK	0.46
SAOUHSC_00125	CapL	0.5
SAOUHSC_00126	CapM	0.5
SAOUHSC_00127	CapN	0.5
SAOUHSC_00300	Geh product = glycerol ester hydrolase	0.41
SAOUHSC_02260	Hld product = delta-hemolysin	0.38
SAOUHSC_02709	HlgC product = gamma-hemolysin component C	0.47
SAOUHSC_02710	HlgB product = gamma-hemolysin component B	0.41
Hypothetical protein genes		
SAOUHSC_02384	Hypothetical protein	2
SAOUHSC_02523	Hypothetical protein	2
SAOUHSC_02858	Hypothetical protein	2
SAOUHSC_01296	Hypothetical protein	2.46
SAOUHSC_01729	Hypothetical protein	2.14
SAOUHSC_01991	Hypothetical protein	2.82
SAOUHSC_00202	Hypothetical protein	2
SAOUHSC_02850	Hypothetical protein	2
SAOUHSC_03047	Hypothetical protein	2
SAOUHSC_01032	Hypothetical protein	2.14
SAOUHSC_01072	Hypothetical protein	2.14
SAOUHSC_00704	Hypothetical protein	2.29
SAOUHSC_02886	Hypothetical protein	2.29
SAOUHSC_01557	Hypothetical protein	0.38
SAOUHSC_02521	Hypothetical protein	0.5
SAOUHSC_02838	Hypothetical protein	0.38
SAOUHSC_00091	Hypothetical protein	0.5
SAOUHSC_00094	Hypothetical protein	0.44
SAOUHSC_01675	Hypothetical protein	0.5
SAOUHSC_01918	Hypothetical protein	0.5
SAOUHSC_02781	Hypothetical protein	0.47
SAOUHSC_02788	Hypothetical protein	0.33
SAOUHSC_02805	Hypothetical protein	0.36
SAOUHSC_00401	Hypothetical protein	0.5
SAOUHSC_00413	Hypothetical protein	0.47
SAOUHSC_01956	Hypothetical protein	0.47
SAOUHSC_00414	Hypothetical protein	0.47
SAOUHSC_00257	Hypothetical protein	0.5
SAOUHSC_01109	Hypothetical protein	0.47
SAOUHSC_01956	Hypothetical protein	0.47
SAOUHSC_02796	Hypothetical protein	0.088

[a] CoA, coenzyme A.
[b] PTS, phosphotransferase system.
[c] WT, wild type.

100 mM, which is similar to the normal K^+ concentration in host cells. Three groups of wild-type bacteria were initially cultivated in CDM with 0.2 mM K^+ to an OD_{600} of 0.3, and then K^+ was added to two groups of these to reach final K^+ concentrations of 4 mM and 100 mM, respectively. In order to integrate the time factor, we cultivated the bacteria under specific K^+ conditions for different times and then measured the transcript levels of *kdpD* and *kdpE* by using real-time RT-PCR analysis. As shown in Fig. 3A and B, when the external K^+ concentration was 4 mM, the transcript levels of *kdpD* and *kdpE* remarkably decreased compared with those of the cells cultivated under 0.2 mM K^+ conditions; when the external K^+ concentration was 100 mM, this tendency to decrease became much more obvious. These data suggest that the transcription of both *kdpD* and *kdpE* can be influenced by alterations in the external K^+ concentrations. Besides this, it was notable that these influences always existed whether the cultivation time was short (10 min and 40 min) or long (about 3 h), indicating that the external K^+ concentration has an instant effect on the transcription of *kdpD* and *kdpE*.

Furthermore, we measured *kdpD* transcript levels in the *kdpE* mutants growing in CDM at different external K^+ concentrations, and the results showed that inactivation of *kdpE* did not affect the influence of external K^+ concentration on the transcription of *kdpD* (Fig. 3C), suggesting that alterations in the transcript level of *kdpD* in response to changes in the external K concentration are not dependent on KdpE.

As mentioned above, our data showed that KdpE exhibited no

regulatory effects on the transcription of *kdpD* in the LB medium. To exclude the specific influence of the high K^+ concentration of the LB medium, we compared the transcript levels of *kdpD* between the wild type and the *kdpE* mutant under different K^+ conditions. As shown in Fig. 3D, when the external K^+ concentration was 0.2 mM, the transcript level of *kdpD* in the *kdpE* mutant was much higher than that of the wild type; however, when the external K^+ concentration was above 4 mM, this difference was no longer apparent. These results suggest that KdpE can repress the transcription of *kdpD*, but only when the cells are under the lowest external K^+ conditions.

KdpDE is a global regulator of virulence genes. To characterize the gene transcriptional profiling influence of KdpDE, DNA microarray assays were performed using the parental strain NCTC8325 and the *kdpDE* deletion mutant strain. The cells were grown in LB medium to an OD_{600} of 2.0. A 2-fold induction ratio was used as the cutoff limit for comparing the transcriptional profiling of the wild type and the *kdpDE* mutant strain. Microarray data indicated that 48 genes were induced and 58 genes were repressed in the *kdpDE* mutant strain (Table 2). Of importance, the transcript levels of a range of virulence factor genes, such as *spa*, *cap*, *hla*, *aur*, *geh*, and *hlgB*, were altered in the *kdpDE* mutant strain (3, 8, 10, 13, 15, 25, 37, 42, 45, 46). Real-time RT-PCR experiments were conducted to further analyze the regulatory effect of KdpDE on the transcription of these genes. Our previous work showed that the inactivation of *kdpDE* resulted in a decreased transcript level of *cap* operon (36). Here, our results showed that the transcript levels of *spa*, *hla*, *aur*, *geh*, and *hlgB* displayed apparent alterations in the *kdpDE* mutant compared with those in the parental strain (Fig. 4A). Among these genes, *spa* and *cap* belong to the group of genes encoding cell wall-associated proteins and polysaccharides that play roles in bacterial colonization, while *hla*, *aur*, *geh*, and *hlgB* belong to the group of genes encoding the toxin proteins that facilitate local invasion. Interestingly, our results showed that the transcript levels of *spa* and *cap* decreased, whereas the transcript levels of *hla*, *aur*, *geh*, and *hlgB* all increased in the *kdpDE* mutant compared to those in the parental strain.

In addition, the transcript levels of *spa*, *hla*, *aur*, *geh*, and *hlgB* in the wild type and the *kdpE* mutant were also compared using real-time RT-PCR analysis. The results showed that KdpE displayed the same regulatory tendency as did KdpDE on the transcription of these virulence genes (Fig. 4A). Since KdpE is a DNA-binding protein, we further performed EMSAs to investigate whether or not KdpE can regulate the transcription of these genes by directly binding to their promoter regions. As shown in Fig. 4B, KdpE can specifically bind to the promoter regions of all of these genes, except *hla*, *in vitro*. Meanwhile, we also measured the transcript levels of these virulence factors in the *kdpFABC* mutant, and the results showed that the transcription of these genes (*spa*, *cap*, *hla*, *aur*, *geh*, and *hlgB*) almost did not change in the mutant compared with the wild type (data not shown). These results suggest that, in *S. aureus*, KdpE is a global regulator which can bind to many virulence targets and regulate their transcription.

Modulatory effect of KdpDE on expression of Spa. According to the above data, KdpDE can modulate the transcription of *spa*. Since Spa is a representative cell wall-associated exoprotein and a major determinant of virulence in *S. aureus* (14, 16, 45), it was of importance to further investigate this modulatory effect.

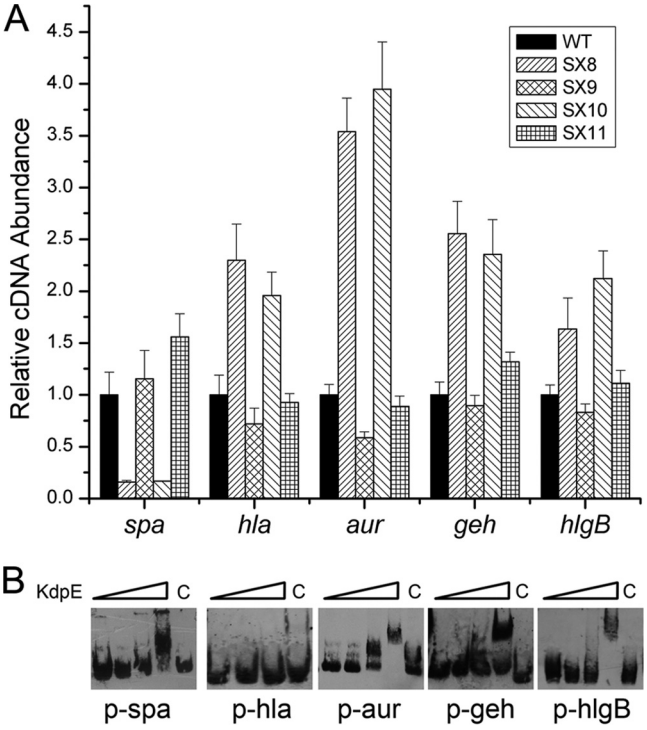

FIG. 4. KdpDE is a global virulence regulator. (A) Comparative measurements of a range of virulence gene transcripts by real-time RT-PCR in WT, SX8 (*kdpDE* mutant), SX9 (*kdpDE* mutant with a plasmid encoding KdpDE), SX10 (*kdpE* mutant), and SX11 (*kdpE* mutant with a plasmid encoding KdpE). All of the strains were grown in LB medium to an OD_{600} of 1.7. The relative transcription of each gene compared to that of the constitutively expressed 16S rRNA gene in SX8, SX9, SX10, and SX11 was compared with that in the wild type, to which we assigned a value of 1. All the real-time PCR assays were repeated four times with similar results. Error bars indicate standard deviations. (B) The ability of KdpE to bind to these gene promoters as determined by EMSAs. The concentrations of KdpE used for p-hla EMSA were 0 μM, 0.5 μM, 1 μM, and 2 μM (from left to right, respectively); the concentrations of KdpE used for other EMSAs with other promoters were 0 μM, 0.5 μM, 1 μM, 2 μM, and 2 μM (from left to right, respectively). Tenfold unlabeled probes were used for the negative-control assays.

First, we examined this effect during the different growth phases of this bacterium. Three groups of strains (the wild type, the *kdpDE* mutant, the *kdpDE* mutant with the complementing plasmid, the *kdpE* mutant, and the *kdpE* mutant with the complementing plasmid) were cultivated in LB medium to OD_{600}s of 1, 2, and 3, respectively. The results of real-time RT-PCR revealed that the transcript levels of *spa* in the *kdpDE* mutant and the *kdpE* mutant were decreased compared to those in the wild type and the strains with the complementing plasmids, no matter which OD the cells were grown to (Fig. 5A), indicating that *kdpDE* can strongly activate the transcription of *spa* throughout the whole growth phase. We also performed Western blot assays to compare the protein levels of Spa between the *kdpDE* mutant and the wild type when they were grown to different growth phases, and the results confirmed that the expression of Spa in the *kdpDE* mutant was always lower than that in the wild type (see Fig. S1 in the supplemental material). In addition, previous studies showed that the expression of Spa enhanced bacterial virulence in a mouse bacteremia model and in macrophages due to its anti-

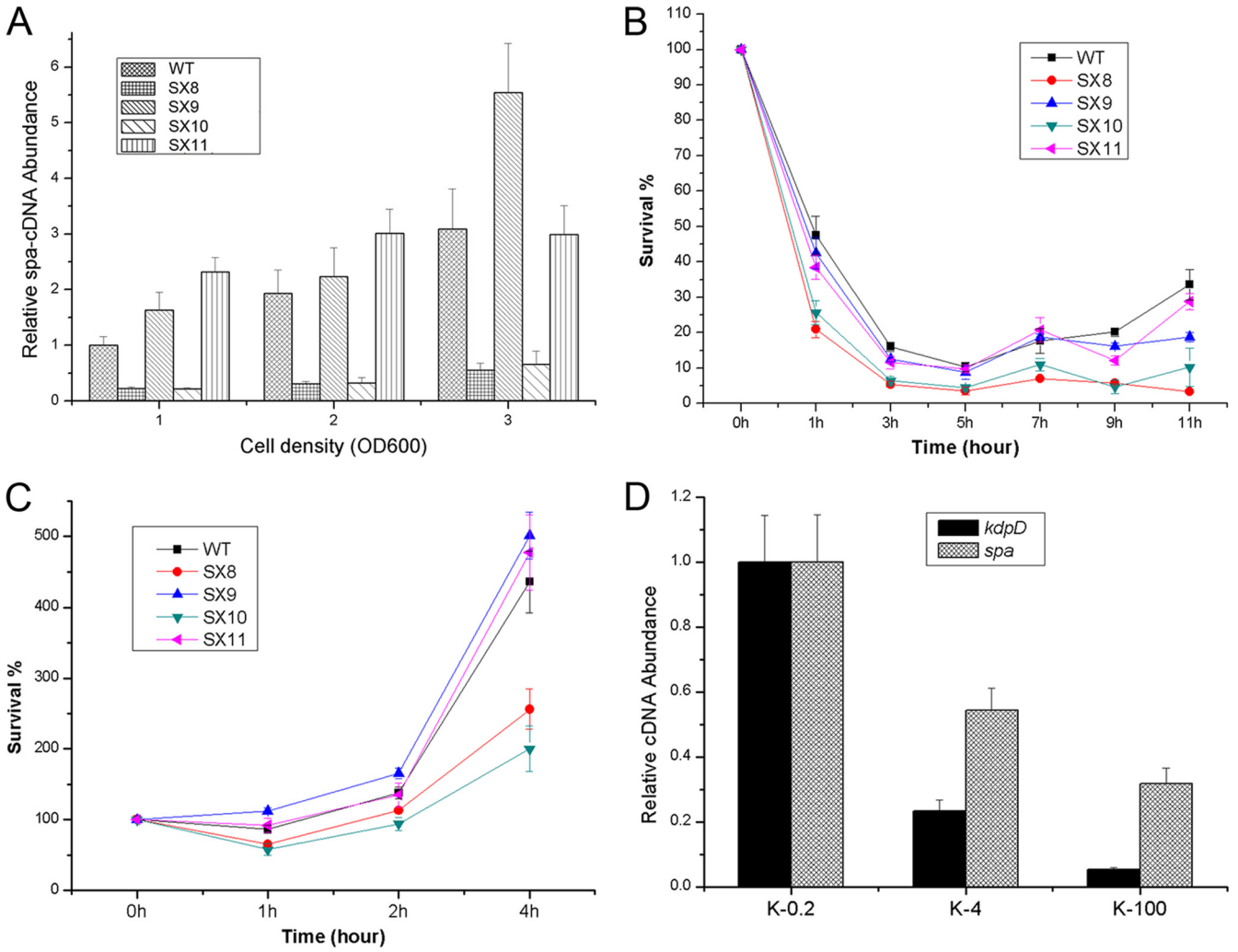

FIG. 5. Regulatory effect of KdpDE on *spa* expression. (A) Analysis of the transcriptional regulation of *spa* by KdpDE. The transcript levels of *spa* were compared using real-time RT-PCR in WT, SX8 (*kdpDE* mutant), SX9 (*kdpDE* mutant with a plasmid encoding KdpDE), SX10 (*kdpE* mutant), and SX11 (*kdpE* mutant with a plasmid encoding KdpE). Three groups of strains were grown in LB medium to OD_{600}s of 1, 2, and 3, respectively. (B) Comparative measurements of survival rates of WT, SX8, SX9, SX10, and SX11 in heparinized human blood. Results are from five separate blood donors. (C) Comparative measurements of survival rates of WT, SX8, SX9, SX10, and SX11 when cultured with U937 monocytic cells. The percentage of *S. aureus* CFU that survived was determined as described in Materials and Methods. (D) Influence of K^+ stimuli on the transcription of *spa* and *kdpD*. The transcript levels of *kdpD* and *spa* in WT were tested in cells grown under different K^+ conditions for different times. Three groups of wild-type bacteria were cultivated in CDM with 0.2 mM, 4 mM, and 100 mM K^+. All the real-time PCR assays were repeated four times with similar results. Error bars indicate standard deviations.

phagocytic nature. Therefore, we examined the influences of the inactivation of *kdpDE* or *kdpE* on the survival of *S. aureus* in human whole blood and human U937 monocytic cells. As shown in Fig. 5B and C, the *kdpDE* and *kdpE* mutants exhibited significantly lower survival rates than did the wild type and the strain with the complementing plasmid in both human whole blood and human U937 monocytic cells, further demonstrating that inactivation of *kdpDE* repressed the expression of Spa.

As noted above, the transcription of *kdpDE* decreased with an increase in the external K^+ concentration. We also assessed the transcript levels of *spa* under different external K^+ conditions. Interestingly, the real-time RT-PCR data showed that the changing trend in the transcription of *spa* was the same as that of *kdpD* in transition from low to high external K^+ conditions (Fig. 5D). This observation, together with the regulatory effect of KdpDE on the transcription of *spa* as investigated previously, further demonstrated that KdpDE in *S. aureus* can regulate the transcription of *spa* in response to K^+ stimuli in the environment and that KdpDE will not always stimulate the transcription of *spa* when the external K^+ concentration increases.

Identification of the KdpE binding sequence. To further identify the precise KdpE binding sequence, DNase I footprinting was performed. As shown in Fig. 6A, one region from −51 to −73 bp, relative to the translation start site of the *kdpF* gene, is protected, which is indicated by the disappearing nucleotide peaks in Fig. 6A, panel a, compared to Fig. 6A, panel b. These footprinting results demonstrated that KdpE binds to a 23-bp region (GCATACACATCTTAATGATTTCT) of the *kdpF* promoter, which is probably near the −35 to −10 transcriptional box of *kdpF* (Fig. 6B). This evidence led us to

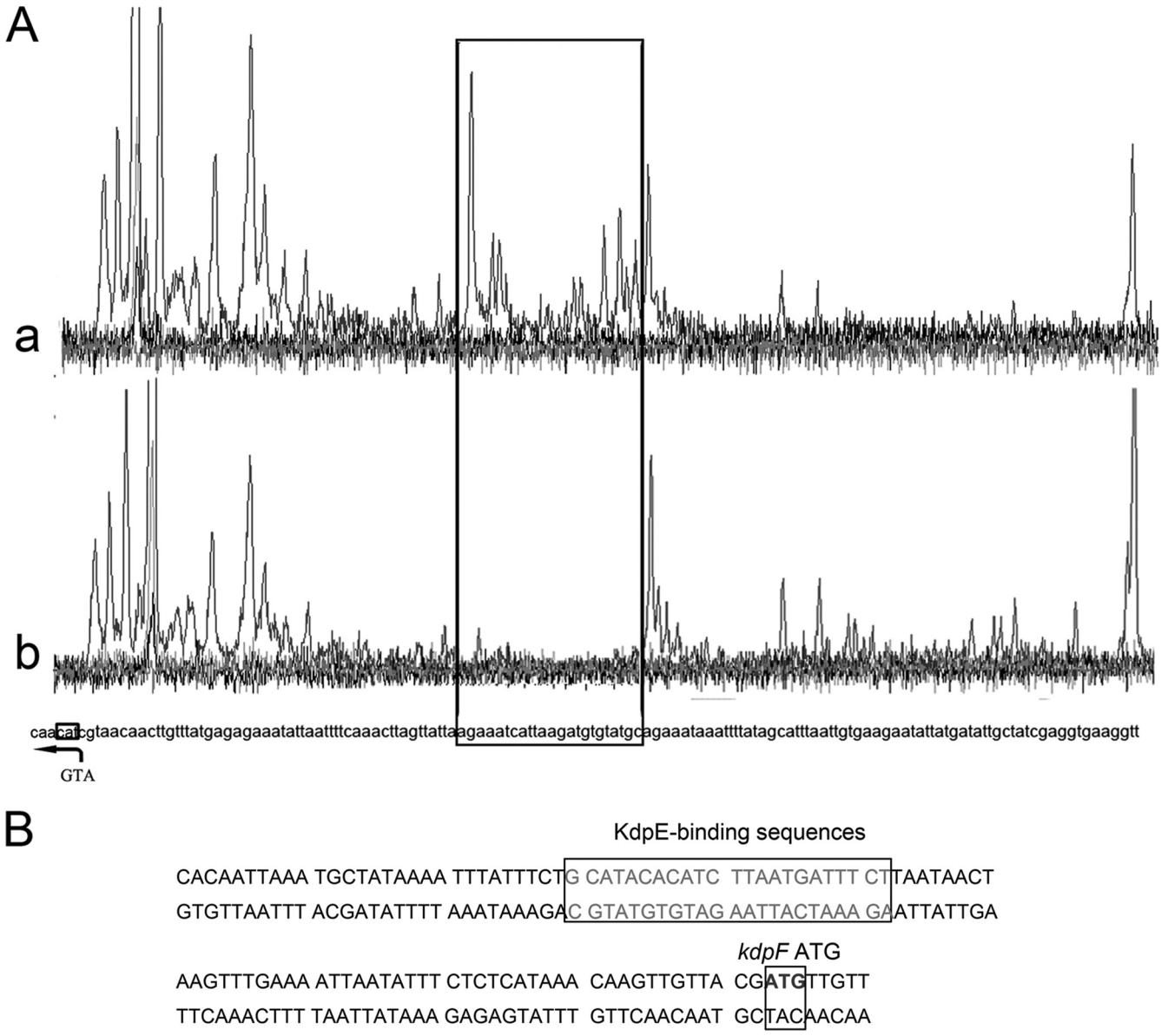

FIG. 6. Identification of the KdpE-binding sequences by DNase I footprinting. (A) Identification of the KdpE-binding site on the promoter of *kdpFABC* by DNase I footprinting assays. The black frame indicates the DNA region protected from DNase I by KdpE. (B) KdpE-binding sequences on *kdpF* promoter regions.

conclude that the sustained repression effect of KdpE on *kdpFABC* transcription is most likely due to the fact that KdpE binds to the −35 to −10 transcriptional box of *kdpFABC*, thereby competitively inhibiting the binding of its transcriptional factors, such as σ factor and RNA polymerase.

Agr/RNAIII activates the transcription of *kdpDE* by Rot. We observed that the transcript levels of both *kdpD* and *kdpE* significantly increased when the cells were grown to the post-exponential phase in LB medium (Fig. 7A), suggesting that the transcription of *kdpDE* might be associated with the Agr quorum sensing system. In order to determine whether or not Agr/RNAIII is involved in the regulation of *kdpDE* transcription, we first made the *agr* mutant, the RNAIII mutant, and the strains with the complementing plasmids and subsequently compared the transcript levels of *kdpDE* of these strains with that of the NCTC8325 parental strain. The results showed that the transcript levels of *kdpDE* in the *agr* mutant and the RNAIII mutant were much lower than that of the parental strain, while the strains with the complementing plasmids were restored to the parental phenotype (Fig. 7B), indicating that the Agr system can activate *kdpDE* transcription through RNAIII. We also cultivated the wild type and the *agr* mutant in CDMs with different K^+ concentrations and tested the transcript levels of *kdpD* of the two strains, and the results showed that the transcript level of *kdpD* of the *agr* mutant was also always much lower than that of the wild type under different K^+ conditions (see Fig. S2A in the supplemental material). Since RNAIII cannot directly activate target gene transcription, there certainly exists an intermediate component in this

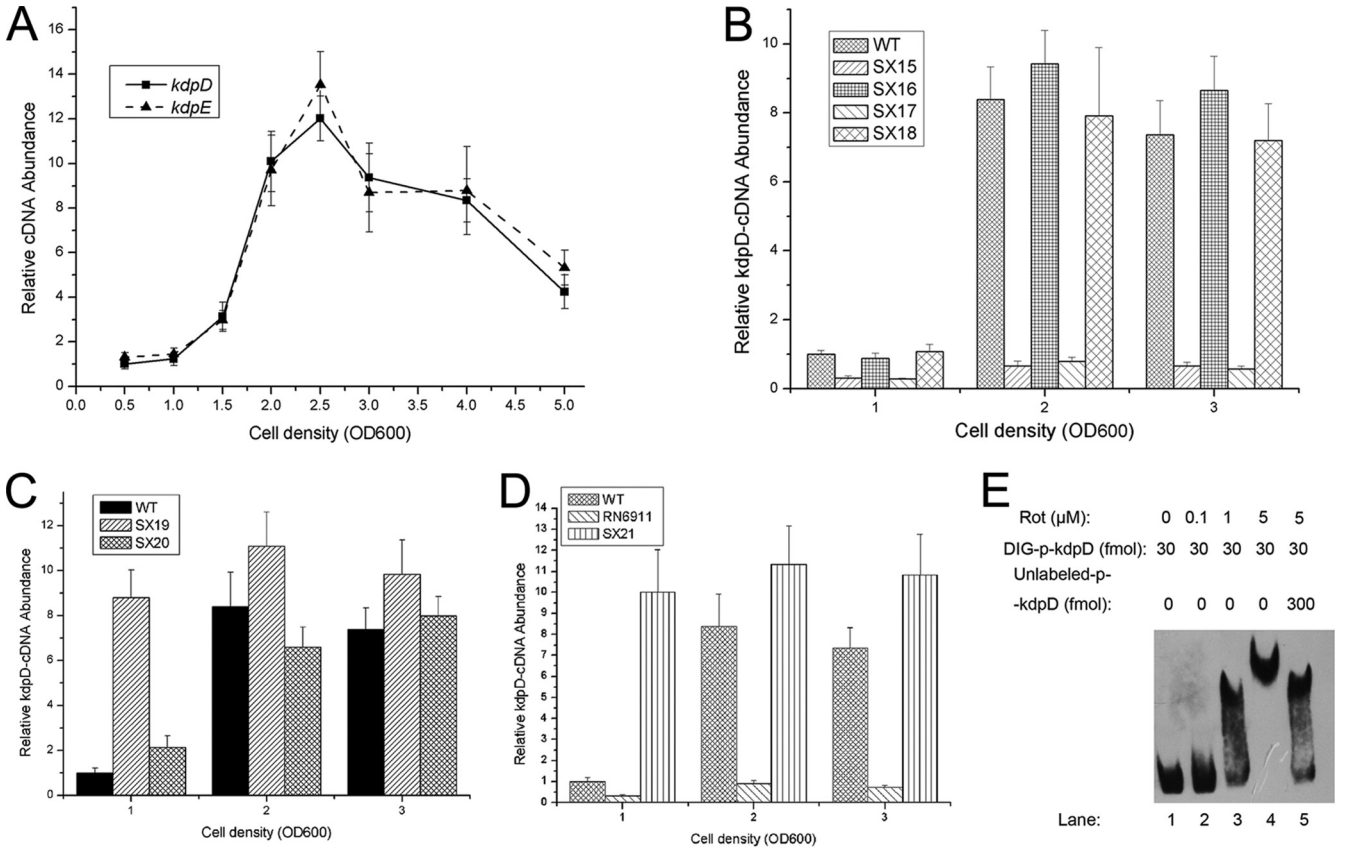

FIG. 7. Agr/RNAIII activates the transcription of *kdpDE* by Rot. (A) Transcript levels of *kdpD* and *kdpE* in different growth phases. (B) Characterization of the regulatory effect of Agr/RNAIII on *kdpDE* transcription. The transcript levels of *kdpD* were measured using real-time RT-PCR in WT, SX15 (*agr* mutant), SX16 (*agr* mutant with a plasmid encoding the Agr system), SX17 (RNAIII mutant), and SX18 (RNAIII mutant with a plasmid encoding RNAIII). Three groups of strains were grown in LB medium to OD_{600}s of 1, 2, and 3, respectively. (C) Effect of Rot on *kdpDE* transcription. The transcript levels of *kdpD* were measured using real-time RT-PCR in WT, SX19 (*rot* mutant), and SX20 (*rot* mutant with a plasmid encoding Rot). Three groups of strains were grown in LB medium to OD_{600}s of 1, 2, and 3. (D) Effect of Rot on *kdpDE* transcription. The transcript levels of *kdpD* were measured using real-time RT-PCR in WT, RN6911 (*agr* mutant), and SX21 (*agr rot* double mutant). Three groups of strains were grown in LB medium to OD_{600}s of 1, 2, and 3. All the real-time RT-PCR assays were repeated four times with similar results. Error bars indicate standard deviations. (E) The ability of Rot to bind to the *kdpDE* promoter as determined by EMSAs.

regulatory pathway. As described previously, the mask of *rot* translation by RNAIII is a key feature of the Agr function (23, 42). Therefore, we proposed that Agr upregulates *kdpDE* transcription probably through repressing *rot* translation. Furthermore, we tested the transcript levels of *kdpD* in the wild-type strain NCTC8325, the *rot* mutant, and the *rot* mutant with complementing plasmid when they were in different growth phases. The transcript level of *kdpD* in the *rot* mutant was much higher than that in the wild type when the cells were grown to an OD_{600} of 1 (early exponential phase), indicating that Rot strongly represses *kdpDE* transcription in this growth phase. The difference in transcript levels between the wild type and the *rot* mutant was not that apparent (Fig. 7C) when the cells were grown to the exponential phase (OD_{600} of 2 and 3), which was in accordance with the phenomenon that huge amounts of the RNAIII transcript accumulate in the wild type during the transition from the early exponential to the exponential phase. The accumulation of the RNAIII transcript inhibited the translation of *rot*, which, in return, diminished the Rot effect on *kdpDE* transcription. As shown in Fig. 7D, we also tested the transcript levels of *kdpD* in the wild type, RN6911 (*agr* mutant), and RN6911 with *rot* deletion (*agr rot* double mutant) when they were in different growth phases. The transcript level of *kdpD* in the *agr rot* double mutant was similar to that in the *rot* mutant but different from that in the *agr* mutant. These data demonstrated that Agr regulates *kdpDE* transcription through Rot. Furthermore, our EMSAs confirmed that Rot can specifically bind to the promoter of *kdpD in vitro* (Fig. 7E).

DISCUSSION

All of the bacterial *kdp* operons investigated previously were found to be repressed during growth in media with a high external K^+ concentration and activated when the external K^+ concentration becomes lower than a threshold value (6, 26, 41). These events are controlled by the KdpDE two-component system. For instance, in *E. coli*, when the external K^+ concentration falls below 2 mM, KdpD is autophosphorylated and activates KdpE, forming a product which binds to the promoter region of *kdpFABC* and activates its transcription (38, 48). In contrast, our results showed that, in *S. aureus*, KdpDE always repressed the transcription of *kdpFABC*,

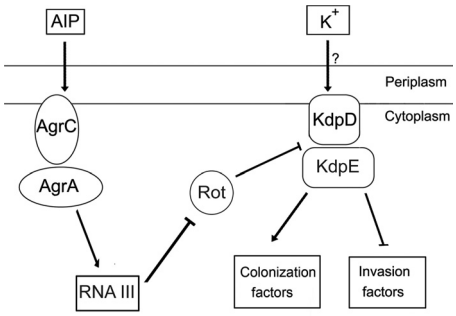

FIG. 8. Proposed regulation scheme of KdpDE. The pathogenesis of *S. aureus* is determined by the coordinated gene regulation in response to the self-secreting signal and other specific stimuli in the environment, and KdpDE, as a two-component system which can respond to both, is the concrete embodiment of this coordination.

whether under high or low external K^+ conditions. In *E. coli*, KdpFABC is the major K^+ transporter when the cells are subjected to K^+ limitation. Therefore, inactivation of *kdpFABC* in *E. coli* would result in notable growth inhibition of the cells when the external K^+ concentration is low (43). However, we found that the growth rates of the *S. aureus kdpFABC* mutant and the parental strain showed no apparent differences when the cells were grown in CDM with different K^+ concentrations. In addition, by carrying out atomic absorption spectrometry assays, we observed that the internal potassium concentration of the *S. aureus kdpFABC* mutant was almost equal to that of the parental strain whether the K^+ concentration of the medium was low or high. Thus, we conclude that KdpFABC is not a major K^+ transporter in *S. aureus* and that another highly efficient K^+ transporter which is functional in K^+ transport must exist in this bacterium. This would also help to explain why the transcriptions of *kdpFABC* were always repressed by KdpE to quite low levels in *S. aureus* and indicates that the regulatory effect of KdpDE on KdpFABC ATPase in *S. aureus* is extremely different from that in *E. coli*. However, our real-time RT-PCR data showed that the transcription of *kdpDE* exhibits notable changes in response to fluctuations of the external K^+ concentrations. Taking all of these data into account, we suggest that the *S. aureus* KdpDE functions in other aspects through sensing the external K^+ concentration.

The pathogenic mechanisms of *S. aureus* infections are highly complex (9, 14, 31). It is very likely that distinct networks of multiple virulence genes are expressed in response to distinct host signals, including those found in blood and specific target tissues and those related to innate host defense factors that emerge during the infectious process. A high K^+ concentration is also a host-specific signal which differs from potassium signals in the natural environment. In this study, we found that the transcript levels of *kdpDE* decreased as the external K^+ concentration increased. When the external K^+ concentration was 0.2 mM, which is close to the usual K^+ concentration found in the natural environment, the transcript levels of *kdpD* were relatively high. Comparatively, when the external K^+ concentration increased to 4 mM, which is almost equal to the K^+ concentration in host blood and tissue fluid, or to 100 mM, which is similar to the K^+ concentration in the host cells, the transcription of *kdpD* was largely repressed. As noted above, we showed that KdpE can activate the transcription of genes encoding cell wall-associated proteins and polysaccharides and that it can repress the transcription of toxin genes. Collectively, these interesting data led us to propose that the two-component system KdpDE might be an important virulence gene regulator in response to changes in the environment. In the natural environment, a high level of KdpDE transcription helps to activate the expression of cell wall proteins and polysaccharides, which are beneficial to colonization. However, in transition from the natural environment to the host, which has a higher K^+ concentration, transcript levels of *kdpDE* decrease, causing a low expression of cell wall proteins but a high production of extracellular toxins and enzymes which facilitate local invasion. The capability of KdpDE in regulating alterations in the gene expression pattern indicates that KdpDE plays an important role in the pathogenesis of *S. aureus*.

It is interesting that the Agr system activates the transcription of *kdpDE*. Agr is the best-characterized quorum sensing system in *S. aureus* and regulates specific physiological functions when the population density of the community reaches a threshold (32, 39, 53). Thus, we can conclude that the high transcript level of *kdpDE* is also dependent on high cell density. This phenomenon is of special significance for the pathogenesis of *S. aureus* because the pathogenic processes are unproductive when undertaken by an individual bacterium acting alone but become beneficial when carried out simultaneously by a large number of cells. Only when the cell number in the community reaches a high level does it become meaningful for KdpDE to regulate virulence gene expression via sensing of the external K^+ concentration in the environment. We cultivated the wild type and the *agr* mutant in CDM at different K^+ concentrations and measured the transcript levels of *kdpD* and *spa* of the two strains, and the results showed that the transcript level of *kdpD* in the *agr* mutant was also always much lower than that of the wild type under different K^+ conditions (see Fig. S2A in the supplemental material). However, the transcript level of *spa* in the *agr* mutant showed a fold increase of tens to hundreds compared with that in the wild type, suggesting that Agr acts as a main regulator of the *spa* transcription. We suggest that because the transcription of *kdpD* in the *agr* mutant could not be activated by the Agr system, the influence of Kdp on *spa* transcription was not obvious under this condition.

Collectively, we think that the pathogenesis of *S. aureus* is determined by the coordinated gene regulation in response to the self-secreting signal and other specific stimuli in the environment and that KdpDE, as a two-component system that can respond to both, is the partial embodiment of this coordination (Fig. 8).

ACKNOWLEDGMENTS

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