The Trk Potassium Transporter Is Required for RsmB-Mediated Activation ofVirulence in the Phytopathogen Pectobacterium wasabiae

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ABSTRACT
Pectobacterium wasabiae (previously known as Erwinia carotovora) is an important plant pathogen that regulates the production of plant cell wall-degrading enzymes through an N-acyl homoserine lactone-based quorum sensing system and through the GacS/GacA two-component system (also known as ExpS/ExpA). At high cell density, activation of GacS/GacA induces the expression of RsmB, a noncoding RNA that is essential for the activation of virulence in this bacterium. A genetic screen to identify regulators of RsmB revealed that mutants defective in components of a putative Trk potassium transporter (trkH and trkA) had decreased rsmB expression. Further analysis of these mutants showed that changes in potassium concentration influenced rsmB expression and consequent tissue damage in potato tubers and that this regulation required an intact Trk system. Regulation of rsmB expression by potassium via the Trk system occurred even in the absence of the GacS/GacA system, demonstrating that these systems act independently and are both required for full activation of RsmB and for the downstream induction of virulence in potato infection assays. Overall, our results identified potassium as an essential environmental factor regulating the Rsm system, and the consequent induction of virulence, in the plant pathogen P. wasabiae.

IMPORTANCE
Crop losses from bacterial diseases caused by pectolytic bacteria are a major problem in agriculture. By studying the regulatory pathways involved in controlling the expression of plant cell wall-degrading enzymes in Pectobacterium wasabiae, we showed that the Trk potassium transport system plays an important role in the regulation of these pathways. The data presented further identify potassium as an important environmental factor in the regulation of virulence in this plant pathogen. We showed that a reduction in virulence can be achieved by increasing the extracellular concentration of potassium. Therefore, this work highlights how elucidation of the mechanisms involved in regulating virulence can lead to the identification of environmental factors that can influence the outcome of infection.
the expression of csrB (a functional rsmB homologue) in *E. coli* or *Salmonella enterica* subsp. *enterica* serovar Typhimurium at low pH, but the stimuli that activate the system at neutral pH have not been identified (12, 13).

To identify the physiological stimuli involved in the activation of the Gac/Rsm system and to understand how this system regulates virulence in the well-characterized *P. wasabiae* strain SCC3193, we performed a genetic screen to identify mutants affected in the expression of rsmB, the main target of the Gac/Rsm system in this bacterium. The screen revealed 5 mutants defective in genes coding for components of a putative homologue of the *E. coli* Trk potassium uptake system (10). Therefore, we investigated the importance of potassium and the Trk system to the regulation of RsmB. Our results demonstrated that extracellular potassium is a critical environmental factor influencing virulence in *Pectobacterium* spp.

**MATERIALS AND METHODS**

**Bacterial strains, plasmids, and media.** All of the strains and plasmids used in this study are listed in Table S1 in the supplemental material. *P. wasabiae* strains were grown from the wild-type (WT) strain SCC3193 (14). Strains were grown at 30°C with aeration in Luria-Bertani (LB) broth, M9 minimal medium (MM), or minimal potassium medium (15) with 0.4% (wt/vol) glucose. When specified, the medium was supplemented with 0.4% polygalacturonic acid (PGA; Sigma P3850) to induce the expression of PCWDEs. Where mentioned, KCl was added at various final concentrations. Antibiotics were used at the following concentrations: 100 mg liter⁻¹ ampicillin (Amp), 50 mg liter⁻¹ streptomycin (Str), 50 mg liter⁻¹ kanamycin (Kan), 50 mg liter⁻¹ spectinomycin (Spec), and 25 mg liter⁻¹ chloramphenicol (Cm). To assess bacterial growth, the optical density at 600 nm (OD₆₀₀) was determined by measuring absorbance at 600 nm in the Bioscreen C reader system (multiplate reader; Oy Growth Curves Ab Ltd.).

**Genetic and molecular techniques.** Primers used in this study are listed in Table S2 in the supplemental material. The plasmid carrying the rsmB promoter fused to the green fluorescence protein (GFP) (P_rsmB::gfp) was constructed by using a modified version of the promoterless::gfp vector pCMW1 (16). First, the chloramphenicol resistance gene (cm) was amplified by PCR from pKD3 (17) using P0276-pKD3/4(BamH) and P0277-pKD3/4(BamH) primers and introduced into pCMW1, yielding PRS59. Next a 392-bp fragment containing the promoter region of *rsmB* was amplified by PCR using the P0528-rsmB(SphI) and P0529-rsmB-(SalI) primers and was ligated into PRS59, yielding pRSV206. Deletion mutants were constructed by chromosomal gene replacement with an antibiotic marker using the λ Red recombinase system (17, 18). The DNA region of gacA, including approximately 500 bp upstream and 500 bp downstream of the gene, was amplified by PCR and cloned into pUC18 (19) using SalI and SacI. This construct, containing the gacA gene and its flanking regions, was divergently amplified by PCR using primers to introduce an XhoI restriction site in the 5′ and 3′ regions of the gene. The streptomycin resistance gene (str) was amplified from pNG101 (20) with primers containing the XhoI restriction site. The amplified fragment containing the gene for streptomycin resistance was digested with XhoI and was introduced into the XhoI-digested PCR fragment. The final construct contained the antibiotic resistance marker flanked by the upstream and downstream regions of gacA. The 500-bp–str–500-bp fragment was amplified by PCR, and approximately 3 ng of DNA was electroporated into a strain expressing the λ Red recombinase system from pKD46 to allow recombination (17). To construct the trkA⁻ and *trkH⁻* complementation plasmids, a 400-bp–trkA–400-bp fragment and a 400-bp *trkH* fragment were amplified from SCC3193 and were cloned into pOM18 using SalI and SacI or PstI and XbaI, respectively. pOM18 was constructed by amplifying the multiple cloning site of pUC18 and by cloning it into the BglII and SacI or PstI and XbaI, respectively. pOM18 was constructed by amplifying the multiple cloning site of pUC18 and by cloning it into the BglII

**Analysis of expression of P_rsmB::gfp.** Analysis of *rsmB* expression was performed by flow cytometry. Mutant and WT strains of *P. wasabiae* SCC3193 containing the *rsmB* reporter fusion were grown overnight in MM supplemented with Km and Kan and were then inoculated into fresh medium in multiwell plates at a starting OD₆₀₀ of 0.02. Aliquots were collected at an OD₆₀₀ of 0.3 or 0.4, as indicated in the figure legends. Cells were diluted 1:10 into phosphate-buffered saline (PBS), and the fluorescence intensity of GFP per cell was assessed immediately in the flow cytometer (LSRFortessa; BD). Results were analyzed with Flowing Software v2.5.1. A minimum of 5,000 GFP-positive single cells were acquired per sample and were analyzed for their *rsmB* expression. *rsmB* expression of the mutants with the P_rsmB::gfp fusion is reported as the median GFP expression of the GFP-positive single cells in arbitrary units (a.u.).

**Pel activity assay.** Overnight cultures of bacterial strains were diluted to an OD₆₀₀ of 0.02 in fresh LB supplemented with PGA in multiwell plates. Bacteria were subcultured to an OD₆₀₀ of 0.4, at which time cell-free supernatants were harvested by centrifugation. Extracellular Pel activity was measured using the previously described TBA colorimetric method (22). Briefly, supernatants were incubated for 3 h with the substrate mixture at 37°C. The reaction was stopped by acetic acid, TBA (Sigma T5500) was added, and the reaction mixture was boiled for 1 h. The pink coloration was measured at 548 nm using a multilabel counter (Victor²; Perkin-Elmer). The absorbance values obtained at 548 nm were divided by the OD₆₀₀ of the corresponding cultures.

**Potassium Regulates RsmB-Mediated Virulence Activation**

**P. wasabiae virulence assay.** Virulence was determined using a modified protocol to assess maceration of potato tubers (25). Potatoes were washed and surface sterilized by soaking for 10 min in 10% bleach followed by 10 min in 70% ethanol. To prepare bacteria for inoculation, overnight cultures were washed twice in PBS, which contained a total of 4.5 mM potassium, or in PBS with various concentrations of KCl as indicated. Thirty microliters of cells at an OD₆₀₀ of 0.05 was inoculated into previously punctured potatoes and incubated at 28°C with relative humidity above 90% for 48 h. After incubation, potatoes were sliced and macerated tissue was collected and weighed. To quantify the inoculum, serial dilutions of this bacterial suspension were performed in PBS and were plated, and bacterial growth was quantified by the number of CFU present in 30 μl.

**Statistical analysis.** Data were analyzed using Graphpad Prism 6 software and R program v3.0.2. The Mann-Whitney test was performed to
which is involved in potassium uptake in E. coli (26). As shown in Fig. 1, all five mutants had an approximately 2-fold reduction in the expression of the *rsmb*-gfp fusion compared to WT levels. Three of those mutants (RSV108, RSV141, and RSV154) had transposon insertions in the WSS_4400 gene, whose product is annotated as the potassium uptake protein TrkH. This protein has 89% sequence identity with one of the potassium uptake channel proteins (TrkH) from *E. coli*. This bacterium has two redundant Trk channel proteins, TrkH and TrkG; disruption of the two proteins is required for the abolishment of potassium uptake by the Trk system. In contrast, *P. wasabiae* has only one putative Trk channel protein, similar to most other bacteria containing the Trk system. In contrast, other two mutants (RSV124 and RSV236) had transposon insertions in the WSS_4132 gene, whose product is annotated as the potassium uptake protein TrkA. This protein shares 85% sequence identity with the TrkA protein from *E. coli*, a regulator of the Trk potassium uptake system. In *E. coli*, disruption of *trkA* results in a lower rate of potassium uptake by the Trk system (26). To investigate the role of the Trk system in the regulation of RsmB, one mutant with a transposon insertion in the *trkH* gene (RSV141) and another with an insertion in the *trkA* gene (RSV236) were selected for further characterization.

We tested the *trkH::Tn5* and *trkA::Tn5* mutants for complementation in trans with the *trkH* or *trkA* gene, respectively, for *rsmb* expression and Pel activity (Fig. 2). Due to the growth defect for both *trkH* and *trkA* mutant strains (see Fig. S1 in the supplemental material), cells were collected and analyzed at the same cell density (OD<sub>600</sub> = 0.4). *P. wasabiae* expression was restored to WT levels in *trkH* and *trkA* mutants when the respective genes were expressed under the control of their own promoter, but it remained low in mutants carrying the empty vector (Fig. 2A). When tested for their ability to produce PCWDEs, these mutants had a >2-fold decrease in extracellular Pel activity, which was restored to WT levels upon expression of either *trkH* or *trkA* in trans (Fig. 2B). These data showed that the reduction in *rsmb* expression and the consequent effect upon the downstream induction of PCWDEs observed were a consequence of the disruption of *trkH* and *trkA* by the transposon insertion, and they suggested that the Trk system could affect regulation of virulence in *P. wasabiae*. 

**RESULTS**

Mutants with mutations in the Trk potassium uptake system are impaired in *rsmb* expression and production of PCWDEs. To identify regulators of the Gac/Rsm system we generated and screened a library of 15,126 *P. wasabiae* SCC3193 transposon mutants for changes in Pel activity. We obtained 58 mutants with reduced Pel activity compared to that of the WT strain. *rsmb* expression levels were then tested in these mutants following the introduction of a plasmid-encoded *rsmb* promoter-GFP fusion construct (pRSV206-P<sub>rsmb</sub>-gfp) into the mutants. Of these, 29 independent mutants showed <75% *rsmb* expression in comparison to the WT strain (see Table S3 in the supplemental material). From those 29 mutants, five were found to have transposon insertions in genes annotated as components of a putative Trk system, which is involved in potassium uptake in *E. coli* (26). As shown in Fig. 1, expression of a *P. wasabiae* SCC3193 transposon mutant strains was measured by fluorescence flow cytometry of cells grown for 24 h in MM. Error bars represent standard deviations (SD); n = 3.

Effect ofPotassium Uptake Mutations on *rsmb* Expression and Pel Activity

**FIG 1** Trk mutants have low *rsmb* expression. Expression of *P. wasabiae* SCC3193 transposon mutant strains was measured by fluorescence flow cytometry of cells grown for 24 h in MM. Error bars represent standard deviations (SD); n = 3.

**FIG 2** Complementation of the *trkH* and *trkA* mutants. (A) Expression of a *P. wasabiae* SCC3193 transposon mutant carrying either the pOM18 empty vector or the vector expressing *trkH*, p(*trkH*)<sup>gfp</sup>, and the *trkA::Tn5* mutant carrying either the pOM18 empty vector or the vector expressing *trkA*, p(*trkA*)<sup>gfp</sup>. Fluorescence was measured in cells collected from cultures grown to an OD<sub>600</sub> of 0.4 in MM. (B) Pel activity was measured in cell-free supernatants from cultures of the bacterial strains indicated above grown in LB supplemented with PGA at an OD<sub>600</sub> of 0.4. Error bars represent standard errors of the means (SEM); n = 6.

*P. wasabiae* strains were grown for 24 h in MM. Determination of statistical significance, and P values were adjusted using the Holm-Bonferroni correction for multiple comparisons. An adjusted P value of <0.05 was used as the cutoff for statistical significance.
wasabiae. We therefore investigated the ability of the selected trk mutants to cause tissue damage in potatoes. As shown in Fig. 3, the mutant strains were impaired in virulence, showing an approximately 40% reduction in the mass of macerated potato tissue compared to that in tubers infected with WT bacteria.

Extracellular potassium levels influence rsmB expression. The requirement for a functional Trk potassium uptake system for full activation of rsmB expression indicated that induction of virulence might in fact be regulated by potassium. Therefore, we analyzed rsmB expression levels in WT P. wasabiae grown in different concentrations of potassium. Bacteria grown in 0.25, 2.5, or 25 mM potassium induced rsmB expression by responding positively to increasing concentrations of potassium. However, in cells grown in 250 mM potassium, induction was as low as that observed in cells with 0.25 mM potassium (Fig. 4A).

To verify that this regulation was dependent upon the Trk system, we analyzed rsmB expression in the trkA and trkH mutants cultured in the same range of potassium concentrations. As neither mutant grew in the lowest concentration tested (0.25 mM) (Fig. 4B), it was not possible to determine the level of rsmB expression in these mutants under these conditions. Importantly, this lack of growth shows that the Trk system is required for the growth of P. wasabiae in low concentrations of potassium, supporting the predicted function of these genes in potassium uptake. Furthermore, as shown in Fig. 4A, the two mutants yielded distinct phenotypes with regard to potassium-dependent regulation of rsmB expression, which is in agreement with the putative functions assigned by similarity to the Trk system in E. coli. In the trkH mutant, where based on knowledge from E. coli, we would expect Trk-dependent potassium uptake to be absent, rsmB expression was low when it was grown in the tested potassium concentrations. This result showed that no potassium-dependent regulation of rsmB expression was observed upon disruption of the putative Trk potassium channel protein. In contrast, trkA mutants retained some ability to induce rsmB expression in response to changes in potassium availability, though 100-fold higher concentrations (250 mM) were needed to reach the level of activation seen in the WT cultured with 2.5 mM potassium. This suggests that Trk-dependent potassium uptake is less efficient in P. wasabiae trkA mutants than in the WT strain, which is similar to what was reported for E. coli (26).

Previous studies of the Gac/Rsm system in pectobacteria and other bacterial species have established GacA as the key response regulator responsible for activating the expression of rsmB. To verify whether potassium-dependent regulation of RsmB occurs via this two-component system, we measured rsmB expression in mutants lacking gacA and determined the effect of potassium in the absence of this response regulator. In line with current literature, disruption of gacA resulted in reduced expression of rsmB compared to that of WT bacteria (Fig. 4A). Nonetheless, rsmB expression in the gacA mutant can still be induced with intermediate levels of potassium, which is similar to what was observed with WT bacteria. Furthermore, deletion of gacA in a trkA mutant background (gacA trkA::Tn5 double mutant) resulted in the same potassium-dependent activation of rsmB expression observed for the trkA single mutant. Therefore, potassium-dependent regulation of RsmB is not mediated by the GacS/GacA signal transduction pathway.

Overall, our results demonstrate that rsmB expression is regulated by extracellular levels of potassium and that this regulation requires the Trk system but is independent of the GacS/GacA system.

Virulence in P. wasabiae is regulated by the extracellular concentration of potassium. Next, we tested whether the potassium-dependent effect on rsmB expression observed in the in vitro studies described above had consequences for virulence in vivo using the maceration of potato tubers. We tested whether supplementation of the inoculum with different concentrations of potassium affected the outcome of infection. In agreement with the in vitro results for rsmB expression, high concentrations of extracellular potassium (250 mM) had an inhibitory effect on the virulence of WT bacteria; less tissue maceration was observed in potatoes inoculated with cells resuspended in buffer with 250 mM potassium than in potatoes infected with bacteria prepared in buffer supplemented with 4.5 mM potassium or no potassium (squares in Fig. 5). As for the mutants, the virulence of the trkH mutant was low at all potassium concentrations tested while that of the trkA mutant was also low at low potassium concentrations, though supplementation of high concentrations of potassium (250 mM) resulted in an increase in virulence to near WT levels (Fig. 5). The results for the trkH and trkA mutant strains are in full agreement with their respective phenotypes for RsmB induction obtained in vitro (Fig. 4A).

Nonetheless, no increase in the virulence of the WT bacteria occurred when potatoes were inoculated with cells prepared in 4.5 mM potassium compared to the virulence in those prepared without the addition of potassium. However, bacterial inocula were grown in LB (a medium that contains approximately 8 mM potassium [15]) prior to infections, and because our data showed that concentrations higher than 2.5 mM were sufficient to induce rsmB expression in WT bacteria in vitro (Fig. 4), we reasoned that such induction might sustain rsmB expression, and therefore virulence, during infection. To address this possibility and to determine if extracellular potassium at the site of infection is essential
to induce virulence, WT *P. wasabiae* was grown under noninducing conditions in minimal potassium medium supplemented with low potassium (0.25 mM) and was then resuspended in buffer with different potassium concentrations before inoculation into potatoes. The *trkH* and *trkA* mutant strains were not tested under these conditions due to their lack of growth in low concentrations of potassium (Fig. 4B). As shown in Fig. 6, WT bacteria grown in 0.25 mM caused some tissue maceration even when no potassium was added to the inoculum, but importantly, an increase in virulence was observed with the addition of 4.5 mM potassium (Fig. 6). These results demonstrate that the addition of potassium at the time of infection induced virulence in cells that were grown under noninducing conditions. Again, high concentrations of potassium (250 mM) inhibited the induction of virulence; the mass of macerated tissue was as low under these conditions as when no potassium was added to the inoculum (Fig. 6).

**DISCUSSION**

The small RNA RsmB has a major role in the signaling network controlling virulence in *P. wasabiae* by preventing RsmA-medi-
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FIG 5 Regulation of virulence by extracellular potassium concentration. The virulence of WT bacteria and trkH::Tn5 and trkA::Tn5 mutants was measured by quantification of the mass of macerated tissue 48 h after inoculation of potato tubers. Cells were cultured overnight in LB (approximately 8 mM potassium) and were harvested and resuspended in potassium-free PBS (WT only) or PBS supplemented with a final concentration of either 4.5 mM or 250 mM potassium. Potatoes were inoculated with approximately 3 × 10^6 cells of the respective strain at the different potassium concentrations. Error bars represent SEM; n = 6; **, P < 0.01; ns, not significant. This is a representative experiment from three independent experiments.

FIG 6 Induction of virulence by extracellular potassium. Virulence of WT bacteria was measured by determining the mass of damaged tissue 48 h after the infection of potato tubers. Potatoes were inoculated with approximately 3 × 10^6 cells of the respective strain grown overnight in minimal potassium medium supplemented with a final potassium concentration of 0.25 mM and resuspended in potassium-free PBS (0 mM), in PBS (4.5 mM potassium), or in PBS supplemented with potassium to a final concentration of 250 mM. Error bars represent SEM; n = 6; **, P < 0.01. This is a representative experiment from two independent experiments.

ated repression of PCWDE expression. Consequently, activation of rsmB transcription is essential for the production of these enzymes, and thus unraveling the signals and mechanisms involved in this regulation is a key step in understanding the environmental factors influencing virulence in Pectobacterium spp.

In a screen for regulators of RsmB, we isolated five mutants disrupted in the genes involved in the putative Trk potassium uptake system (trkH and trkA). We demonstrated that these mutants presented a reduced rsmB expression and that the Trk system is important for production of PCWDEs. These results led us to investigate the role of potassium in the expression of rsmB and virulence. We showed that intermediate concentrations of potassium (2.5 to 25 mM) were required to induce RsmB, but high potassium concentrations (250 mM) inhibited the expression of this regulatory RNA. The conclusion that potassium and Trk participate in RsmB regulation is further supported by the identification of another mutant isolated in our screen, which had the transposon inserted in the third gene of the annotated sapABCDF operon (RSV238) (see Table S3 in the supplemental material). This operon, in particular sapD, has been implicated in potassium transport via the Trk system in E. coli, as sapD mutants present no potassium uptake by the Trk system (27). It is thus possible that a transposon insertion in sapC also affects the Trk system.

Our analysis of the trk mutants showed that in P. wasabiae the Trk potassium system functions similarly to the E. coli Trk system. However, although in E. coli the trk system seems to be important mainly at intermediate concentrations of potassium, in P. wasabiae this system appeared to be relevant in a broader range of potassium concentrations. In E. coli, an inducible high-affinity potassium system, the Kdp system, is the major system responsible for potassium uptake at concentrations lower than 5 mM (reviewed in reference 28), but in P. wasabiae the trk mutants had a strong growth defect at a low potassium concentration (2.5 mM) and did not even grow at 0.25 mM. These results showed that the Trk system is important at low potassium concentrations. This, together with the fact that we could not find any kdp-like gene in the genome of P. wasabiae, indicates that P. wasabiae, in contrast to E. coli, may lack a high-affinity potassium transporter and may rely solely on Trk at low potassium concentrations.

Our results showed that the Trk system regulated rsmB expression and provided strong support for the hypothesis that extracellular levels of potassium are important for virulence in P. wasabiae. Accordingly, we observed that extracellular potassium was required to fully induce maceration of plant tissue in WT bacteria and that this induction required an intact Trk system. Moreover, we observed that virulence was inhibited at high extracellular concentrations of potassium (250 mM). Importantly, this inhibition of virulence in WT bacteria at a concentration of 250 mM potassium is unlikely to be a consequence of growth inhibition because, at this potassium concentration in vitro, WT bacteria still grow better than the trkA mutant. In addition, despite its growth defect, the trkA mutant can cause almost as much tissue maceration with 250 mM potassium as the maximal levels observed with the WT strain (Fig. 4E and 5; see also Table S4 in the supplemental material). We also determined that the supplementation of a high concentration of potassium had no effect on the viability of the inoculum applied to the potato tubers in any of strains tested as shown in Fig. S2 in the supplemental material.

The mechanism by which the extracellular levels of potassium regulate rsmB expression via the Trk system is still unclear. As rsmB transcription is activated by the GacS/GacA system, we investigated whether the observed effects of potassium were also linked with or dependent upon the function of this two-component system. Although disruption of the gacA gene decreased the extent to which rsmB expression was induced, surprisingly potassium-dependent and Trk-dependent regulation was still observed in a gacA mutant. Two additional regulators in Pectobacterium spp., KdgR and RsmC, have been shown to repress RsmB expression (7, 29). However, disruption of these regulators had no effect on potassium-dependent regulation of rsmB expression (data not shown). These results provide evidence for additional players in the regulation of RsmB. The identity of such regulators could
come from a genetic approach to isolate mutants that no longer respond to changes in extracellular levels of potassium. For example, it is possible that an additional two-component system is involved in such regulation. In fact, some transport systems have been associated with the activation of two-component systems (reviewed in reference 30). In E. coli, the DcuS/DcuR two-component system, which is activated by C₄-carboxylates in the periplasm, is also regulated by the DcuB antiporter, which takes up C₄-carboxylates. It has been proposed that, in the absence of C₄-carboxylates, protein-protein interactions between DcuB and DcuS repress the autophosphorylation activity of the DcuS sensor kinase. Upon transport of these compounds, this repression is absent presumably because DcuB releases DcuS, which can then be activated by the periplasmic levels of C₄-dicarboxylates (31). A similar interaction might happen between the Trk transport proteins and an unknown two-component system to regulate rsmB. Alternatively, it is possible that the observed regulation of rsmB is not responding to potassium flux through the Trk system but that it is instead sensitive to changes in intracellular potassium concentrations, as has been shown for the induction of biofilm formation in Bacillus subtilis as a response to potassium leakage (32). In this case, it was proposed that in the presence of natural compounds that cause potassium leakage, the membrane kinase KinC responds to transient decreases in cytoplasmic potassium concentrations, activating a phosphorylation cascade that results in induction of exopolysaccharide production and biofilm formation.

The Trk system is widespread among bacteria, and it is the potassium transporter found in the largest number of species. This transport system is crucial for many of the intracellular functions of potassium, such as the maintenance of cell turgor pressure, the regulation of intracellular pH, adaptation to osmotic stress, and the function of many cytoplasmic enzymes that require potassium (reviewed in reference 28), but importantly it has also been implicated in the regulation of virulence in diverse bacteria. Trk mutants of Vibrio vulnificus, Salmonella enterica subsp. enterica serovar Typhimurium, and Franciscella tularensis are impaired in virulence; however, the molecular mechanisms involved in such regulation have not been identified (15, 33, 34). As both V. vulnificus and S. enterica also use homologues of the Rsm system (Csr) to regulate virulence, it is conceivable that Trk regulation of virulence in these organisms might also take place through the regulatory RNAs of the Csr system. Therefore, it is possible that the mechanisms identified here are conserved among the other pathogens that have the Gac/Rsm system. As the Gac/Rsm system has been shown to modulate carbon fluxes (10, 11), we propose that for bacteria that use the Trk transporter to regulate components of the Gac/Rsm pathway cells, we might benefit from coupling the information obtained from perceiving changes in potassium concentrations with the information on the metabolic state of the cell to modulate functions that go beyond maintaining the physiological functions of potassium to control activities that contribute to host colonization. For instance, when short-chain fatty acids accumulate during fermentation, bacterial cells typically use potassium transporters to manipulate intracellular potassium levels to cope with changes in cytoplasmic pH and to control turgor pressure (reviewed in reference 35). It is interesting that the Gac/Rsm homologue system in E. coli has been shown to respond under certain conditions to these weak organic acids (12), and thus, it is tempting to speculate that the link between the regulation of potassium transport and the regulation of the Gac/Rsm system might be related to the need to adapt to the presence of short-chain fatty acids. The benefit of regulating virulence in response to changes in potassium concentrations might also be related to the environmental changes typically experienced by pathogens during host invasion. For example, plant pathogens, such as P. wasabiae, are typically found in the soil, where potassium concentrations are in the 10 to 100 µM range, contrary to the 100 mM concentration found in eukaryotic cells (36). Hence, upon arrival of the bacteria to a wounded host, the local increased potassium concentration might provide a cue for the activation of the production of PC-WDEs. The action of these enzymes will further disrupt the host cells with the consequent leakage of intracellular potassium, and the bacteria will have to adapt to the increasing potassium levels, which can ultimately reach the levels found inside the eukaryotic plant cells.

Crop losses resulting from bacterial diseases continue to cause significant agricultural and economic concerns. Understanding the regulatory networks responsible for the activation of virulence genes will help to define and improve control strategies that target this problem. The results presented here identify potassium as an important environmental factor in the regulation of virulence in the plant pathogen P. wasabiae and show that a reduction in WT virulence can be achieved by increasing the extracellular concentration of potassium (Fig. 5 and 6). Although additional work is required to fully characterize the molecular mechanisms behind the regulation of virulence by potassium via RsmB, our study highlights how potassium levels in the soil could affect the outcome of virulence in the plant pathogen P. wasabiae.

**ACKNOWLEDGMENTS**

We thank Joana Amaro for technical assistance and Jessica Thompson and Pol Nadal for helpful discussions. We are very grateful to Andres Mæ (Institute of Molecular and Cell Biology) for sharing protocols and strains and to Tapio Palva (University of Helsinki) for providing strains used in this study.

**FUNDING INFORMATION**

Howard Hughes Medical Institute (HHMI) provided funding to Rita S. Valente and Karina B. Xavier under grant number HHMI 55007436. Science and Technology Foundation, Ministry of Education and Science (Fundação para a Ciência e a Tecnologia) provided funding to Ruta S. Valente under grant number SFRH/BD/33570/2008. Science and Technology Foundation, Ministry of Education and Science (Fundação para a Ciência e a Tecnologia) provided funding to Karina B. Xavier under grant number PTDC/BIA-BCM/101585/2008.

The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

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