Identification of *Dickeya zae* as a Causal Agent of Bacterial Soft Rot in Banana in China

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Abstract

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Bacterial soft rot of banana was first noticed in 2009 in Guangzhou city, China. The disease also was observed on various banana cultivars of different genotypes in several other cities. Symptoms of the disease included leaf wilting, collapse of pseudostems, and unusual odor. Five isolated strains that fulfilled Koch's postulates were used for biochemical testing. The five strains were most similar to *Dickeya dadantii* or *D. zae*, but were much less similar to *D. paradisiaca* when using several phenotype characteristics. Sequence analysis of 16S rDNA, *dnaX*, *gryB*, and *recA* of a reference strain revealed a similarity of 99% with

the sequences of *D. zae*, rather than *D. paradisiaca*. Phylogenetic analysis of concatenated sequences of *dnaX*, *gryB*, and *recA* indicated that the banana strain constituted a distinguishable clade with several *D. zae* strains involving rice pathogens *D. zae* EC1 and ZJU1202 from Guangdong province, but the banana pathogen had several characteristics that distinguished it from the rice pathogens. Therefore, the banana pathogen was determined to be *D. zae*. This is the first report of banana soft rot caused by *D. zae* in China; however, the pathogen can infect other important crops.

The herbaceous, flowering banana (*Musa* spp.) plant is native to tropical Asia. It has been widely domesticated in at least 107 countries for its fruit, fiber, wine, and ornamental uses, and is the fourth most cultivated food crop in the world after rice, wheat, and maize. Until 2007, the area planted to banana was approximately 4.4 million ha with an output of more than 80.0 million metric tons. In south China, banana is also one of the most important economic fruits, with banana plantations encompassing more than 0.3 million ha annually. Banana plays an increasingly important role in the tropical economic regions of China, including Guangdong, Guangxi, Hainan, and Fujian provinces.

In September 2009, there was a bacterial soft rot outbreak in banana in Guangzhou, China, resulting in 82% yield losses in 10 ha of surveyed fields (17). Soft rot of banana has been reported to be caused by *Pectobacterium carotovora* (*Erwinia carotovora*) (20) in Korea, Iran, and India (1,6,26). However, one symptom caused by *P. carotovora* is stunted growth (28), which was different from the symptoms observed in Guangzhou. In this outbreak, the first disease symptom observed was yellowing of petioles on emerging leaves, followed by the collapse of petioles, leaf wilting, and then death. The vascular tissue of the pseudostem of infected banana turned brown or black, and a bacterial ooze was observed on the pseudostem and carpopodium (Fig. 1). Isolates of *Dickeya* sp. were

obtained from infected plants from the previously mentioned locations, but it was difficult to determine the exact classification of the pathogen (17) because of the complex classification and taxonomic history of the genus *Dickeya* (24).

Since discovery of this disease in 2009, its incidence and severity have increased. From 2010 to 2012, 6,000 ha of bananas were affected in Guangdong province. The mean disease incidence ranged from 20 to 70%, but in some plantations incidences as high as 90% were observed. By 6 to 8 days after the first symptoms were observed, infected plants died. Therefore, the control of this disease including seedling quarantine is important for the Chinese banana industry. Identifying the species that causes banana soft rot in China is essential for effective quarantine and disease management. Traditional phytobacterial identification includes characterization of symptoms and biochemical properties (24), NGM medium (14), selective crystal violet polypectin medium (CVP) (9), and species-specific polymerase chain reaction (PCR). However, the PCR method used for detecting *Dickeya* sp. could not identify the pathogen at the species level, including primers for the pectinase genes *pelADE* (21), and *pelZ* (13). To identify the causal agent at the species level, representative strains were subjected to phenotypic assays and genetic analysis of multi-locus sites, as compared to known reference species of *Dickeya* sp.

Materials and Methods

Isolation of causal agent. In three plantations in Guangzhou, banana plants with soft rot symptoms were collected at different locations. The pseudostem bases of 65 infected plants were sampled and stored separately in an ice box, followed by immediate pathogen isolation. Direct isolation was performed on rotting pseudostem tissues from symptomatic plants. Pseudostem tissues from the margins between diseased and healthy areas were cut into 1.5-cm pieces and surface-sterilized by dipping in 75% ethanol for 30 s, 1% NaOCl for 1 min, and rinsing with sterile distilled water three times. The sterilized sections were macerated in drops of sterile water, and the tissue extract was streaked onto nutrient agar (3 g/liter beef extract, 0.5 g/liter yeast extract paste, 5 g/liter pep-

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The GenBank accession numbers for 16S rDNA, *dnaX*, *gryB*, and *recA* of *Dickeya zae* MS1 are JQ301888, JQ284038, JQ284039, and KC793203, respectively.

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tone, and 10 g/liter agar, pH 7.0) and cultured for 48 h at 32°C. Single colonies were chosen for subculturing three times to obtain strains.

Biochemical characterization. Forty-five strains from diseased plants were confirmed by Koch's postulates, and five of them were selected for biochemical characterization. The five strains were assessed for their ability to utilize 95 sugars, alcohols, acids, amines, and other substrates using the BIOLOG automated microbial analysis system (Version 4.20.05, Hayward, CA 94545 USA). One hundred fifty microliters of a bacterial suspensions containing 10^8 CFU/ml of each strain was added to each well of a BIOLOG GN micro-plate according to the manufacturer's instruction.

Amplification and sequencing of 16S rDNA, *dnaX*, *gyrB*, and *recA* genes. Bacteria were grown at 30°C for 24 h in LB medium (10 g/liter bacto tryptone, 5 g/liter yeast extract, and 10 g/liter NaCl, pH 7.0) in an incubator shaker at 100 RPM. Total DNA was extracted from bacterial suspensions (10^8 CFU/ml) with a TIANamp Bacterial DNA kit (Tiangen Biotech, Beijing, China) according to manufacturer's directions. A 1,503 bp region of the 16S ribosomal DNA (rDNA) gene, 536 bp of DNA polymerase III gamma subunit gene (*dnaX*), 2,412 bp of DNA gyrase gene (*gyrB*), and 700 bp of recombinase A gene (*recA*) were amplified using primer pairs fd2/rP1 (30), dnaXf/dnaXr (25), gyrBf1/gyrBr1 designed with published *gyrB* gene sequences of *Dickeya* genus in

Fig. 1. Symptoms of bacterial soft rot of banana. **A**, Cross-sections of pseudostem of diseased banana; **B**, incidence of soft rot in a banana plantation; **C**, symptoms of a whole plant.

Fig. 2. Culturing characteristics of three *Dickeya* strains at 48 h. MS1 produced gray colonies on nutrient agar compared to other strains. **A**, MS1 was isolated from banana; **B**, EC1 was *D. zeae* strain from rice; **C**, PA1 was *Dickeya* sp. strain from phalaenopsis.

the National Center for Biotechnology Information (NCBI) database, and *recA*/*recAr* (29), respectively (Table 1). For PCR amplification, 50 µl of reaction mixtures contained 10× TransStart FastPfu Buffer, 250 µM dNTPs, 0.4 µM of each primer, 2.5 U *TransStart FastPfu* DNA Polymerase (TransGen Biotech), and 2 µl of template DNA. PCR reactions were performed using the following thermal profile: initial denaturation for 2 min at 95°C, followed by 30 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 2 min, followed by a final extension at 72°C for 7 min. PCR products were separated on a 1% agarose gel and purified with a PCR purification kit (TransGen Biotech) according to the manufacturer's instructions. After purification, the products were cloned into the pEASY-T vector (TransGen Biotech); transformed into *Escherichia coli* DH5α competent cells, and transformants were selected on LB agar amended with 100 µg/ml ampicillin and 50 µg/ml kanamycin. Transformants were validated by colony PCR using common primers M13F (5'-GTAAAACGACGGCCAGT-3') and M13R (5'-CAGGAAACAGCTATGAC-3'), and the positive clones were grown in LB at 37°C for 12 h. Plasmids were extracted with a TIANprep Mini Plasmid kit (Tiangen Biotech) and sequenced in Sangon Co. (Shanghai, China).

Phylogenetic analyses. Each 16S rDNA, *dnaX*, *gryB*, and *recA* sequence was subjected to BLAST analysis against sequences in the National Center for Biotechnology Information (NCBI). *Dickeya* species with complete *dnaX*, *gryB*, and *recA* sequences in NCBI GenBank were used for phylogenetic analyses, including 15 whole-genome sequenced *Dickeya* strains, of which six were type *Dickeya* strains in a previous study (22). A preliminary automatic alignment of the sequences was generated using CLUSTALW (8) with a gap penalty of 15 and then clipped to the length of consensus sequences. The concatenated, informative sequences of the *dnaX*, *gryB*, and *recA* gene sequences were used for phylogenetic analysis using MEGA 5.1 (27) with a neighbor-joining method and bootstrapping test of 1,000 replicates.

Pathogenicity test. Pathogenicity tests were conducted by injecting 100-µl bacterial suspensions (10⁸ CFU/ml) of five representative strains of the pathogen into each of 10 banana seedlings (cultivar of Musa Cavendish var. Brazil) at the trefoil stage. Ten banana seedlings inoculated with sterile water served as negative controls. Plants were placed in a greenhouse at 30°C and 90% relative humidity for 1 week. The seedlings showing soft rot symptoms were photographed and used for bacterial reisolation. The strains were reisolated as described above, and PCR assays using primer pairs fD2/rP1 for 16S rDNA were performed for pathogen

confirmation. 16S rDNA sequence data from the reisolated strains were compared to those of the original strains isolated from banana. In another case, banana leaves were detached from seedlings at the five- or six-leaf stage. Entire carpododums filled with immature fruits were also detached from the plants and kept at high relative humidity for the pathogenicity test. Fifty microliters of bacterial suspension (10⁸ CFU/ml) of five representative strains was injected into each banana leaf and fruit, and injections of sterile water served as negative control treatments. The injected leaves and fruits were incubated at 30°C, and the injection sites were wrapped with wet cotton balls moisturized with sterile water.

Pathogenicity of the strains was confirmed on seedlings of tobacco, maize, rice, and sugarcane, and the potato tubers, cucumber, tomato, watermelon, Chinese cabbage, towel gourd, onion, carrot, papaya, apple, and pineapple. Inoculation was conducted as described above for banana leaves and fruits.

Prevalence survey. Between September and November 2010, banana plantations were surveyed for prevalence of soft rot in Guangdong province (Panyu district and Nansha district of Guangzhou city, Dongguan city, Zhongshan city, Zhuhai city, Gaozhou city, Huazhou city, Laozhou island, Donghai island, and Xuwen county of Zhanjiang city, Jieyang city, and Shantou city), Guangxi province (Nanning city), Fujian province (Zhangzhou city), Hainan province (Chengmai county, Lingao county, Ledong county, Dongfang city, and Chenwang county). Banana plants with soft rot symptoms were collected and used for pathogen isolation and characterization. Plant tissues were assayed directly by PCR using ITS primers LF/LR (LF 5'-TTCGTCTAGAGGCCAGGAC-3' and LR 5'-TCAGCTTGTCCGGATTGTT-3') (16), and bacteria were isolated as describe above.

Results

Characterization of *D. zeae* strains. Strains from diseased bananas produced round or nearly round colonies on nutrient agar that were slightly convex, opaque, wrinkled, and dull. When cultured at 32°C for 24 to 48 h, the colonies appeared gray (Fig. 2). Over time, the gray pigment disappeared, and the bacterium did not produce the gray pigment with subsequent subcultures.

Five representative strains were selected for biochemical analysis. They displayed pectinolytic activity, production of indole, and grew at 39°C. BIOLOG analysis identified the strains as *Pectobacterium chrysanthemi*, and the five strains showed the highest similarities of 0.638, 0.718, 0.729, 0.718, and 0.523 with *P. chrysanthemi*. The five strains from banana catabolized L-arabinose,

Table 1. Polymerase chain reaction (PCR) primer sequences used to analyze banana soft rot strains collected in China and corresponding predicted amplicon sizes

Locus	Primers	Sequence	Product size (bp)	Reference
16S rDNA	fD2 rP1	5'-AGAGTTTGATCATGGCTCAG-3' 5'-GGTTACCTTGTTACGACTTC-3'	1,503	Weisburg et al. (30)
<i>dnaX</i>	<i>dnaXf</i> <i>dnaXr</i>	5'-TATCAGGTYCTTGCCCGTAAGTGG-3' 5'-TCGACATCCARCGCYTTGAGATG-3'	536	Slawiak et al. (25)
<i>gryB</i>	<i>gryBf1</i> <i>gryBr1</i>	5'-ATGTCGAATTCTTATGACTCCTC-3' 5'-TCARATATCRATATTCGCGCTTTC-3'	2,412	
<i>recA</i>	<i>recAf</i> <i>recAr</i>	5'-GGTAAAGGGTCTATCATGCG-3' 5'-CCTTCACCATAcataATTGGA-3'	700	Waleron et al. (29)

Table 2. Different characteristics of six *Dickeya* species^a and the banana soft rot pathogens isolated in China^b

Characteristics	Growth at 39°C	Myo-inositol	cis-Aconitete	D-Melibiose	Mannitol
<i>D. dadantii</i> + <i>D. zeae</i>	+	+	+	+	+
<i>D. chrysanthemi</i> bv. <i>parthenii</i>	+	+	–	+	+
<i>D. chrysanthemi</i> bv. <i>chrysanthemi</i>	+	+	P	+	+
<i>D. dieffenbachiae</i>	+	+	P	–	+
<i>D. dianthicola</i>	–	+	–	P	+
<i>D. paradisiaca</i>	P	–	–	P	–
<i>Dickeya</i> sp. from banana	+	+	+	+	+

^a Data of six *Dickeya* species referred to Samson et al. (24).

^b + Indicated positive, – indicated negative, and P indicated partially positive.

myo-inositol, D-mannose, *cis*-aconitate, D-melibiose, and mannitol, but failed to utilize D-trehalose, D-arabitol, β -gentiobiose, and sorbitol. Compared to the known characteristics of six *Dickeya* species including *D. zeae*, *D. dadantii*, *D. paradisiaca*, *D. chrysanthemi*, *D. dieffenbachiae*, and *D. dianthicola*, the banana pathogen and *D. dadantii* or *D. zeae* grew at 39°C, catabolized myo-inositol, *cis*-aconitate, D-melibiose, and mannitol, while *D. paradisiaca* did not grow at 39°C nor use these four carbon sources. *D. chrysanthemi*, *D. dieffenbachiae*, and *D. dianthicola* also differed by one, two, and three characteristics with the banana pathogen, respectively (Table 2). The phenotypic characteristics of the banana pathogen were most consistent with *D. dadantii* and *D. zeae*.

Phylogenetic analysis based on housekeeping genes. One typical strain of the five banana strains, MS1, was used for genetic analysis, and the sequence data for four conserved genes were deposited in GenBank (GenBank accession numbers JQ301888 for 16S rDNA, JQ284038 for *dnaX*, JQ284039 for *gryB*, and KC793203 for *recA*). BLAST analysis indicated that all of these sequences were 99% similar to the sequences of *D. zeae*.

Twenty-eight strains of different *Dickeya* species with complete sequences of *dnaX*, *gryB*, and *recA* in NCBI GenBank were retrieved for analysis, along with an out group control, *Pectobacterium carotovorum* subsp. *carotovorum* PC1. The combined sequences of these three conserved genes of *Dickeya* strains consisted of 1,715 nucleotides (Supplementary Figure 1), of which 1,305 were constant, 410 were variable (388 were parsimony informative), and 22 were singleton. The phylogenetic analysis of concatenated sequences yielded six well-separated clades in the subgroup of *Dickeya* sp. (Fig. 3), and all the clades represented respective *Dickeya* species with each type strain, of which *D. para-*

disiaca clade was distinct from the other five species. In the *D. zeae* clade, MS1 clustered with *D. zeae* NCPPB3532, *D. zeae* NCPPB2538, *D. zeae* EC1, and *D. zeae* ZJU1202; strains EC1 and ZJU1202 were isolated from rice in Guangdong province. They formed a strongly supported subclade with 100% bootstrap values, which was significantly different from other *Dickeya* species. The phylogenies strongly supported the lineages of each gene leading to the classification of the banana pathogen as *D. zeae*.

Pathogenicity studies of *D. zeae* from banana. All five strains of the banana pathogen caused vascular tissue collapse or parenchyma necrosis. Banana leaves, fruits, and seedlings showed soft rot symptoms within 48 to 72 h of inoculation with bacterial strains isolated from banana. Negative control plants did not develop symptoms. The injection of bacterial suspensions into leaf veins caused the leaves to appear water-soaked and wilted. The injection of bacterial suspensions into the stem base resulted in rotting of the whole plant and emission of a foul odor. Fruit inoculation caused the internal fruit rot. The symptoms caused by MS1 are shown in Figure 4A, B, and C. The results for other strains were similar; therefore, their data were not presented. *D. zeae* was reisolated from infected seedlings and confirmed to be identical to the original pathogen based on 16S rDNA sequence comparison.

Dickeya species have a wide host range and induce soft rot on different plants species. *D. zeae* infected most of the plant species that were inoculated, including potato (Fig. 4D), cucumber (Fig. 4E), tomato (Fig. 4F), watermelon, cabbage (Fig. 4G), onion, carrot (Fig. 4H), towel gourd (Fig. 4I), tobacco, rice, maize, and sugarcane. In most cases, the rot was accompanied by a sharp odor. Moreover, inoculated rice plants showed severe foot rot symptoms 1 week after inoculation (Fig. 4J). Inoculated corn and sugarcane

Fig. 3. Phylogenetic analysis of MS1 and *Dickeya* strains based on concatenated sequences of *dnaX*, *gryB*, and *recA* genes. Confidence values on the branches were obtained for 1,000 bootstrap replicates with Mega version 5.1 software. Twenty-eight *Dickeya* strains were used for phylogenetic analyses, and the sequences of 15 strains were retrieved from whole-genome sequences as indicated with asterisks. Type *Dickeya* strains (22) are shown in boldface. *D. dadantii* Ech586, *D. zeae* Ech1591, and *D. dadantii* Ech703 were suggested to be reclassified as *D. zeae* Ech586, *D. chrysanthemi* Ech1591, and *D. paradisiaca* Ech503, respectively (19).

seedlings showed stalk rot or foot rot symptoms. However, the pathogen did not induce soft rot on papaya, apple, and pineapple fruits (Table 3).

Disease prevalence in mainland China. Banana soft rot was first observed in September 2009 in Guangzhou city, and spread

rapidly through the banana-growing regions of China. The disease usually had a peak incidence between September and November. Sixty-four symptomatic banana plants were sampled from 16 locations of south China. Of these, *Dickeya* strains were detected in 56 plants by PCR using specific ITS primers (Table 4). Thirty-nine

Fig. 4. Symptoms of soft rot of different crops after inoculation with bacterial suspensions of MS1. **A**, Banana leaf 48 h after inoculation; **B**, banana fruits 48 h after inoculation; **C**, banana seedlings 72 h after inoculation; **D**, potato slices 48 h after inoculation; **E**, cucumbers 24 h after inoculation; **F**, tomatoes 16 h after inoculation; **G**, Chinese cabbages 48 h after inoculation; **H**, carrots 24 h after inoculation; **I**, towel gourds 24 h after inoculation; **J**, rice seedlings a week after inoculation. Uninfected materials were inoculated with sterile water as controls.

Table 3. Pathogenicity of the banana soft rot pathogen *Dickeya zeae* MS1 on various plant species^a

Plant	Pathogenicity	Plant	Pathogenicity	Plant	Pathogenicity
Potato tube	+	Towel gourd	+	Carrot	+
Cucumber	+	Onion	+	Papaya	–
Tomato	+	Tobacco	+	Pineapple	–
Watermelon	+	Rice	+	Sugarcane	+
Chinese cabbage	+	Maize	+	Apple	–

^a + Indicated positive, – indicated negative.

Table 4. Isolation and polymerase chain reaction (PCR) validation of strains of the banana soft rot pathogen from various locations in China

Location	Cultivar	Gene type	Date	Quantity of samples	Positive by PCR detection	Positive by pathogen isolation ^a
Dongguan Guangdong	Musa Cavendish var. Brazil	AAA	2010.09.08	3	3	2
Panyu Guangzhou Guangdong	Guangfen-1 (dwarf banana)	ABB	2010.09.15	2	2	2
Nansha Guangzhou Guangdong	Guangfen-1 (dwarf banana)	ABB	2010.09.22	5	4	3
Zhongshan Guangdong	Guangfen-1 (dwarf banana)	ABB	2010.09.28	2	2	2
Zhuhai Guangdong	Guangfen-1 (dwarf banana)	ABB	2010.10.08	3	3	2
Jieyang Guangdong	Musa Cavendish var. Brazil	AAA	2010.10.08	1	1	1
Shantou Guangdong	Musa Cavendish var. Brazil	AAA	2010.10.08	1	1	0
Gaozhou Guangdong	Musa Cavendish var. Brazil	AAA	2010.10.28	3	3	2
Huazhou Guangdong	Musa Cavendish var. Brazil	AAA	2010.10.28	2	2	0
Zhanjiang Laozhou Island Guangdong	Musa Cavendish var. Brazil	AAA	2010.10.28	8	7	4
Xuwen Guangdong	Musa Cavendish var. Brazil	AAA	2010.10.28	3	3	3
Zhangzhou Fujian	Tianbao	AAA	2010.10.20	8	7	4
Zhangzhou Fujian	Musa Cavendish var. B. F.	AAA	2010.10.20	3	3	1
Dongfang Hainan	Musa Cavendish var. Brazil	AAA	2010.11.3	9	6	5
Chenmai Hainan	Musa Cavendish var. Brazil	AAA	2010.11.3	5	3	3
Chenwang Hainan	Unnamed dwarf banana	ABB	2010.11.3	2	2	2
Nanning Guangxi	Guangfen-1 (dwarf banana)	ABB	2010.11.15	4	4	3
Total				64	56	39

^a All banana plants from which *Dickeya* strains could be isolated were detected as positive samples through PCR.

strains were isolated from these diseased plants, and all strains produced colonies with specific gray pigment that distinguished them from other *Dickeya* species. The disease was present in most of the surveyed locations, including Dongguan, Panyu Guangzhou, Nansha Guangzhou, Zhongshan, Zhuhai, Jieyang, Gaozhou, Laozhou island, Zhanjiang, and Xuwen of Guangdong province, Zhangzhou of Fujian province, Nanning of Guangxi province, and Dongfang, Chenmai, and Chenwang of Hainan province. The disease affected different banana cultivars, including AAA type, Musa Cavendish var. Brazil, local cultivars Tianbao (from Fujian) and Musa Cavendish var. B. F. (from Taiwan), ABB type, dwarf cultivar Guangfen-1 (from Guangzhou), and an unnamed dwarf banana cultivar (from Hainan).

Discussion

Dickeya sp., formerly named *Erwinia chrysanthemi* and *Pectobacterium chrysanthemi* (7), is a recently established genus (24) that includes necrotrophic pathogens responsible for soft rot in a wide range of hosts (18). According to host range assays, biochemical characteristics, phylogenetic analyses, and serological reactions, these gram-negative pathogens were divided into six species: *D. chrysanthemi*, *D. dianthicola*, *D. dieffenbachiae*, *D. paradisiaca*, *D. dadantii*, and *D. zeae* (4,24). However, there was no diagnostic test that allows accurate clarification of taxonomy until now (24). Since different species are closely related, or lack strains with standard biochemical or genetic characteristics, it is not sufficient to distinguish them based on biochemical reactions alone or single gene sequences analysis (12,19). Therefore, this study combined biochemical characteristics and multi-gene sequence analysis to determine the identity of the banana pathogen, because multi-gene sequence analysis can provide a better understanding of phylogeny information in bacteria (5). In this study, we analyzed the sequences of four genes including 16S rDNA, *dnaX*, *gyrB*, and *recA* to identify the banana pathogen. For *Dickeya* spp., *dnaX* gene sequences were used as identification tags for the classification of complex biovars (25). The *gyrB* gene is a recommended identification tag to describe the phylogenetic relationships among *Enterobacteriaceae* (2). The *gyrB* gene can also distinguish different strains at the species level (10,11,31); therefore, we designed a set of PCR primers to amplify the *gyrB* gene for the banana strains. The phylogenetic analysis based on *recA* sequences also provides an effective method for determining *Dickeya* strains to the species and intraspecies level (22). In virtue of multi-locus sequence analysis (MLSA) of *dnaA*, *gyrB*, and *recA*, different *Dickeya* strains formed six distinct subclades representing respective *Dickeya* species, supported with each type strain. However, it was also noteworthy that *D. dadantii* Ech586, *D. zeae* Ech1591, and *D. dadantii* Ech703 were not placed in their respective clades. The same conclusion was also illustrated in a previous study (19), and they were recommended to be reclassified as *D. zeae* Ech586, *D. chrysanthemi* Ech1591, and *D. paradisiaca* Ech503, respectively.

Through the use of biochemical characteristics and phylogenetic analysis of multi-locus sequences, the pathogen isolated from banana was determined to be *D. zeae*. First, the pathogen showed the most similar biochemical characteristics to *D. zeae* and *D. dadantii*, and differed in several characteristics from other species, especially *D. paradisiaca*. *Dickeya* species (*E. chrysanthemi*) that cause bacterial soft rot on banana were usually reported to be *D. paradisiaca*, as initially observed in Columbia (3,24). However, the strain affecting banana in Guangdong differed from the typical *D. paradisiaca* strains as indicated by biochemical characteristics and sequence analysis of conserved genes. According to BLAST analysis, *D. paradisiaca* did not appear in the list of the top 100 most similar sequences. Secondly, the banana strain clustered with *D. zeae* EC1 (23) and *D. zeae* ZJU1202 (15), both of which were isolated from rice in Guangdong province, and formed a distinguishable clade based on concatenated sequences of *dnaX*, *gyrB*, and *recA*. Interestingly, *D. zeae* from banana had some unique properties compared to *D. zeae* from rice in Guangdong province,

indicating that it is a specific *Dickeya* strain. For example, it has gray colonies (Fig. 2A), prefers anaerobic conditions, and lacks phytotoxins. The phytotoxin gene cluster, *zmsA*, was not detected in *D. zeae* MS1 using specific primers (32), and it did not produce an antibiotic effect against *E. coli* on LB medium (Supplementary Figure 2), while EC1 inhibited the growth of *E. coli* (32). The pathogenicity of MS1 is different from the rice strains, the diversity of strains colonizing different hosts. The difference between the banana and rice strains was also supported by grouping of the concatenated sequences. The banana strain and other *D. zeae* strains comprised a clade that was separate from *D. zeae* EC1, ZJU1202.

D. zeae has been shown to cause soft rot of banana in Columbia, Ivory Coast, Panama, and several Caribbean islands (24), but to our knowledge, this is the first report of banana soft rot caused by *D. zeae* in China. Recently, the disease has been found in most banana-growing areas in China, and our results indicate the causal pathogen has many potential hosts in the grasses, Crucifer, Solanaceae, Cucurbitaceae, and Liliaceae, which are important crops. Therefore, the pathogen should be studied to develop effective management strategies. Our findings will help to develop molecular tools for detection and ultimately effective control strategies for bacterial soft rot of banana.

Acknowledgments

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