AMERICAN SOCIETY FOR MICROBIOLOGY | Applied and Environmental Microbiology®

PLANT MICROBIOLOGY

Virulence Factor Identification in the Banana Pathogen *Dickeya zeae* MS2

Luwen Feng,[a] Amy L. Schaefer,[b] Ming Hu,[c] Ruiyi Chen,[a] E. Peter Greenberg,[a,b] Jianuan Zhou[c]

[a]Guangdong Province Sociomicrobiology Basic Science and Frontier Technology Research Team & Integrative Microbiology Research Center, South China Agricultural University, Guangzhou, People's Republic of China

[b]Department of Microbiology, University of Washington, Seattle, Washington, USA

[c]Integrative Microbiology Research Centre, Guangdong Province Key Laboratory of Microbial Signals and Disease Control, South China Agricultural University, Guangzhou, People's Republic of China

ABSTRACT The phytopathogen *Dickeya zeae* MS2 is a particularly virulent agent of banana soft rot disease. To begin to understand this banana disease and to understand the role of quorum sensing and quorum-sensing-related regulatory elements in *D. zeae* MS2, we sequenced its genome and queried the sequence for genes encoding LuxR homologs. We identified a canonical LuxR-LuxI homolog pair similar to those in other members of the genus *Dickeya*. The quorum-sensing signal for this pair was *N*-3-oxo-hexanoyl-homoserine lactone, and the circuit affected motility, cell clumping, and production of the pigment indigoidine, but it did not affect infections of banana seedlings in our experiments. We also identified a *luxR* homolog linked to a gene annotated as encoding a proline iminopeptidase. Similar linked pairs have been associated with virulence in other plant pathogens. We show that mutants with deletions in the proline iminopeptidase gene are attenuated for virulence. Surprisingly, a mutant with a deletion in the gene encoding the LuxR homolog shows normal virulence.

IMPORTANCE *Dickeya zeae* is an emerging banana soft rot pathogen in China. We used genome sequencing and annotation to create an inventory of potential virulence factors and virulence gene regulators encoded in *Dickeya zeae* MS2, a particularly virulent strain. We created mutations in several genes and tested these mutants in a banana seedling infection model. A strain with a mutated proline iminopeptidase gene, homologs of which are important for disease in the *Xanthomonas* species phytopathogens, was attenuated for soft rot symptoms in our model. Understanding how the proline iminopeptidase functions as a virulence factor may lead to insights about how to control the disease, and it is of general importance as homologs of the proline iminopeptidase occur in dozens of plant-associated bacteria.

KEYWORDS genome sequence, LuxR homolog, iminopeptidase, quorum sensing

Members of the bacterial genus *Dickeya* cause serious soft rot diseases of crops, fruits, and ornamental plants. As a group, *Dickeya* species are considered one of the most important bacterial phytopathogens worldwide (1, 2). Most of the *Dickeya* species have a broad host range and can infect both dicotyledons and monocotyledons (1, 3). Characteristic *Dickeya* infections show invasion at the center or a portion of the rhizome, resulting in maceration and rotting (4). The plant growing point is destroyed, internal material decays, and vascular discoloration is obvious.

The past decade has seen the emergence of severe banana soft rot disease in Guangdong Province, China, and the causative agent has been identified as a variant of *Dickeya zeae* (1, 5), formerly known as *Erwinia chrysanthemi* pv. zeae (6). This bacterial species has been isolated from diseased pseudostem tissues of the banana *Musa* ABB.

Citation Feng L, Schaefer AL, Hu M, Chen R, Greenberg EP, Zhou J. 2019. Virulence factor identification in the banana pathogen *Dickeya zeae* MS2. Appl Environ Microbiol 85:e01611-19. https://doi.org/10.1128/AEM.01611-19.

Editor Gladys Alexandre, University of Tennessee at Knoxville

Copyright © 2019 American Society for Microbiology. All Rights Reserved.

Address correspondence to E. Peter Greenberg, epgreen@uw.edu, or Jianuan Zhou, jianuanzhou@scau.edu.cn.

Received 16 July 2019
Accepted 16 September 2019
Accepted manuscript posted online 20 September 2019
Published 14 November 2019

Little is known about the banana-disease-causing *D. zeae*, but the genomic sequence of one isolate (MS1) has been published previously (5). This isolate and a second isolate (MS2) have been surveyed for their ability to cause disease in a range of plants (1). Interestingly, *D. zeae* MS2, but not MS1, produces an antimicrobial agent (1).

In various *Dickeya* soft rot diseases, plant cell-wall-degrading enzymes (PCWDEs) (for a review, see reference 7), siderophores (for a review, see reference 8), type III secretion systems (9, 10), and the blue pigment indigoidine (11, 12) have been implicated as virulence factors. In *D. zeae* EC1, isolated from infected rice, a phytotoxin called zeamine has been identified, and this compound also has antibacterial and nematicidal activity (13, 14).

To better understand the banana pathogens and the potential involvement of quorum sensing or quorum-sensing-related transcription factors in virulence, we report here the sequence and annotation of the *D. zeae* MS2 genome. We further focus this report on LuxR homologs and genes known to be controlled by LuxR homologs in related bacteria. Like other members of the genus *Dickeya* (5, 15, 16), we found that strain MS2 possesses genes coding for an acyl-homoserine lactone (AHL) quorum-sensing circuit, that is, a gene coding for a LuxI homolog (an acyl-homoserine lactone synthase) linked to a gene coding for a LuxR homolog (a putative AHL signal receptor). By analogy to other members of the genus *Dickeya*, we call these genes *expI* and *expR* (15, 17, 18). We also identified a gene coding for a so-called orphan or solo LuxR homolog. This gene is linked to a gene coding for a putative proline iminopeptidase and, thus, falls into a group of *luxR* homologs with similar genomic contexts (19–21). These orphan LuxR homologs have been shown by others to activate virulence and proline iminopeptidase functions in several plant pathogens, and rather than responding to AHLs, they respond to plant-derived signals (22–24). We have studied one such system, the *pipR-pipA* system, in an endophyte found in the roots of cottonwood trees (25). By analogy to the endophyte system, we call the *D. zeae* MS2 genes *pipR* and *pipA*.

RESULTS AND DISCUSSION

Genome sequencing of *D. zeae* MS2 reveals potential virulence factors and virulence gene regulators, as well as a biosynthetic gene cluster required for antibacterial activity. *D. zeae* MS2 was isolated from diseased banana soft rot tissue on a plant growing in a banana field in southern China. A 16S rRNA gene sequence analysis showed that this bacterium is very closely related to the previously sequenced banana pathogen *D. zeae* MS1 (1). Hu et al. (1) demonstrated that MS2 infects a variety of mono- and dicot plants, including young banana pseudostems, as illustrated in Fig. 1. Seven days after needle inoculation, banana stems showed obvious discoloration, and the disease had spread to the leaves of the seedling whereas a mock-infected seedling showed no sign of disease (Fig. 1; see also Fig. S1 in the supplemental material). To learn more about this potent pathogen, we sequenced the *D. zeae* MS2 genome, which is 4,740,052 bp with a G+C content of 53.44%. We closed the genome and found 4,275 open reading frames (ORFs) with seven copies of the rRNA operon. The *D. zeae* MS2 genome contains genes coding for virulence factors described for other members of the genus, including *D. zeae* MS1 (5), *D. zeae* EC1 (16), *D. zeae* Ech586 (26), and *Dickeya dadantii* 3937 (27) (summarized in Table S1 in the supplemental material). Consistent with previous findings that MS2 produces high levels of PCWDE and extracellular protease activity (1), its genome contains a large number ($n = 14$) of plant cell-wall-degrading enzyme (PCWDE) genes, including those required for pectin and cellulose degradation. These PCWDEs are primarily responsible for the soft rot symptoms in plant hosts (for a review, see reference 7). The type I, II, III, IV, and VI secretion systems are conserved in MS2. These secretion systems have secretory functions important for virulence in other bacteria. Type I secretion is important for export of proteases (16, 28, 29), type II is important for export of pectinases (16, 30, 31), and type III and type VI secretion systems deliver effector proteins to host cells (32–37).

It is often the case that secondary metabolites serve as virulence factors (38, 39). Therefore, we performed an antiSMASH 5.0 analysis (https://antismash

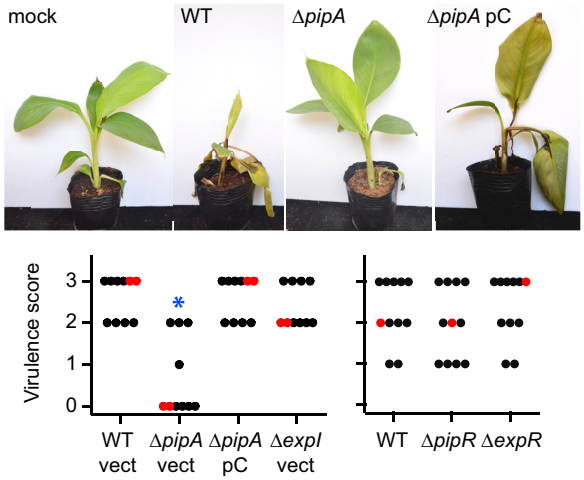

FIG 1 Virulence of *D. zeae* MS2 wild-type and mutant strains. (Top) Photographs of representative infected plants. (Bottom) Virulence scores. For each strain, 10 or 11 banana seedlings with 3 or 4 leaves were infected, and disease was scored 7 days later (see Materials and Methods and Fig. S1 in the supplemental material for details). The red dots indicate the median virulence scores. The blue asterisk indicates that the virulence score distributions between the wild type (WT) and the *pipA* mutant (Δ*pipA*) differed significantly (Mann-Whitney $U = 6$; $n_1 = n_2 = 10$; $P < 0.01$, two-tailed). For Δ*pipA* pC, the pC indicates the infecting bacteria carried the *pipA*-complementing plasmid pCpipA. Bacteria carrying the plasmid control vector pBBR1MCS-5 (vect).

.secondarymetabolites.org) (40) to identify secondary metabolite biosynthetic gene clusters (BGCs) in the MS2 genome. This program identified genes predicted to encode achromobactin and chrysobactin siderophores (8) as well as the blue pigment indigoidine, which is hypothesized to help phytopathogens overcome plant-generated oxidative stress during the host defense response (11, 12). The analysis also confirmed that MS2 lacks biosynthesis genes for the antibacterial and phytotoxic zeamines produced by *D. zeae* EC1 (1, 14) and the antibiotic carbapenem produced by some strains of the related *Pectinobacterium* sp. (41), even though MS2 possesses antibiotic activity against *Escherichia coli* and several fungal strains (1). Interestingly, antiSMASH identified a biosynthetic gene cluster (C1030_04995 to C1030_05185; see Table S1) with sequence similarity to nonribosomal peptide synthetase and bacteriocin clusters. This gene cluster has been found only in the *D. zeae* MS2 genome and in the genomes of two other *Dickeya* sp. (*Dickeya paradisiaca* NCPPB2511 and *D. dadantii* Ech703). We asked whether this cluster was responsible for the previously reported antimicrobial activity (1) by constructing a mutation in the nonribosomal peptide synthase gene. The mutated gene (C1030_05075) codes for a polypeptide missing the putative amino acid adenylation domain (amino acids 674 to 1051). While supernatant fluid from wild-type cultures inhibited growth of *E. coli*, culture fluid from the mutant did not (Fig. 2). Inhibition of *E. coli* by culture fluid was restored in the mutant by complementation with a wild-type peptide synthase gene (Fig. 2).

One might anticipate that coordinating expression of genes coding for the large arsenal of potential virulence factors could involve a number of regulatory proteins. Furthermore, many of the factors that we described are excreted or secreted, and it is common for such factors to be controlled by quorum sensing (42). As mentioned previously, we identified genes coding for homologs of the LuxR quorum-sensing transcriptional activator: *expR*, which is linked to the quorum-sensing signal synthase gene *expI*, and *pipR*, which is linked to *pipA*, a gene coding for a putative proline aminopeptidase (Fig. 3).

The influence of *D. zeae* MS2 *expR* and *expI* mutations on phenotypes regulated by quorum sensing in related bacteria. The *D. zeae* MS2 ExpR and ExpI show 99% amino acid sequence identity to ExpR and ExpI in *D. zeae* EC1. The EC1 AHL is 3OC6-HSL, and in this isolate, ExpR and ExpI control motility, cell aggregation, and, to

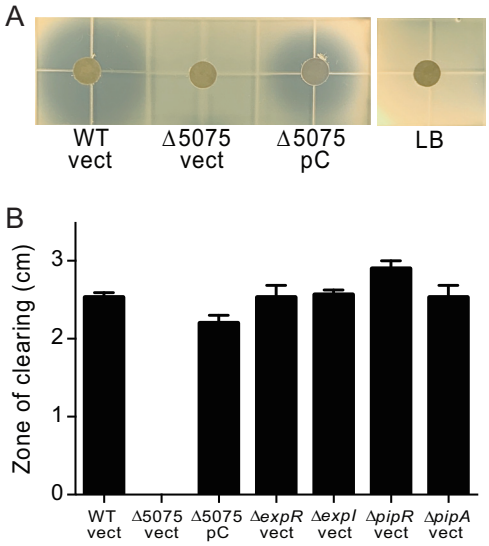

FIG 2 Influence of *D. zeae* MS2 and MS2 mutant culture fluid on growth of *E. coli*. (A) *E. coli* agarose overlay assays (Materials and Methods) with culture fluid from wild-type strain MS2 with the plasmid backbone pBBR1MCS-5 (vect), a strain with a mutation in the nonribosomal peptide synthase gene C1030_05075 (Δ5075) containing either the pBBR1MCS-5 plasmid (vect) or a plasmid containing a copy of C1030_05075 (pC), or fresh LB plus gentamicin (LB). Confluent *E. coli* growth in the agarose appears as a whitish color, while the zones of growth inhibition are a gray-blue color. (B) Sizes of the clearing zones around paper disks saturated with cell-free culture fluid from the indicated strains harboring either pBBR1MCS-5 vector (vect) or pC5075 complementation plasmid (pC). Results are means of three experiments, and error bars are standard deviations.

a minor degree, starch degradation in experiments with potato slices (3). A quantitative radioactivity assay (43) showed a major high-pressure liquid chromatography (HPLC) peak with a retention time matching that of 3OC6-HSL (Fig. 4A). This peak (fractions 19 to 21) contained greater than 50% of the total radioactive carbon in the Fig. 4A peaks. There were also two minor peaks of radioactivity. The radioactivity in fractions 23 and 24 constituted about 24% of the total, and the identity of the compound in this peak remains unidentified. The radioactivity in fractions 33 and 34 constituted about 20% of the total, and this peak

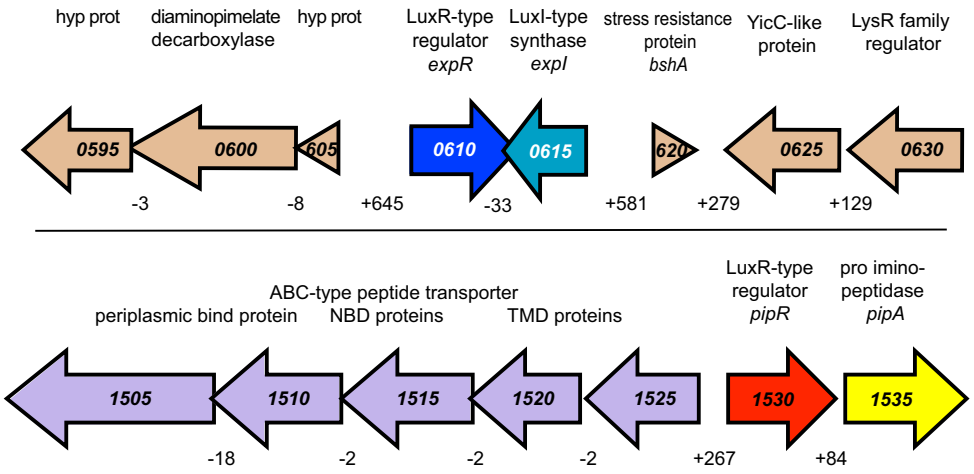

FIG 3 *D. zeae* MS2 genomic regions surrounding the *luxR* homologs *expR* and *pipR*. (Top) The region surrounding *expR* (blue) and *expI* (teal). (Bottom) The region surrounding *pipR* (red). The context of genes surrounding *pipR* is similar to that of other bacteria (25). This includes a flanking gene annotated as a proline iminopeptidase gene (yellow, *pipA*) and genes coding for a transport system (purple). Positive numbers below intergenic regions indicate the number of bases between open reading frames. Negative numbers indicate that the open reading frames overlap. All gene numbers have the prefix "C1030_0."

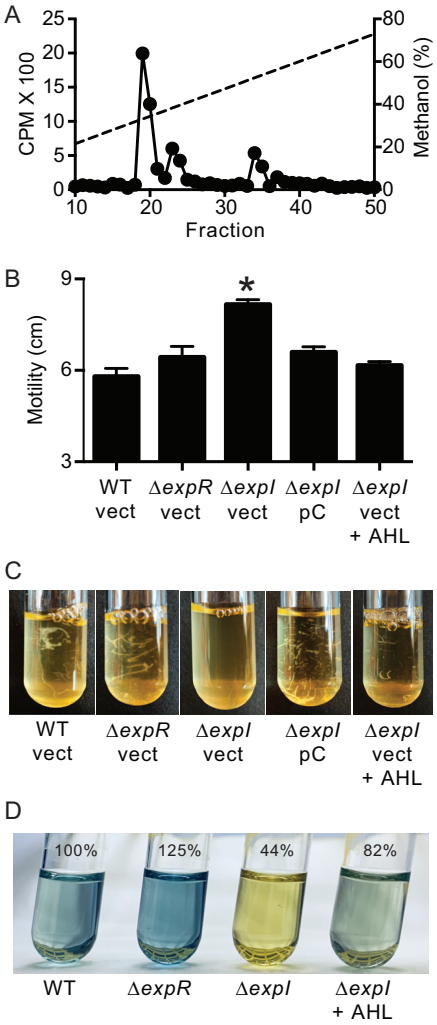

FIG 4 The major AHL produced by *D. zeae* MS2 and effects of *expR* and *expI* mutations on motility, aggregation, and indigoidine production. (A) HPLC profiles of ^{14}C-AHLs synthesized by *D. zeae* MS2. The *x* axis indicates the fraction numbers collected over a 10 to 100% methanol-in-water gradient. The left *y* axis denotes the counts per minute (CPM) of radiolabel in each fraction (black circles), and the right *y* axis indicates the methanol concentration of the HPLC run (dashed line). (B) Motility on 0.3% agar plates. Data are means of three replicates, and the error bars are the standard deviations. The black asterisk indicates that the difference between the wild type (WT) and Δ*expI* mutant is statistically significant ($P < 0.01$) using a two-tailed *t* test. (C) Cellular aggregation. (D) Indigoidine production. The amount of indigoidine produced relative to the wild type is given as a percent (means of three replicates). Strains are *D. zeae* MS2 wild type (WT) and Δ*expR* and Δ*expI* mutants; where indicated, strains harbored the pBBR1MCS-5 vector control (vect) or pCexpI (pC) or were grown with 2 μM 3OC6-HSL (AHL).

matches the retention time of 3OC8-HSL. These results indicate that like other *Dickeya* spp., MS2 produces 3OC6-HSL and lesser amounts of 3OC8-HSL (3, 18).

We then constructed ExpR and ExpI mutants. Growth of the mutants in lysogeny broth (LB) was indistinguishable from that of the wild type (see Fig. S2 in the supplemental material). By using a 3OC6-HSL bioassay, we estimate that wild-type MS2 produced the equivalent of about 1.5 μM 3OC6-HSL. We did not detect 3OC6-HSL in cultures of the ExpI mutant (limit of detection, 5 nM), and the ExpR mutant produced about 350 nM 3OC6-HSL. Similar to strain EC1, the MS2 ExpI mutant exhibited a small increase in motility (Fig. 4B) and a decrease in cell aggregation in the early logarithmic phase (Fig. 4C). Both phenotypes were recovered by complementing the mutation with an *expI* plasmid or by addition of 3OC6-HSL to the culture medium (Fig. 4). We also noticed that the indigoidine biosynthetic cluster and the *expRI* genes were located within a 35-kb region of the chromosome. Often *luxR* homologs are located near

biosynthetic gene clusters under their regulatory control (44); thus, we extracted indigoidine from the quorum sensing mutants and found that the AHL synthase mutant, but not the receptor mutant, produced lower levels of blue pigment. Pigment production was restored by exogenous addition of 3OC6-HSL (Fig. 4D). This AHL effect is modest (about 2-fold), perhaps because there are multiple regulatory inputs into indigoidine production. In other *Dickeya* species, indigoidine production is influenced by carbon source, oxidative stress, and the PecS global repressor (11).

Our results are consistent with the notion that ExpR functions as a repressor of motility, aggregation, and pigment production as is the case for *D. dadantii* 3937 (45, 46), where, in the absence of AHL, the LuxR homolog binds to operator DNA and blocks transcription. When bound to the cognate AHL, the transcription factor is released from the operator and transcription commences.

We found that both the ExpR and ExpI mutants remained fully virulent in syringe-needle inoculations of banana pseudostems (Fig. 1). Also, neither mutant displayed any reduction in PCWDE production (see Fig. S3 in the supplemental material) or *E. coli* killing (Fig. 2). The ExpR-ExpI quorum sensing system is conserved in nearly all sequenced *Dickeya* (46 of 49) and *Pectobacterium* (57 of 58) strains and has been found to be important in infection and/or plant macerating enzyme production in nearly all strains tested (18), with the exception of *D. dadantii* 3937 and *D. zeae* MS2. Whether the lack of AHL control in banana virulence is a general feature of the *Dickeya* banana pathogens unique to MS2 or perhaps an experimental limitation (needle inoculation of the plant, which may not require normal motility) requires further experiments. As AHL-quorum-sensing systems have been targeted for infection control in several pectinolytic phytopathogens (47, 48), it seems important to investigate such questions.

***Dickeya zeae* MS2 possesses a predicted plant-responsive *luxR* homolog (*pipR*) adjacent to a *pipA* homolog, but this regulator is not required for plant virulence.** As described above, *D. zeae* MS2 also encodes a second LuxR homolog with 49% amino acid identity to the PipR regulator from *Pseudomonas* sp. GM79 (25). We have called the *D. zeae* MS2 genes *pipR* and *pipA*. The MS2 *pipR* possesses the two conserved amino acid substitutions (M65 and W69) distinguishing the plant-responsive LuxR homologs from AHL-responsive LuxR homologs (19–21). We created a PipR mutant and found it had no defect in *E. coli* killing (Fig. 2), indigoidine production (data not shown), motility, or PCWDE production (see Fig. S4 in the supplemental material). However, as PipR homologs of other bacteria require a plant-derived signal (22, 49), it is possible that the MS2 PipR is not active in laboratory cultures. However, the MS2 PipR mutant was fully virulent in syringe needle inoculations of banana pseudostems (Fig. 1), suggesting that the MS2 PipR is not required to activate any banana virulence genes.

The *D. zeae* MS2 *pipA* product has proline iminopeptidase activity and is required for banana plant disease. Although we found that the MS2 PipR mutant has no apparent phenotype (see discussion above), we wondered if the gene adjacent to *pipR*, *pipA*, played any role in MS2 pathogenesis because in two *Xanthomonas* sp. pathogens, mutants in the *pipA* homolog are impaired in plant virulence (22, 24). The *pipA* gene of *Pseudomonas* GM79 codes for an aminopeptidase that shows a preference for cleavage of N-terminal prolines but can also cleave N-terminal alanines (25). This gene product shares 64% amino acid identity with the *D. zeae* MS2 *pipA* gene product. We first asked if the MS2 *pipA* product is a proline iminopeptidase. We purified the MS2 PipA enzyme as a hexahistidine-tagged fusion protein and assayed its ability to cleave N-terminal amino acids coupled to a fluorescent substrate (β-naphthylamide). The purified protein was most active against the proline substrate but also showed some activity with the hydroxyproline and alanine substrates. There was relatively little activity with any of the other substrates tested (Table 1). These results are similar to those reported for the *Pseudomonas* GM79 *pipA* product (25).

We next screened the MS2 Δ*pipA* strain for a variety of phenotypes. Relative to the parent, there was no effect on growth in LB (Fig. S2) nor did the mutation affect *E. coli* killing (Fig. 2), cellular aggregation (data not shown), motility, or maceration in the potato slice assay (Fig. S4). However, the MS2 *pipA* mutant was greatly impaired in its

TABLE 1 Substrate specificities of purified MS2 PipA-His_6 enzyme

Substrate	Relative PipA-His_6 activity[a]
L-Proline-β-naphthylamide	100.0 ± 0.7
L-Alanine-β-naphthylamide	34.5 ± 2.6
L-Hydroxy-proline-β-naphthylamide	5.8 ± 0.3
L-Serine-β-naphthylamide	1.4 ± 0.1
L-Histidine-β-naphthylamide	0.3 ± 0.1
L-Leucine-β-naphthylamide	0.7 ± 0.0
L-Glutamic acid-β-naphthylamide	ND[b]
L-Lysine-β-naphthylamide	ND

[a]Enzyme purification and assay conditions were as described in Materials and Methods. Naphthylamide substrate results were measured as relative fluorescence units per minute per milligram of protein and normalized to activity exhibited by His_6-PipA with L-proline-β-naphthylamide as substrate ± the standard deviation. Results are the averages of 4 to 8 assays ± the standard deviation.
[b]ND, not detected; not above background of the no enzyme addition control.

ability to cause banana leaf rot (Fig. 1). Full virulence of the PipA mutant was restored by a *pipA*-complementing plasmid (Fig. 1). These data demonstrate that PipA is important for banana virulence as has been reported for *Xanthomonas* PipA homologs in cabbage black rot disease and bacterial pustule disease in soybeans (22, 24).

The MS2 PipR mutant has no defect in plant disease (Fig. 1), which suggests that it is not needed for sufficient *in planta pipA* expression as has been reported in other bacteria (22–24). We note that in the biocontrol strain *Pseudomonas protegens* Pf-5, the PipR homolog (PsoR) does not activate its downstream *pipA* homolog (21). We have not determined how the *pipA* product might be involved in *D. zeae* MS2 virulence. This also remains an open question for other phytopathogens where *pipA* homologs have been shown to be involved in virulence (22, 24), although recently it has been reported that the PipA homolog in *Xanthomonas campestris* pv. campestris 8004 is a type III effector that can elicit a hypersensitive response in nonhost plants and suppress plant immunity when expressed transgenically in *Arabidopsis* (50). Whether PipA is a type III effector protein in MS2 will be tested in future experiments; interestingly, many bacteria harboring PipR-PipA-type systems do not even encode type III secretion systems. Any understanding of how PipA functions at the molecular level to affect plant health could be important.

Unlike *expR* and *expI*, which are highly conserved in the genus *Dickeya*, *pipR* and *pipA* are found only in 5 of the 11 sequenced *D. zeae* genomes (strains MK19, NCPPB 2539, NCPPB 3532, MS1, and MS2). We note *pipR* and *pipA* are in the genomes of both banana isolates (MS1 and MS2). It will be interesting to learn how similar other *D. zeae* banana isolates are to strain MS2, whether or not *pipA* is present and plays a role in banana virulence, and how *D. zeae* has evolved to become an emerging pathogen of bananas in Southeast Asia.

MATERIALS AND METHODS

Bacterial strains, plasmids, growth conditions, and chemicals. Bacterial strains and plasmids are described in Tables 2 and 3. *D. zeae* MS2 and its derived strains were grown in LB (51) or in M9 minimal medium (52) with 10 mM glucose. Bacteria were grown with shaking (200 rpm) at 30°C. *Escherichia coli* strains were grown in LB at 37°C with shaking. Antibiotics were used at the following final concentrations: kanamycin, 50 μg/ml; ampicillin, 100 μg/ml; streptomycin, 50 μg/ml; chloramphenicol, 35 μg/ml; and gentamicin, 20 μg/ml. Plating was on LB or minimal medium solidified with 1.5% agar. Chemically synthesized AHL compounds used in this work included 3OC6-HSL [*N*-(β-ketocaproyl)-L-homoserine lactone, item K3007; Sigma-Aldrich, St. Louis, MO] and 3OC8-HSL [*N*-(β-oxo-octanoyl)-L-homoserine lactone, item 10011206; Cayman Chemical, Ann Arbor, MI].

Genome sequencing. DNA was isolated from *D. zeae* MS2 grown to mid-logarithmic phase in LB. Genomic sequencing, assembly, and electronic annotation were performed by Total Genetics Solution Company (Shenzhen, China). Genome sequencing was performed using PacBio RSII (250-fold coverage) and Illumina HiSeq 2500 (400-fold coverage). The whole-genome sequencing approach resulted in 3,495,253 high-quality filtered reads with an average paired-end read length of 150 bp and 110-fold sequencing coverage on average. De novo assembly was with SMRT Analysis version 2.3.0 (Pacific Biosciences, Menlo Park, CA) and SOAPaligner version 2.21 (https://github.com/aquaskyline/SOAPdenovo2). Functional annotation was done by RefSeq Prokaryotic Genome Annotation Project (RGAP) and BLAST. The genome was closed and consists of 4,740,052 bp. The genome was annotated by using

TABLE 2 Bacterial strains and plasmids

Strain or plasmid	Relevant characteristic(s)	Source/reference
D. zeae strains		
MS2	Wild type; isolated from infected banana	1
Δ5075	C1030_05075 deletion mutant (annotated as nonribosomal peptide synthetase gene) of MS2	This work
Δ*expR*	*expR* deletion mutant of MS2	This work
Δ*expI*	*expI* deletion mutant of MS2	This work
Δ*pipR*	*pipR* deletion mutant of MS2	This work
Δ*pipA*	*pipA* deletion mutant of MS2	This work
E. coli strains		
DH5α	*fhuA2* Δ(*argF-lacZ*)*U169 phoA glnV44* Φ80 Δ(*lacZ*)M15 *gyrA96 recA1 relA1 endA1 thi-1 hsdR17*	Invitrogen
C118 λpir	Δ(*ara-leu*) *araD* Δ*lacX74 galE galK phoA20 thi-1 rpsE rpoB argE* (Am) *recA1 λpir*	63
SM10 λpir	*thi-1 thr leu tonA lacY supE recA*::RP4-2-Tc::Mu *λpir* Kan^r	56
DH12S(pHV402, pLC42)	3OC6-HSL bioassay reporter; Cam^r Kan^r	64
Plasmids		
pKNG101	Suicide vector; *strAB sacB oriR6K monRK2*; Str^r	65
pKNG5075	Knockout plasmid for 5075-mutant strain construction; Str^r	This work
pKNGexpR	Knockout plasmid for ExpR-mutant strain construction; Str^r	This work
pKNGexpI	Knockout plasmid for ExpI-mutant strain construction; Str^r	This work
pKNGpipR	Knockout plasmid for PipR-mutant strain construction; Str^r	This work
pKNGpipA	Knockout plasmid for PipA-mutant strain construction; Str^r	This work
pBBR1MCS-5	Broad-host-range vector; Gen^r	62
pC5075	pBBR1MCS-5 expressing P_{lac} C1030_05075; Gen^r	This work
pCexpI	pBBR1MCS-5 expressing P_{lac} *expI*; Gen^r	This work
pCpipA	pBBR1MCS-5 expressing P_{lac} *pipA*; Gen^r	This work
pBBR1MCS-4	Broad-host-range vector; Amp^r	62
pPipAhis	C-terminal PipA-His_6 expression plasmid; Amp^r	This work

the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (53), and putative biosynthetic gene clusters were identified by using antiSMASH 5.0 (40) with default settings. LuxI and LuxR homologs were identified by searching for their associated PFAM domains (pfam00765 and pfam03472/pfam00196, respectively) (54). To identify the genes for secretion systems and PCWDEs, we performed tBLASTn analyses of those previously described systems in the related *Dickeya* genomes, including *D. zeae* MS1 (5), *D. zeae* EC1 (16), *D. zeae* Ech586 (26), and *D. dadantii* 3937 (27).

Mutant and plasmid construction. All plasmids and primer sequences are described in Tables 2 and 3, respectively. For knockout plasmids used to construct the ExpR and PipR mutants (Δ*expR* and Δ*pipR*), about 500 bp of upstream and downstream flanking DNA was synthesized (IGE Biotechnology Ltd,

TABLE 3 Primers used in this study

Primer	Sequence (5′–3′)	Description
pKNG-F	CGTATTCAGTGTCGCTGATTTGTA	Suicide vector backbone
pKNG-R	GGTACCATAAGTAGAAGCAGCAAC	Suicide vector backbone
expI-KO-UP-F	AACAACTTCAGACAATACAAATCAGCGACACTGAATACGTCACCCTGAACGGACGTGATC	Construction of Δ*expI* mutant
expI-KO-UP-R	CGAAATGAACCTGATCAAGCTCCCCCTATCCTTAACTTCATGTC	Construction of Δ*expI* mutant
expI-KO-DN-F	GAAGTTAAGGATAGGGGGAGCTTGATCAGGTTCATTTCGACC	Construction of Δ*expI* mutant
expI-KO-DN-R	GGTAAAGCTACTTGGGTTGCTGCTTCTACTTATGGTACCGTGCCGTCATGATCAGATTGC	Construction of Δ*expI* mutant
pipA-KO-UP-F	AAACAACTTCAGACAATACAAATCAGCGACACTGAATACGGATGCGGTGATTTACGACTACAC	Construction of Δ*pipA* mutant
pipA-KO-UP-R	GGTGTTAGCAACCATTAACAGGTGTACATGAGTGACGTTTACC	Construction of Δ*pipA* mutant
pipA-KO-DN-F	AAACGTCACTCATGTACACCTGTTAATGGTTGCTAACACCAGG	Construction of Δ*pipA* mutant
pipA-KO-DN-R	TGGTAAAGCTACTTGGGTTGCTGCTTCTACTTATGGTACCCAAGAGCGCATAGGTAGTCATTAG	Construction of Δ*pipA* mutant
5075-KO-UP-F	AAACAACTTCAGACAATACAAATCAGCGACACTGAATACGGTGCTGCTGGATACCTTCG	Construction of Δ5075 mutant
5075-KO-UP-R	CGCCATGCCAAAAGTTTTACCAGTCATCCACCACGTTGATC	Construction of Δ5075 mutant
5075-KO-DN-F	ATCAACGTGGTGGATGACTGGTAAAACTTTTGGCATGGCGC	Construction of Δ5075 mutant
5075-KO-DN-R	TGGTAAAGCTACTTGGGTTGCTGCTTCTACTTATGGTACCAAACGCGGACTGAAAGTTGG	Construction of Δ5075 mutant
pipA-F	CCCAAGCTTGTAACTAGCATTTTTGCTAGTG	Construction of pPipAhis
pipA-R	AACTGCAGAGCAACCATTAGTGATGATGATGATGATGACAATACTGCGTC	Construction of pPipAhis
pBBR-F	GCGCTTGGCGTAATCATGGTC	pBBR1MCS-5 backbone
pBBR-R	TCGTGACTGGGAAAACCCTGG	pBBR1MCS-5 backbone
C5075-F	AAACAGCTATGACCATGATTACGCCAAGCGCGCAAACGTTACCGCACAACCAGAG	Construction of pC5075
C5075-R	TGTAGCCGCCTCGCCGCCCTATACCTTGTCTGCCTTAGCTGACTGGAGGCAATGC	Construction of pC5075
CexpI-F	ACACAGGAAACAGCTATGACCATGATTACGCCAAGCGCTCCCAGCGGGAGTTATCTCAC	Construction of pCexpI
CexpI-R	GATTAAGTTGGGTAACGCCAGGGTTTTCCCAGTCACGACTAAACACGCCATTCGGCTTG	Construction of pCexpI
CpipA-F	CCCAAGCTTATAACTAGCATTTTTGCTAG	Construction of pCpipA
CpipA-R	AACTGCAGCCTACCTGGTGTTAGCAACC	Construction of pCpipA

Guangzhou, China) and then cloned into the suicide plasmid pKNG101 by standard restriction enzyme-based cloning. To construct the ΔC1030_05075, Δ*expI*, and Δ*pipA* knockout plasmids, we used genomic DNA as a template to PCR amplify about 750 bp of upstream and downstream DNA of the intended deletion with overlapping homology, including that of the suicide plasmid pKNG101. We used *E. coli* DH5α for *in vivo* plasmid assembly as described elsewhere (55). All constructs were confirmed by DNA sequencing and introduced in the conjugal donor strain *E. coli* SM10 λpir by electroporation. Plasmids were moved to strain MS2 by conjugal transfer (56) and double-crossover transconjugants were selected on M9 minimal agar plates containing 10% sucrose for *sacB* counterselection. To create the complementing plasmids, *expI* (including 175 bp of DNA upstream of the translational start codon) or *pipA* (including 85 bp upstream of the start codon) was PCR amplified and cloned in pBBR1MCS-5 by using *E. coli*-mediated DNA assembly (55) or standard restriction enzyme-based techniques. All plasmid constructions were confirmed by DNA sequencing and introduced into MS2 via electroporation. Growth of mutants in LB was indistinguishable from that of the wild type (see Fig. S2 in the supplemental material).

Banana virulence assays. Young banana plants (*Musa* ABB) were obtained from the Fruit Research Institute, Guangdong Academy of Agricultural Sciences (Guangzhou, China). Efforts were made to obtain plants of similar size and characteristics. Banana plants were grown in plastic pots with garden soil and acclimated in a greenhouse at about 25°C with 12-h alternating light-dark cycles for 2 weeks prior to infection. For infections, bacteria were grown overnight in LB with shaking at 28°C and then diluted 1:100 into 5 ml fresh LB and grown to mid-log phase (optical density at 600 nm [OD_{600}] of 1.0). When appropriate, antibiotics were added for plasmid maintenance. Plants were infected via needle injection (0.1 ml of culture in 1-ml syringe with 26-gauge needle) into the central pseudostem. Plants were incubated at 30°C with 12-h alternating light-dark cycles for 7 days, and then disease was assessed by using a modified virulence score (57) as described in Fig. S1 in the supplemental material.

***E. coli* inhibition assay.** To survey *D. zeae* strains for the ability to inhibit growth of *E. coli*, we used a method similar to one described previously (14, 58). Briefly, 20 ml of LB agar (1.5% agar) plus gentamicin was solidified in square plastic plates (10 cm by 10 cm). The plates were overlaid with 15-ml of 1% agarose cooled to 50°C and inoculated with *E. coli* pBBR1MCS-5 (10^8 CFU). Cell-free culture fluid from the indicated *D. zeae* strains was prepared from overnight LB cultures filtered through a 0.2-μm filter. A paper disk (6 mm from Becton, Dickinson and Company, NJ, USA) was saturated with 40 μl of culture fluid and placed on top of the *E. coli*-impregnated agarose using sterilized tweezers. Plates were incubated at 37°C for 24 h. When the plates were imaged using transmitted light from a "bucket of light" (59), confluent *E. coli* growth in the agarose appeared as a whitish color while zones of growth inhibition appeared as a gray/blue color emanating from the paper disk (Fig. 2A). The diameters of these gray/blue-colored zones of clearing were measured using a ruler.

Detection of AHL signals. To detect and identify AHL signals, we utilized a ^{14}C-radiolabel assay (43) as follows. Bacteria were grown in 4 ml of minimal medium to log phase and then incubated for 16 h in the presence of 5 μCi (about 90 nM) of [1-^{14}C]-methionine (American Radiolabeled Chemicals, Inc., St. Louis, MO). The ^{14}C-labeled AHLs were extracted with acidified ethyl acetate (0.1 ml of glacial acetic acid per liter of solvent) and fractionated by C_{18} reverse-phase HPLC. Radioactivity in each fraction was determined by scintillation counting. To determine the retention times of synthetic AHL standards, we used the same HPLC protocol and monitored AHL elution by UV absorbance at 210 nm. We utilized the bioassay with the reporter strain *E. coli* DH12S(pHV402, pLC42) to measure 3OC6-HSL in culture extracts with a standard curve generated using synthetic 3OC6-HSL [*N*-(β-ketocaproyl)-L-homoserine lactone, item K3007; Sigma-Aldrich, St. Louis, MO] according to a protocol described previously (60).

Motility assays, cell aggregation assays, and measurement of indigoidine. Bacterial motility was assessed by using soft-agar motility plates (0.3% agar) as described previously (3), except that 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) was omitted from the medium. Cell aggregation was assessed by eye at 3 to 4 h postinoculation (early logarithmic phase) into 4 ml of LB broth incubated at 30°C with shaking. Indigoidine was extracted with dimethyl sulfoxide (DMSO) from agar-grown cells as described previously (61). Blue pigment was measured by absorption at A_{615}, and values were normalized relative to the wild-type *D. zeae* MS2.

Purification and enzyme activity of PipA-His_6. To obtain purified PipA, the *pipA* gene was amplified from genomic DNA and cloned into pBBR1MCS-4 (62) to create pPipAhis. The reverse primer contained a C-terminal hexahistidine tag to aid in protein purification (Table 3). PipA-His_6 overexpression, purification, and enzyme activity assays were performed as described previously (25) except that nickel resin was used in place of cobalt resin.

Data availability. The genome sequence has been deposited at the National Center for Biotechnology Information (NCBI accession number CP025799 and BioProject number PRJNA429264).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AEM.01611-19.

SUPPLEMENTAL FILE 1, PDF file, 1.4 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.2 MB.

ACKNOWLEDGMENTS

We thank Ajai Dandekar for helpful statistical analysis discussions.

This research was sponsored by grants from the National Natural Science

Foundation of China (31972230), National Key Project for Basic Research of China (2015CB150600), Team R&D Project of Guangdong Province (key areas of research and development program 2018B020205003), and the China Scholarship Council fellowship program grant (201808440406) to L.F.

REFERENCES

1. Hu M, Li J, Chen R, Li W, Feng L, Shi L, Xue Y, Feng X, Zhang L, Zhou J. 2018. *Dickeya zeae* strains isolated from rice, banana and clivia rot plants show great virulence differentials. BMC Microbiol 18:136. https://doi.org/10.1186/s12866-018-1300-y.
2. Mansfield J, Genin S, Magori S, Citovsky V, Sriariyanum M, Ronald P, Dow M, Verdier V, Beer SV, Machado MA, Toth I, Salmond G, Foster GD. 2012. Top 10 plant pathogenic bacteria in molecular plant pathology. Mol Plant Pathol 13:614–629. https://doi.org/10.1111/j.1364-3703.2012.00804.x.
3. Hussain BM, Zhang HB, Xu JL, Liu Q, Jiang Z, Zhang LH. 2008. The acyl-homoserine lactone-type quorum-sensing system modulates cell motility and virulence of *Erwinia chrysanthemi* pv. zeae. J Bacteriol 190:1045–1053. https://doi.org/10.1128/JB.01472-07.
4. Perombelon MCM, Kelman A. 1980. Ecology of the soft rot erwinias. Annu Rev Phytopathol 18:361–387. https://doi.org/10.1146/annurev.py.18.090180.002045.
5. Zhang JX, Lin BR, Shen HF, Pu XM. 2013. Genome sequence of the banana pathogen *Dickeya zeae* strain MS1, which causes bacterial soft rot. Genome Announc 13:e00317-13. https://doi.org/10.1128/genomeA.00317-13.
6. Samson R, Legendre JB, Christen R, Fischer-Le Saux M, Achouak W, Gardan L. 2005. Transfer of *Pectobacterium chrysanthemi* (Burkholder et al. 1953) Brenner et al. 1973 and *Brenneria paradisiaca* to the genus *Dickeya* gen. nov. as *Dickeya chrysanthemi* comb. nov. and *Dickeya paradisiaca* comb. nov. and delineation of four novel species, *Dickeya dadantii* sp. nov., *Dickeya dianthicola* sp. nov., *Dickeya dieffenbachiae* sp. nov. and *Dickeya zeae* sp. nov. Int J Syst Evol Microbiol 55:1415–1427. https://doi.org/10.1099/ijs.0.02791-0.
7. Hugouvieux-Cotte-Pattat N, Condemine G, Nasser W, Reverchon S. 1996. Regulation of pectinolysis in *Erwinia chrysanthemi*. Annu Rev Microbiol 50:213–257. https://doi.org/10.1146/annurev.micro.50.1.213.
8. Expert D. 1999. Withholding and exchanging iron: interactions between *Erwinia* spp. and their plant hosts. Annu Rev Phytopathol 37:307–334. https://doi.org/10.1146/annurev.phyto.37.1.307.
9. Wu X, Zeng Q, Koestler BJ, Waters CM, Sundin GW, Hutchins W, Yang CH. 2014. Deciphering the components that coordinately regulate virulence factors of the soft rot pathogen *Dickeya dadantii*. Mol Plant Microbe Interact 27:1119–1131. https://doi.org/10.1094/MPMI-01-14-0026-R.
10. Yap MN, Yang CH, Barak JD, Jahn CE, Charkowski AO. 2005. The *Erwinia chrysanthemi* type III secretion system is required for multicellular behavior. J Bacteriol 187:639–648. https://doi.org/10.1128/JB.187.2.639-648.2005.
11. Reverchon S, Rouanet C, Expert D, Nasser W. 2002. Characterization of indigoidine biosynthetic genes in *Erwinia chrysanthemi* and role of this blue pigment in pathogenicity. J Bacteriol 184:654–665. https://doi.org/10.1128/JB.184.3.654-665.2002.
12. Pait IGU, Kitani S, Kurniawan YN, Asa M, Iwai T, Ikeda H, Nihira T. 2017. Identification and characterization of *lbpA*, an indigoidine biosynthetic gene γ-butyrolactone signaling system of *Streptomyces lavendulae* FRI-5. J Biosci Bioeng 124:369–375. https://doi.org/10.1016/j.jbiosc.2017.04.020.
13. Hellberg JE, Matilla MA, Salmond GP. 2015. The broad-spectrum antibiotic, zeamine, kills the nematode worm *Caenorhabditis elegans*. Front Microbiol 6:137. https://doi.org/10.3389/fmicb.2015.00137.
14. Zhou J, Zhang H, Wu J, Liu Q, Xi P, Lee J, Liao J, Jiang Z, Zhang L-H. 2011. A novel multidomain polyketide synthase is essential for zeamine production and the virulence of *Dickeya zeae*. Mol Plant Microbe Interact 24:1156–1164. https://doi.org/10.1094/MPMI-04-11-0087.
15. Barnard AM, Salmond GP. 2007. Quorum sensing in *Erwinia* species. Anal Bioanal Chem 387:415–423. https://doi.org/10.1007/s00216-006-0701-1.
16. Zhou J, Cheng Y, Lv M, Liao L, Chen Y, Gu Y, Liu S, Jiang Z, Xiong Y, Zhang L. 2015. The complete genome sequence of *Dickeya zeae* EC1 reveals substantial divergence from other *Dickeya* strains and species. BMC Genomics 16:571. https://doi.org/10.1186/s12864-015-1545-x.
17. Andersson RA, Eriksson AR, Heikinheimo R, Mae A, Pirhonen M, Koiv V, Hyytiainen H, Tuikkala A, Palva ET. 2000. Quorum sensing in the plant pathogen *Erwinia carotovora* subsp. carotovora: the role of $expR_{Ecc}$. Mol Plant Microbe Interact 13:384–393. https://doi.org/10.1094/MPMI.2000.13.4.384.
18. Crepin A, Beury-Cirou A, Barbey C, Farmer C, Helias V, Burini JF, Faure D, Latour X. 2012. *N*-acyl homoserine lactones in diverse *Pectobacterium* and *Dickeya* plant pathogens: diversity, abundance, and involvement in virulence. Sensors 12:3484–3497. https://doi.org/10.3390/s120303484.
19. Gonzalez JF, Venturi V. 2012. A novel widespread interkingdom signaling circuit. Trends Plant Sci 18:167–174. https://doi.org/10.1016/j.tplants.2012.09.007.
20. Patankar AV, Gonzalez JE. 2009. Orphan LuxR regulators of quorum sensing. FEMS Microbiol Rev 33:739–756. https://doi.org/10.1111/j.1574-6976.2009.00163.x.
21. Subramoni S, Gonzalez JF, Johnson A, Pechy-Tarr M, Rochat L, Paulsen I, Loper JE, Keel C, Venturi V. 2011. Bacterial subfamily of LuxR regulators that respond to plant compounds. Appl Environ Microbiol 77:4579–4588. https://doi.org/10.1128/AEM.00183-11.
22. Chatnaparat T, Prathuangwong S, Ionescu M, Lindow SE. 2012. XagR, a LuxR homolog, contributes to the virulence of *Xanthomonas axonopodis* pv. *glycines* to soybean. Mol Plant Microbe Interact 25:1104–1117. https://doi.org/10.1094/MPMI-01-12-0008-R.
23. Ferluga S, Venturi V. 2009. OryR is a LuxR-family protein involved in interkingdom signaling between pathogenic *Xanthomonas oryzae* pv. oryzae and rice. J Bacteriol 191:890–897. https://doi.org/10.1128/JB.01507-08.
24. Zhang L, Jia Y, Wang L, Fang R. 2007. A proline iminopeptidase gene upregulated in planta by a LuxR homologue is essential for pathogenicity of *Xanthomonas campestris* pv. *campestris*. Mol Microbiol 65:121–136. https://doi.org/10.1111/j.1365-2958.2007.05775.x.
25. Schaefer AL, Oda Y, Coutinho BG, Pelletier DA, Weiburg J, Venturi V, Greenberg EP, Harwood CS. 2016. A LuxR homolog in a cottonwood tree endophyte that activates gene expression in response to a plant signal or specific peptides. mBio 7:e01101-16. https://doi.org/10.1128/mBio.01101-16.
26. Pritchard L, Humphris S, Saddler GS, Parkinson NM, Bertrand V, Elphinstone JG, Toth IK. 2012. Detection of phytopathogens of the genus *Dickeya* using a PCR primer prediction pipeline for draft bacterial genome sequences. Plant Pathol 62:587–596. https://doi.org/10.1111/j.1365-3059.2012.02678.x.
27. Glasner JD, Yang C-H, Reverchon S, Hugouvieux-Cotte-Pattat N, Condemine G, Bohin J-P, Van Gijsegem F, Yang S, Franza T, Expert D, Plunkett G, San Francisco MJ, Charkowski AO, Py B, Bell K, Rauscher L, Rodriguez-Palenzuela P, Toussaint A, Holeva MC, He SY, Douet V, Boccara M, Blanco C, Toth I, Anderson BD, Biehl BS, Mau B, Flynn SM, Barras F, Lindeberg M, Birch PRJ, Tsuyumu S, Shi X, Hibbing M, Yap M-N, Carpentier M, Dassa E, Umehara M, Kim JF, Rusch M, Soni P, Mayhew GF, Fouts DE, Gill SR, Blattner FR, Keen NT, Perna NT. 2011. Genome sequence of the plant-pathogenic bacterium *Dickeya dadantii* 3937. J Bacteriol 193:2076–2077. https://doi.org/10.1128/JB.01513-10.
28. Binet R, Letoffe S, Ghigo JM, Delepelaire P, Wandersman C. 1997. Protein secretion by Gram-negative bacterial ABC exporters–a review. Gene 192:7–11. https://doi.org/10.1016/S0378-1119(96)00829-3.
29. Delepelaire P, Wandersman C. 1991. Characterization, localization and transmembrane organization of the three proteins PrtD, PrtE and PrtF necessary for protease secretion by the Gram-negative bacterium *Erwinia chrysanthemi*. Mol Microbiol 5:2427–2434. https://doi.org/10.1111/j.1365-2958.1991.tb02088.x.
30. Ferrandez Y, Condemine G. 2008. Novel mechanism of outer membrane targeting of proteins in Gram-negative bacteria. Mol Microbiol 69:1349–1357. https://doi.org/10.1111/j.1365-2958.2008.06366.x.
31. Lindeberg M, Boyd CM, Keen NT, Collmer A. 1998. External loops at the C terminus of *Erwinia chrysanthemi* pectate lyase C are required for species-specific secretion through the out type II pathway. J Bacteriol 180:1431–1437.

32. Alfano JR, Collmer A. 1997. The type III (Hrp) secretion pathway of plant pathogenic bacteria: trafficking harpins, Avr proteins, and death. J Bacteriol 179:5655–5662. https://doi.org/10.1128/jb.179.18.5655-5662.1997.
33. Bauer DW, Bogdanove AJ, Beer SV, Collmer A. 1994. *Erwinia chrysanthemi hrp* genes and their involvement in soft rot pathogenesis and elicitation of the hypersensitive response. Mol Plant Microbe Interact 7:573–581. https://doi.org/10.1094/MPMI-7-0573.
34. Bingle LE, Bailey CM, Pallen MJ. 2008. Type VI secretion: a beginner's guide. Curr Opin Microbiol 11:3–8. https://doi.org/10.1016/j.mib.2008.01.006.
35. Pedron J, Mondy S, Des Essarts YR, Van Gijsegem F, Faure D. 2014. Genomic and metabolic comparison with *Dickeya dadantii* 3937 reveals the emerging *Dickeya solani* potato pathogen to display distinctive metabolic activities and T5SS/T6SS-related toxin repertoire. BMC Genomics 15:283. https://doi.org/10.1186/1471-2164-15-283.
36. Silverman JM, Brunet YR, Cascales E, Mougous JD. 2012. Structure and regulation of the type VI secretion system. Annu Rev Microbiol 66:453–472. https://doi.org/10.1146/annurev-micro-121809-151619.
37. Yang CH, Gavilanes-Ruiz M, Okinaka Y, Vedel R, Berthuy I, Boccara M, Chen JW, Perna NT, Keen NT. 2002. *hrp* genes of *Erwinia chrysanthemi* 3937 are important virulence factors. Mol Plant Microbe Interact 15:472–480. https://doi.org/10.1094/MPMI.2002.15.5.472.
38. Bender CL, Alarcon-Chaidez F, Gross DC. 1999. *Pseudomonas syringae* phytotoxins: mode of action, regulation, and biosynthesis by peptide and polyketide synthetases. Microbiol Mol Biol Rev 63:266–292.
39. Pierson LS, III, Pierson EA. 2010. Metabolism and function of phenazines in bacteria: impacts on the behavior of bacteria in the environment and biotechnological processes. Appl Microbiol Biotechnol 86:1659–1670. https://doi.org/10.1007/s00253-010-2509-3.
40. Blin K, Medema MH, Kottmann R, Lee SY, Weber T. 2017. The antiSMASH database, a comprehensive database of microbial secondary metabolite biosynthetic gene clusters. Nucleic Acids Res 45:D555–D559. https://doi.org/10.1093/nar/gkw960.
41. McGowan SJ, Sebaihia M, Porter LE, Stewart G, Williams P, Bycroft BW, Salmond GPC. 1996. Analysis of bacterial carbapenem antibiotic production genes reveals a novel beta-lactam biosynthesis pathway. Mol Microbiol 22:415–426. https://doi.org/10.1046/j.1365-2958.1996.00125.x.
42. Schuster M, Lostroh CP, Ogi T, Greenberg EP. 2003. Identification, timing, and signal specificity of *Pseudomonas aeruginosa* quorum-controlled genes: a transcriptome analysis. J Bacteriol 185:2066–2079. https://doi.org/10.1128/JB.185.7.2066-2079.2003.
43. Schaefer AL, Harwood CS, Greenberg EP. 2018. "Hot stuff": the many uses of a radiolabel assay in detecting acyl-homoserine lactone quorum-sensing signals. Methods Mol Biol 1673:35–47. https://doi.org/10.1007/978-1-4939-7309-5_3.
44. Brotherton CA, Medema MH, Greenberg EP. 2018. *luxR* homolog-linked biosynthetic gene clusters in *Proteobacteria*. mSystems 3:e00208-17. https://doi.org/10.1128/mSystems.00208-17.
45. Castang S, Reverchon S, Gouet P, Nasser W. 2006. Direct evidence for the modulation of the activity of the *Erwinia chrysanthemi* quorum-sensing regulator ExpR by acylhomoserine lactone pheromone. J Biol Chem 281:29972–29987. https://doi.org/10.1074/jbc.M601666200.
46. Reverchon S, Bouillant ML, Salmond G, Nasser W. 1998. Integration of the quorum-sensing system in the regulatory networks controlling virulence factor synthesis in *Erwinia chrysanthemi*. Mol Microbiol 29:1407–1418. https://doi.org/10.1046/j.1365-2958.1998.01023.x.
47. Dong YH, Wang LH, Xu JL, Zhang HB, Zhang XF, Zhang LH. 2001. Quenching quorum-sensing-dependent bacterial infection by an *N*-acyl homoserine lactonase. Nature 411:813–817. https://doi.org/10.1038/35081101.
48. Faure D, Dessaux Y. 2007. Quorum sensing as a target for developing control strategies for the plan pathogen *Pectobacterium*. Eur J Plant Pathol 119:353–365. https://doi.org/10.1007/s10658-007-9149-1.
49. Wang L, Zhang L, Geng Y, Xi W, Fang R, Jia Y. 2011. XerR, a negative regulator of XccR in *Xanthomonas campestris* pv. campestris, relieves its repressor function in planta. Cell Res 21:1131–1142. https://doi.org/10.1038/cr.2011.64.
50. Kan J, An L, Wu Y, Long J, Song L, Fang R, Jia Y. 2018. A dual role for proline iminopeptidase in the regulation of bacterial motility and host immunity. Mol Plant Pathol 19:2011–2024. https://doi.org/10.1111/mpp.12677.
51. Sambrook J, Russell DW. 2001. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
52. Miller JH. 1972. Experiments in molecular genetics. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
53. Tatusove T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569.
54. Schaefer AL, Lappala CR, Morlen RP, Pelletier DA, Lu TY, Lankford PK, Harwood CS, Greenberg EP. 2013. LuxR- and LuxI-type quorum-sensing circuits are prevalent in members of the *Populus deltoides* microbiome. Appl Environ Microbiol 79:5745–5752. https://doi.org/10.1128/AEM.01417-13.
55. Kostylev M, Otwell AE, Richardson RE, Suzuki Y. 2015. Cloning should be simple: *Escherichia coli* DH5a-mediated assembly of multiple DNA fragments with short end homologies. PLoS One 10:e0137466. https://doi.org/10.1371/journal.pone.0137466.
56. Simon R, Priefer U, Puhler A. 1983. A broad host range mobilization system for *in vivo* genetic engineering: transposon mutagenesis in Gram negative bacteria. Nat Biotechnol 1:784–791. https://doi.org/10.1038/nbt1183-784.
57. Down JM, Clarke BR, Milligan DE, Tang JL, Daniels MJ. 1990. Extracellular proteases from *Xanthomonas campestris* pv. campestris, the black rot pathogen. Appl Environ Microbiol 56:2994–2998.
58. Liao L, Cheng Y, Liu S, Zhou J, An S, Lv M, Chen Y, Gu Y, Chen S, Zhang LH. 2014. Production of novel antibiotics zeamines through optimizing *Dickeya zeae* fermentation conditions. PLoS One 9:e116047. https://doi.org/10.1371/journal.pone.0116047.
59. Parkinson JS. 2007. A "bucket of light" for viewing bacterial colonies in soft agar. Methods Enzymol 423:432–435. https://doi.org/10.1016/S0076-6879(07)23020-4.
60. Puri AW, Schaefer AL, Fu Y, Beck DA, Greenberg EP, Lidstrom K. 2017. Quorum sensing in a methane-oxidizing bacterium. J Bacteriol 14:e00773-16. https://doi.org/10.1128/JB.00773-16.
61. Lee YA, Yu CP. 2006. A differential medium for the isolation and rapid identification of a plant soft rot pathogen, *Erwinia chrysanthemi*. J Microbiol Methods 64:200–206. https://doi.org/10.1016/j.mimet.2005.04.031.
62. Kovach ME, Elzer PH, Hill DS, Robertson GT, Farris MA, Roop RM, II, Peterson KM. 1995. Four new derivatives of the broad-host-range cloning vector pBBR1MCS, carrying different antibiotic-resistance cassettes. Gene 166:175–176. https://doi.org/10.1016/0378-1119(95)00584-1.
63. Herrero M, de Lorenzo V, Timmis KN. 1990. Transposon vectors containing non-antibiotic resistance selection markers for cloning and stable chromosomal insertion of foreign genes in Gram-negative bacteria. J Bacteriol 172:6557–6567. https://doi.org/10.1128/jb.172.11.6557-6567.1990.
64. Antunes LC, Schaefer AL, Ferreira RB, Qin N, Stevens AM, Ruby EG, Greenberg EP. 2007. Transcriptome analysis of the *Vibrio fischeri* LuxR-LuxI regulon. J Bacteriol 189:8387–8391. https://doi.org/10.1128/JB.00736-07.
65. Kaniga K, Delor I, Cornelis GR. 1991. A wide-host-range suicide vector for improving reverse genetics in Gram-negative bacteria: inactivation of the *blaA* gene of *Yersinia enterocolitica*. Gene 109:137–141. https://doi.org/10.1016/0378-1119(91)90599-7.