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Isolation, Characterization, and Genomic Investigation of a Phytopathogenic Strain of *Stenotrophomonas maltophilia*

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ABSTRACT

Stenotrophomonas maltophilia is ubiquitous in diverse environmental habitats. It merits significant concern because of its increasing incidence of nosocomial and community-acquired infection in immunocompromised patients and multiple drug resistance. It is rarely reported as a phytopathogen except in causing white stripe disease of rice in India and postharvest fruit rot of Lanzhou lily. For this study, *Dickeya zeae* and *S. maltophilia* strains were simultaneously isolated from soft rot leaves of *Clivia miniata* in Guangzhou, China, and were both demonstrated to be pathogenic to the host. Compared with the *D. zeae* strains, *S. maltophilia* strains propagated faster for greater growth in lysogeny broth medium and produced no cellulases or polygalacturonases, but did produce more proteases and fewer extracellular polysaccharides. Furthermore, *S. maltophilia* strains swam and swarmed dramatically less on semisolid media, but formed a great many more biofilms. Both *D. zeae* and *S. maltophilia* strains isolated from clivia caused rot symptoms on other monocot hosts, but not on dicots. Similar to previously reported *S. maltophilia* strains isolated from other sources, the strain JZL8 survived under many antibiotic stresses. The complete genome sequence of *S. maltophilia* strain JZL8 consists of a chromosome of 4,635,432 bp without a plasmid. Pan-genome analysis of JZL8 and 180 other *S. maltophilia* strains identified 50 genes that are unique to JZL8, seven of which implicate JZL8 as the potential pathogen contributor in plants. JZL8 also contains three copies of Type I Secretion System machinery; this is likely responsible for its greater production of proteases. Findings from this study extend our knowledge on the host range of *S. maltophilia* and provide insight into the phenotypic and genetic features underlying the plant pathogenicity of JZL8.

Keywords: complete genome, host range, pathogenicity, *Stenotrophomonas maltophilia*

Stenotrophomonas maltophilia is the type species (species typica) in genus *Stenotrophomonas*, which was initially described as *Pseudomonas maltophilia* (Hugh and Ryschenkow 1961) and then as *Xanthomonas maltophilia* (Swings et al. 1983). It was eventually reclassified into *Stenotrophomonas* because it is nonpathogenic on plants, as are most species in the *Xanthomonadaceae* (Palleroni and Bradbury 1993). Although *S. maltophilia* was cosmopolitan in a wide range of environmental habitats, with soil and plants as its main biotope, locating preferentially in the plant rhizosphere (Berg et al. 1996; Juhnke and Desjardin 1989) and endophytically in plant roots and stems (Germida and Siciliano 2001; Krechel et al. 2002; Ryan et al. 2009; Taghavi et al. 2009). It can be used as a bioremediation agent for degrading complex compounds and pollutants (Binks et al. 1995; Oves et al. 2013; Ribitsch et al. 2012), as a biocontrol agent in the promotion of plant growth and health, and as a suppressor of plant pathogens (Berg and Ballin 1994; Messiha et al. 2007; Nakayama et al. 1999; Schmidt et al. 2012). In the genus *Stenotrophomonas*, *S. maltophilia* is the only species that was reported to cause human disease (Coenye et al. 2004; Ryan et al. 2009). The species *S. rhizophila* is not known for any pathogenic associations with humans or clinic environments (Berg and Martinez 2015), and therefore can be used as a harmless alternative for biotechnological application (An and Berg 2018).

Since the early 1980s, *S. maltophilia* has been reported to cause human infections in hospitals and has become a common opportunistic pathogen worldwide. It has increased mortality rates in immunocompromised patients and, in particular, patients with cystic fibrosis, demonstrating its multidrug resistance by forming biofilms on plastic, glass, and metal medical devices (Brooke 2013; Hernández et al. 2011; Lira et al. 2017; Sanchez et al. 2009). Secretion of extracellular enzymes, biofilm formation, hemolysis, and motility are reported as *S. maltophilia*'s main properties (de Abreu Vidipó et al. 2001; Pompilio et al. 2010). Several virulence regulators such as the PhoPQ two-component system, and the Ax21- and the DSF-quorum–sensing signaling systems, were found to mediate various virulence-associated processes in *S. maltophilia* (An and Berg 2018; An and Tang, 2018; Fouhy et al. 2007; Ryan et al. 2015). Up until now, *S. maltophilia* was only reported as a pathogen to two crops: rice (*Oryza sativa*, causing white stripe disease in India and Pakistan; Riaz et al. 2016; Singh et al. 2001) and Lanzhou lily (*Lilium davidii* var. *unicolor*, causing fruit soft rot in China; Ling et al. 2019).

In our previous study, we collected samples of soft rot diseased *Clivia miniata* from the Fangcun flower market in Guangzhou, China in 2017, and isolated *Dickeya zeae* strains (Hu et al. 2018) and *S. maltophilia* strains from the decayed center leaves. Both strains were verified as the pathogens responsible for the disease. This study

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compared the resultant symptoms of the two pathogens on hosts, and their virulence differentials including production of cell wall degrading enzymes (CWDEs), extracellular polysaccharides (EPSs), biofilm formation, cell motility, and nematode-killing capability. Given that *S. maltophilia* is characteristic of metabolic versatility and intraspecific heterogeneity (Berg et al. 1999; Palleroni and Bradbury 1993), we sequenced the complete genome of *S. maltophilia* strain JZL8 to find the specific genes that are associated with plant infection or host specificity on monocots.

MATERIALS AND METHODS

Sample collection of diseased *Clivia miniata* and pathogen isolation. Several pots of *C. miniata* plants with soft rot symptoms at the base of their leaves were collected from the Fangcun flower market in Guangzhou, China in 2017. Diseased leaves were removed from the plants, washed with sterile water, and then cut into ~1-cm^2 tissues. They were then subjected to surface sterilization of ~70% ethanol for 30 s, 5% sodium hypochlorite for 1 min, and sterile water; this surface sterilization was performed three times for each sample gathered. Tissues were placed onto sterile filter paper for drying at room temperature and transferred onto lysogeny broth (LB) agar plates, then inoculated at 28°C for 24 h. Bacterial colonies were picked and individually streaked on fresh LB plates for strain purification. Single colonies were picked and grown in 1 ml of LB medium with shaking at 200 revolutions per min (rpm) at 28°C. After 24 h, 200 µl of bacterial cultures was injected into the base of healthy clivia leaves, and plants inoculated with equivalent volume of LB liquid medium were set as the negative control. Plants were incubated in a growth chamber with conditions of 28 ± 2°C, 75 ± 15% relative humidity, and 12-h white light illumination (Shanghai YiHeng Scientific Instruments Co., Ltd, Shanghai, China; Li et al. 2020a).

Strains causing soft rot symptoms on leaves were further isolated and purified on LB agar plates, then compared with the original inoculated strains; identification was performed using 16S rDNA sequencing with the methods described in Hu et al. (2018). Briefly, the 16S rDNA fragments were amplified using the primer pairs of 27F/1492R (Coenye et al. 1999). Amplicons were purified with the PCR Purification Kit (Tiangen Biotech, Beijing, China). After purification, the products were cloned into the pMD19-T vector (TaKaRa, Dalian, China) and transformed into *Escherichia coli*-DH5α-competent cells (TransGen Biotech, Beijing, China). Transformants were selected from LB agar plates amended with 40 µg/ml of X-gal, 24 µg/ml of IPTG, and 100 µg/ml of ampicillin for PCR validation using the primer pair of M13F/M13R (5′-GTAAAACGACGGCCAGT-3′/5′-CAGGAAACAGCTATGAC-3′; Maxson et al. 2015). Positive clones were sequenced by the Sangon Biotech Company (Shanghai, China). (Sequences were then submitted to the NCBI GenBank database.)

Pathogen identification and phylogenetic analysis. 16S rDNA sequences of the isolated strains and the re-isolated strains were compared using the NCBI's Basic Local Alignment Search Tool (BLAST), and the same sequences were searched against the NCBI 16S ribosomal RNA sequences (Bacteria and Archaea) database with the tool BlastN (via the NCBI web interface). Then the closely related sequences were downloaded from the NCBI database and aligned using the ClustalW program implemented in the software MEGA v.6.06. The sequences were trimmed to 1,506 bp each and used for phylogenetic analysis by employing the neighbor-joining method with a maximum composite likelihood model and 1,000 bootstrap replicates. (The 16S rDNA sequences of JZL6 and JZL8 were deposited to NCBI as GenBank accession numbers MW082806 and MW020097, respectively.)

Measurement of bacterial growth curves. Bacterial strains to be tested were grown in LB medium overnight at 28°C. All the bacterial cultures were adjusted to OD_{600} = 2.0 and diluted into fresh LB medium (1:10). Dilutions were mixed thoroughly and aliquots of 500 µl were transferred into 2.0-ml tubes. Bacteria were grown by shaking at 200 rpm under 28°C. Cell density was measured at 0, 4, 8, 12, 16, 20, 24, 28, and 40 h, respectively. The experiment was repeated three times in triplicate.

Assessment on the aggressiveness of strains JZL7 and JZL8. Given that two species of bacteria were simultaneously isolated from the same samples, we wondered whether there is any synergistic relationship between them. Thus, strains JZL7 and JZL8 were selected to perform pathogenicity tests both individually and collaboratively according to the method described in Hu et al. (2018) and Xue et al. (2021), but with minor modification. Firstly, we visualized these two strains by importing plasmids $pBBP_{gdh}$ (Liao et al. 2018) and pLAFR-GFP (Shi et al. 2019), respectively, using triparental mating with the help of *E. coli* HB101(RK2013). Five microliters of each of the bacterial cultures JZL7pBBP_{gdh}, JZL8pLAFR-GFP, and a mixture of JZL7pBBP_{gdh}+JZL8pLAFR-GFP (all cultures were grown in LB medium until OD_{600} = 1.5), were, respectively, spotted onto the leaf bases regardless of whether or not they had punctured wounds on their surfaces. LB medium was spotted as the negative control. The leaves were placed on Petri dishes, sealed, and kept in a biological incubator as described above and observed and recorded every 24 h. Three days later, the diseased parts were cut off and weighed. Then, 1 ml of phosphate-buffered saline was added. Tissues were ground into homogenate, mixed evenly, and then 5 µl of it was applied to a slide for observation of the luminescence with a ZEN Blue Lite Microscope (Carl Zeiss, Jena, Germany). The homogenate was then diluted in series and 100 µl in each dilution gradient was spread onto fresh LB agar plates and incubated at 28°C overnight. Colonies between 30 and 300 CFUs were counted. Each assay was repeated three times in triplicate.

Pathogenicity assay against monocotyledonous and dicotyledonous plants. The tested strains were grown in LB medium until OD_{600} = 1.5, and different plant organs of monocotyledons and dicotyledons (listed in Table 1) were selected for inoculation, employing the following different inoculation methods: For banana (*Musa sapientum* ABB) and garlic sprout (*Allium sativum*), every 100 µl of bacterial cultures was injected into the basal stems of the seedlings. For cucumber (*Cucumis sativus*), Chinese cabbage (*Brassica pekinensis*), radish (*Raphanus sativus*), potato (*Solanum tuberosum*), carrot (*Daucus carota*), and taro (*Colocasia esculenta*), tubers were washed with tap water and dried with a paper towel, then surface-sterilized with 70% ethanol and sliced evenly into slices of ~5-millimeter (mm) thickness. Each slice was placed in a tray with moistened filter paper. Bacterial cells of 2 µl were applied to the inoculated parts after piercing them with pipette tips. Rice (*O. sativa*) leaves and Lanzhou lily (*L. davidii* var. *unicolor*) bulbs were surface-sterilized and inoculated with 10 µl of bacterial cultures. All trays were kept at 28°C until symptoms appeared. The same volume of LB medium was inoculated as a negative control. Each assay was repeated three times. The area of lesions was measured using the software Image J v.1.52a (the National Institutes of Health, Bethesda, MD).

Measurement of cell wall degrading enzymatic activities. CWDE were measured using a medium described in Zhou et al. (2016) and Lv et al. (2019). Briefly, assay medium was prepared, and 30 ml of each medium was poured into a 13- × 13-cm square plate. Subsequently, wells (5 mm in diameter) were punched after solidification. Samples of 40-µl bacterial cells were added to the wells after they had grown to OD_{600} = 1.8. All plates were incubated at 28°C. Pectate lyase (Pel) and polygalacturonase (Peh) assay plates were covered with 4 molar (M) HCl after 11 and 14 h, respectively. A cellulase (Cel) assay plate was stained with 0.1% (wt/vol) Congo Red for 15 min after 14 h, and treated 2× with 1 M of NaCl. Protease (Prt) activity was indicated by the transparent halos surrounding the wells after 24 h. The experiment was repeated three times in duplicate.

Assay of extracellular polysaccharide production. EPSs are important virulence factors for bacterial phytopathogens, comprising the main toxic factors leading to water-logging and wilting on plants

after infection (Condemine et al. 1999; Hayward 1991; Kao et al. 1992). For measuring the production of EPSs, single colonies of the tested strains were picked and transferred into 10 ml of LB medium for culture overnight at 28°C until OD_{600} = 1.8, then 3 ml of it was applied to 300 ml of LB medium and grown with shaking at 200 rpm for 12 h. Cultures were centrifuged at 8,000 rpm for 40 min, and then at 4,000 rpm for 20 min to obtain 250 ml of supernatants. Double volumes of absolute ethanol were added to the supernatants. They were then mixed thoroughly, and stored at 4°C overnight for precipitation, after which they were subjected to centrifugation at 8,000 rpm for 40 min. Finally, supernatants were discarded, and pellets were weighed after drying at 55°C overnight. The experiment was repeated three times in triplicate.

Measurement of cell motility. To determine the cell motility, media for the swimming (each liter contains 10 g of bactotryptone, 5 g of NaCl, and 3 g of agar) and the swarming (each liter contains 5 g of peptone, 3 g of yeast extract, and 4 g of agar) assays were prepared. One microliter of overnight bacterial culture (OD_{600} = 1.5) was spotted onto the center of a plate containing ~15 ml of each medium (Feng et al. 2019). The plates were incubated at 28°C for 20 h before measurement of the diameters of bacterial motility zone. Each experiment was repeated three times in triplicate.

Biofilm formation assay. The biofilm formation assay was prepared, although with minor modification, according to the procedure used by Chen et al. (2016). Overnight bacterial culture was diluted in 1:1,000 in super optimal broth medium, after which 3 ml was transferred to 14-ml glass tubes and incubated at 28°C for 24 h without shaking. Bacterial biofilm mass was stained with 2 ml of 0.1% crystal violet (wt/vol) for 15 min after pouring off the media gently and washing with water at least three times, and tubes were rinsed with water for three times until all unbound dye was removed. First, for measuring the biofilm mass, stained cells of each tube after dryness were decolorized with 3 ml of 70% ethanol. Then, for measuring the biofilm formation, 100 µl of diluted culture was transferred to each well of 96-well polypropylene microliter plates, and quantified under absorbance at 570 nm. The experiment was repeated three times in triplicate.

Analysis of bacterial antibiotic resistance. The antimicrobial susceptibility of strain JZL8 was tested to further investigate its drug resistance as described in Jorgensen and Ferraro (2009). Determination of minimal inhibitory concentration (MIC) of antibiotics in JZL8 strain was conducted by following the protocol from the Clinical and Laboratory Standards Institute (CLSI 2012), although with slight modification, as described in Liang et al. (2019). Strain JZL8 was cultured overnight (OD_{600} = 1.5) and inoculated into LB medium to obtain ~2.0×10^5 CFU/ml in each well containing antibiotics of twofold dilutions in 96-well plates, which were then cultured at 30°C and 200 rpm for 24 h. Antibiotics including ampicillin, kanamycin, streptomycin, rifampicin, gentamicin, ciprofloxacin, tetracycline, and Polymyxin B were used in this experiment at concentrations of 5, 10, 20, 40, 80, 160, 320, and 400 µg/ml. The minimum antibiotic concentration with no visible cells was defined as MIC. Three replicates were performed for each treatment.

Nematode-killing activity. Wild-type *Caenorhabditis elegans* was maintained according to Huedo et al. (2018), and the medium used for the worm-killing assays was as described by Tan et al. (1999). JZL strains were routinely grown in LB medium at 28°C and the *C. elegans* food-source *E. coli* OP50 at 37°C overnight, and 50 µl of the liquid culture was spotted onto the center of nematode-growth medium (slow-killing) or peptone, glucose, and sorbitol (fast-killing) agar plates and allowed to dry thoroughly. In the slow-killing assay, 50 µM of floxuridine (Fudr, Sigma-Aldrich, St. Louis, MO) was added into nematode-growth medium agar to inhibit hatching of nematode eggs (Houthoofd et al. 2004). *E. coli* OP50 culture was used as a negative control. The plates containing bacteria were incubated at 28°C and 37°C, respectively, overnight and cooled for at least 2 h at room temperature before adding 20 L4 stage or adult hermaphrodite worms. The plates were kept at 20°C, and live worms were scored. Each trial was repeated three times in triplicate.

Statistic analysis. All the experiments were repeated in three passes with duplicates or triplicates. For statistical analysis in Figures 2C, 3, and 4, the software GraphPad Prism (v.8.0, GraphPad Software, San Diego, CA) was used to performed Student's *t* test, where * indicates $P < 0.05$, ** indicates $P < 0.01$, *** indicates $P < 0.001$, and **** indicates $P < 0.0001$.

Genome sequencing, assembly, and annotation. Genomic DNA was extracted from JZL8 in LB medium culture using an EasyPure Bacteria Genomic DNA Kit (Transgen, Beijing, China), which was then subjected to quality control by agarose gel electrophoresis and further analyzed with Qubit Fluorometer Quantitation (Thermo Fisher Scientific, Waltham, MA). The complete genome sequence was determined by Novogene (Tianjin, China) using the PacBio RS II platform in combination with the Hiseq × Ten PE150. For PacBio sequencing, a 10 K SMRT Bell library was constructed using SMRTbell Template Kit (v.1.0, Pacific Biosciences, Menlo Park, CA). DNA fragments were end-repaired and ligated with universal hairpin adapters, and then sequenced in a PacBio RS II instrument. For HiSeq sequencing, genomic DNA was sheared into a series of ~350-bp fragments. The ends were repaired using the Next Ultra DNA Library Prep Kit for Illumina (New England BioLabs, Ipswich, MA). The sample was amplified with PCR and the products were cleaned-up and validated using a model No. 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). The resulting sample was then loaded on a HiSeq instrument (Illumina, San Diego, CA) for sequencing. A hybrid de novo genome assembly using both PacBio long-read and Illumina short-read sequencing data was carried out using the program Unicycler (v.0.4.7). (The sequencing data and genome assembly has been deposited in the NCBI database under the accession no. PRJNA656644.)

The JZL8 genome was annotated with Prokaryotic Genome Annotation Pipeline v.2019-05-13.build3740 (Tatusova et al. 2016). The

TABLE 1. Pathogenicity tests of strains isolated from *Clivia miniata* on some dicotyledonous and monocotyledonous plants

Inoculated plant			Organ	Inoculation amount, time	Diseased area (cm^2)			
Class	Family	Species			JZL1	JZL7	JZL6	JZL8
Dicotyledonous	*Cucurbitaceae*	*Cucumis sativus*	Fruit	2 µl, 24 h	0	0	0	0
	Brassicaceae	*Brassica pekinensis*	Stem	2 µl, 12 h	0	0	0	0
		Raphanus sativus	Tuber root	2 µl, 24 h	0	0	0	0
	Solanaceae	*Solanum tuberosum*	Tuber	2 µl, 24 h	0	0	0	0
	Umbel liferae	*Daucus carota*	Tuber root	2 µl, 24 h	0	0	0	0
Monocotyledons	*Amaryllidaceae*	*Clivia miniata*	Leaf	100 µl, 7 days	9.360 ± 2.000	9.780 ± 0.860	10.650 ± 1.550	11.820 ± 0.620
	Gramineae	*Oryza sativa*	Leaf	10 µl, 5 days	0.270 ± 0.060	0.320 ± 0.040	0.520 ± 0.100	0.530 ± 0.160
	Liliaceae	*Lilium davidii* var. *unicolor*	Bulbs	10 µl, 5 days	0.560 ± 0.070	0.610 ± 0.060	1.900 ± 0.330	1.680 ± 0.590
		Allium sativum	Tuber	100 µl, 5 days	12.361 ± 1.120	9.499 ± 1.725	15.617 ± 1.260	16.177 ± 0.901
	Musaceae	*Musa sapientum*	Stem	100 µl, 7 days	0.850 ± 0.440	1.000 ± 0.160	1.510 ± 0.130	2.000 ± 0.440
	Araceae	*Colocasia esculenta*	Tuber	2 µl, 5 days	1.770 ± 0.130	1.770 ± 0.300	4.650 ± 0.590	5.200 ± 0.500

software AMRFinder v.3.8.4 was used to identify antimicrobial resistance genes (Feldgarden et al. 2019); the software TXSScan was used to identify secretion systems (Abby et al. 2016); and the tool dbCAN2 (Zhang et al. 2018) was used to identify carbohydrate-active enzymes. Proteases were identified through a sequence search against the MEROPS database (Rawlings et al. 2010) with the tool BlastP (v.2.9.0, NCBI). The program Signal v.4.1 (Petersen et al. 2011) was employed to detect proteins with signal peptides.

Pan-genome analysis. Genome sequences of 436 *S. maltophilia* strains were downloaded, in total, from the NCBI RefSeq database, and all of them were evaluated using the tool checkm v.1.0.18 (Parks et al. 2015). After removing strains with genome integrity < 98% or contamination degree > 0.5%, a total of 181 *S. maltophilia* strains, including JZL8, were kept for the pan-genome analysis, and *S. rhizophila* QL-P4 was included as the outgroup. The tool Panaroo v.1.2.3 (Tonkin-Hill et al. 2020) was used in the pan-genome analysis to determine the accessory genome (specific genes found in only one genome) and the core genome (common genes mutually conserved).

The pan-genome analysis identified 1,080 core genes that are present in nearly all the *S. maltophilia* genomes. Translated protein sequences of these 1,080 genes were aligned individually with the software tool MAFFT v.7.455 (Katoh and Standley 2013), and then the default parameters and the corresponding coding sequences were accordantly aligned. Coding sequence alignments of all 1,080 genes were then concatenated for phylogenetic reconstruction using the software IQ-TREE v.1.6.12 (Nguyen et al. 2015). The best-fit model (GTR+G) was determined automatically using the tool ModelFinder, and the reliability of the phylogeny reconstructed was evaluated by 1,000 ultrafast bootstrap replicates. Pairwise average nucleotide identity values between genomes were calculated with the program fastANI v.1.3 (Jain et al. 2018). The program GGTREE (Yu et al. 2017) was used to visualize phylogenetic trees and associated data.

RESULTS

Strains isolated from soft rot clivia plants were classified as *D. zeae* and *S. maltophilia*, based on phylogenetic analysis. In our previous study, we isolated several *D. zeae* strains (JZL1, JZL2, and JZL7) from soft rot clivia plants obtained at the Fangcun flower market in Guangzhou in 2017, and validated them as the causal agents of this disease (Hu et al. 2018). Interestingly, *S. maltophilia* strains JZL6 and JZL8 were also isolated from the same tissues of the decayed leaves. Different from the morphology of *D. zeae* colonies, strains JZL6 and JZL8 produced yellow pigments, and propagated more rapidly than *D. zeae* colonies on LB plates (Fig. 1A). Clivia plants inoculated with strain JZL6 or JZL8 developed soft rot symptoms more quickly than those inoculated with *D. zeae* strains JZL1, JZL2, or JZL7 (Fig. 1B), suggesting that JZL6 and JZL8 are also causal agents of the soft rot disease of clivia. Comparison of the growth rates indicated that strains JZL6 and JZL8 not only grew faster but also reached a higher OD_{600} value (3.390 versus 2.399) than the *D. zeae* strains in LB medium (Fig. 1C).

To identify the taxonomic status of strains JZL6 and JZL8, we amplified their 16S rRNA gene sequences and found that there are only two nucleotides divergent between them, sharing, respectively, 99.87 and 99.93% identity with the *S. maltophilia* strain PEG-305. Phylogenetic analysis showed that strains JZL6 and JZL8 were placed in a clade consisting entirely of *S. maltophilia* strains (Supplementary Fig. S1), suggesting that they belong to this species. Also, their 16S rRNA sequences are, respectively, 98.90 and 99.20% identical to that of *S. maltophilia* strain Pb4-3, the pathogen of postharvest fruit rot in the Lanzhou lily (Ling et al. 2019). To our knowledge, this is the first report indicating clivia as the natural host plant of *S. maltophilia*, and is now the third report mentioning *S. maltophilia* as a phytopathogen in the world.

***D. zeae* and *S. maltophilia* infect clivia non-coordinately.** To evaluate the relationship between the two identified bacteria during disease development, we inoculated *D. zeae* strain JZL7 and *S. maltophilia* JZL8 on the clivia leaf bases individually and collectively, and observed the symptom development in different time points. Results showed similar disease progression of the two bacteria after non-wound inoculation, indicating that both *D. zeae* and *S. maltophilia* infect clivia leaves through natural openings, whereas leaves applied with *S. maltophilia* rotted faster than those inoculated with *D. zeae* at 2 days post-wound inoculation. Additionally, co-inoculation of the combined strains mixture indicated disease development similar to that caused by JZL7pBBP$_{gdh}$ (Fig. 2A). The number of CFUs in the decayed tissues seemed a rare difference between pure inoculation and co-inoculation, except that the tissues applied with JZL8pLAFR-GFP harbored more CFUs after 3 days post-wound inoculation (Fig. 2B and C).

***S. maltophilia* JZL strains only infect monocotyledonous plants with a stronger virulence than *D. zeae* JZL strains.** Our previous study revealed that *D. zeae* JZL1, JZL2, and JZL7 strains could only infect monocot hosts, but not dicotyledonous plants (Hu et al. 2018). To evaluate the host ranges of *S. maltophilia* JZL

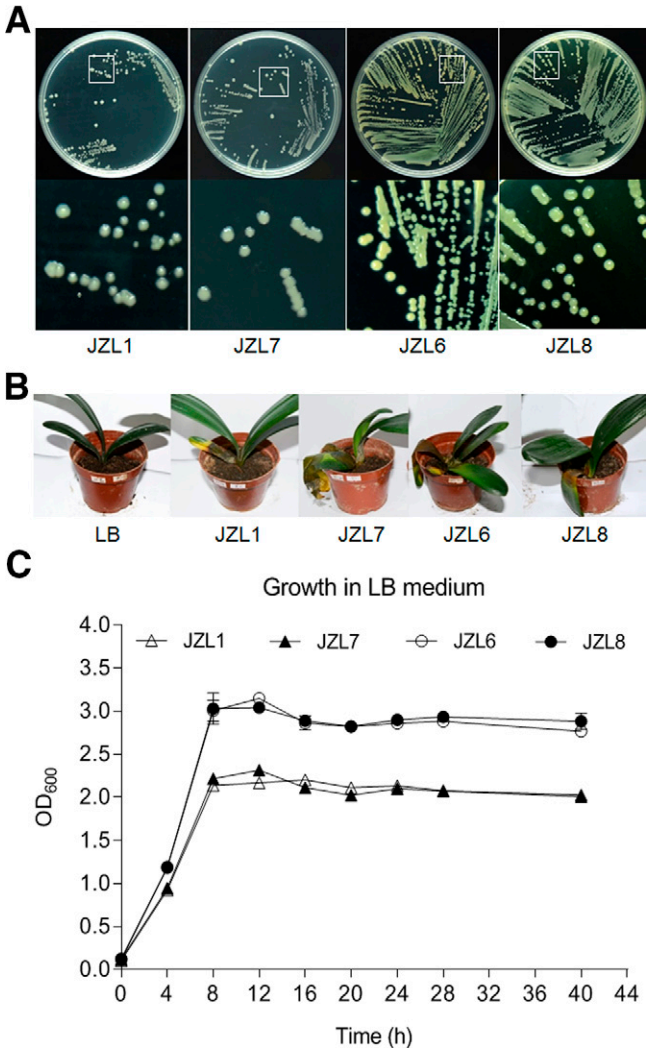

Fig. 1. Characteristics of the pathogenic strains isolated from soft rot clivia. **A,** Morphology of the *Dickeya zeae* JZL1 and JZL7 colonies, and *Stenotrophomonas maltophilia* JZL6 and JZL8 colonies, grown on lysogeny broth agar plates after 24 h. **B,** Symptoms of clivia inoculated with strains *D. zeae* JZL1 and JZL7, and *S. maltophilia* JZL6 and JZL8 after 7 days post-inoculation. The experiment was repeated three times. **C,** Growth curves of strains *D. zeae* JZL1 and JZL7, and *S. maltophilia* JZL6 and JZL8 isolated from clivia in lysogeny broth medium.

strains, we performed pathogenicity tests on various plant materials. Results showed that all *D. zeae* and *S. maltophilia* strains isolated from clivia could only infect monocotyledons, including *C. miniata*, *O. sativa*, *L. davidii* var. *unicolor*, *A. sativum*, *M. sapientum*, and *C. esculenta*, but not dicotyledonous plants including *C. sativus*, *B. pekinensis*, *R. sativus*, *S. tuberosum*, and *D. carota* (Table 1; Supplementary Fig. S2). Furthermore, disease symptoms caused by *S. maltophilia* strains JZL6 and JZL8 developed much faster than those caused by *D. zeae* strains JZL1 and JZL7 (Table 1; Supplementary Fig. S2), indicating that *S. maltophilia* JZL strains are more virulent than *D. zeae* JZL strains.

***S. maltophilia* JZL strains produce very few extracellular enzymes, except for more proteases than *D. zeae* JZL strains.** As an opportunistic pathogen, *S. maltophilia* was mostly reported to be isolated from animals and humans, and there is increasing evidence that extracellular Prts are the key proteins leading the incidence of hosts (Thomas et al. 2014). Our finding that *S. maltophilia* JZL strains could infect monocotyledonous plants (Table 1; Supplementary Fig. S2) raised the question of whether JZL strains produce plant CWDEs such as Cel, Peh, or Pel, for plant host invasion, the same as other phytopathogens such as *D. zeae*. Therefore, we measured the production of CWDEs of all the *S. maltophilia* and *D. zeae* JZL strains. Results showed that *S. maltophilia* JZL6 and JZL8 strains hardly produced any Cel, Peh, or Pel on the testing plates, but produced a large amount of Prt, >2.5-fold more than those produced by *D. zeae* JZL1 and JZL7 strains (Fig. 3).

***S. maltophilia* JZL strains are less motile, and produce more biofilms and EPSs.** The motility of bacterial pathogens is critical for them to establish infection. Thomas et al. (2014) tested the motility of numerous invasive and non-invasive *S. maltophilia* strains from clinical specimens, and found that swimming and swarming, but not twitching, were the prominent modes of motility pattern. They also found that the environmental strain LMG 959 was less motile than the clinical isolates (Thomas et al. 2014). Here, we tested and compared the swimming and swarming motility of *D. zeae* and *S. maltophilia* JZL strains on soft agar plates. Results showed that both *S. maltophilia* JZL strains swam and swarmed little on the plates (Fig. 4, A and B), which is significantly different from the *D. zeae* JZL strains but was similar to the characteristics of the environmental *S. maltophilia* strain LMG 959 (Thomas et al. 2014).

Under certain conditions, bacteria can be adsorbed on a specific solid surface and proliferate to form a membrane structure called a "biofilm" (Branda et al. 2005). *S. maltophilia* strains of both clinical and environmental origins have been reported to adhere to abiotic and living surfaces. Using a scanning electron microscope, Di Bonaventura et al. (2004) found that *S. mestophilia* SM33 could adhere to the plastic surface within 2 h, and formed a complete biofilm within 24 h. Here, we tested and compared the swimming and swarming motility of *D. zeae* and *S. maltophilia* JZL strains on SOBG medium. Results showed that both *S. maltophilia* JZL strains formed dramatically more biofilms than the *D. zeae* JZL strains (Fig. 4C), which is again significantly different from the *D. zeae* JZL strains, but is similar to that of the strain *S. mestophilia* SM33.

Polysaccharides are components of the extracellular matrix of bacterial biofilms that also play a role in resistance to antibiotics (Flores-Treviño et al. 2019). The EPS content of the strains was determined by the ethanol precipitation method. Results showed that *S. maltophilia* JZL strains produced significantly more EPSs than the *D. zeae* JZL strains (Fig. 4D).

***S. maltophilia* JZL strains has stronger nematode-killing ability.** Non-mammalian hosts such as nematodes are commonly used as model systems in the study of human bacterial pathogens, including *S. maltophilia* (Mahajan-Miklos et al. 2000). It has been demonstrated that the disruption of DSF signaling has a drastic effect on *S. maltophilia* E77 and M30, because the *rpfF* mutant shows reduced virulence in a *C. elegans* infection model, indicating that the killing of *C. elegans* by *S. maltophilia* can be exploited to identify novel virulence factors (Huedo et al. 2018). We therefore tested the nematode-killing dynamics of JZL strains. The results showed that all the JZL strains had nematocidal activities. In both slow-killing (Fig. 5A) and fast-killing (Fig. 5B) experiments, *S. maltophilia* JZL6 and JZL8 showed stronger nematode-killing ability than *D. zeae* JZL1 and JZL7 strains, indicating that *S. maltophilia* JZL strains have the same, or similar, animal infective ability as

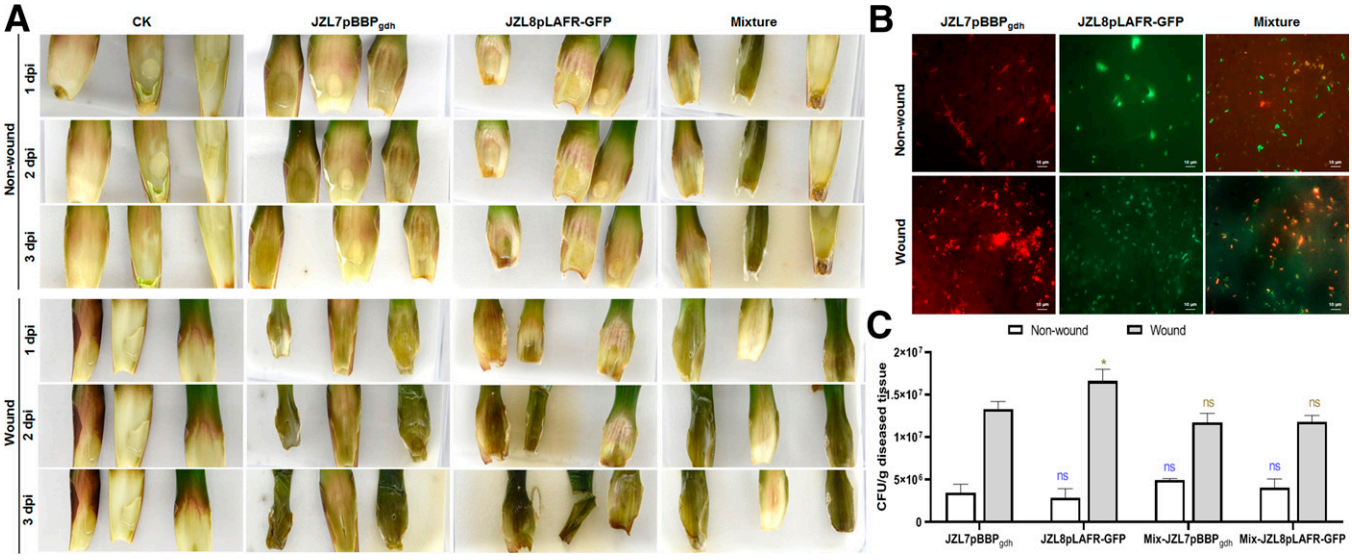

Fig. 2. Assessment on the aggressiveness of strains JZL7 and JZL. **A,** Plasmids pBBPgdh (Liao et al. 2018) and pLAFR-GFP (Shi et al. 2019) were respectively introduced into strains JZL7 and JZL8, and 50 µl of bacterial cultures (OD_{600} = 1.5) of the JZL7pBBPgdh, JZL8pLAFR-GFP, and the JZL7pBBPgdh+ JZL8pLAFR-GFP mixture were respectively spotted onto the leaf bases with non-wound and wound inoculation. Lysogeny broth medium was spotted as the negative control. The leaves were placed on Petri dishes, sealed, and then kept in a biological incubator as already described in the main text, and observed and recorded every 24 h. dpi = days postinoculation. **B,** Visualization of the pathogenic strain cells from the ground homogenate of symptomatic tissues. Symptomatic tissues had been cut off and ground into a homogenate, diluted 1,000×, and then 5 µl of it was applied to a slide for observation of the luminescence with a ZEN Blue Lite microscope (Carl Zeiss, Jena, Germany). **C,** Calculation of CFUs in the diseased tissues. The homogenate was diluted in series, with 200 µl in each dilution gradient spread onto fresh lysogeny broth agar plates and incubated at 28°C overnight. Colonies between 30 and 300 CFUs on plates were counted. Each assay was repeated three times in triplicate. The software GraphPad Prism v.7.0 (GraphPad Software, San Diego, CA) was used to perform the Student's *t*-test. Values were compared with that of JZL7pBBPgdh in the respective group. Here, "ns" indicates not significant; * indicates $P < 0.05$.

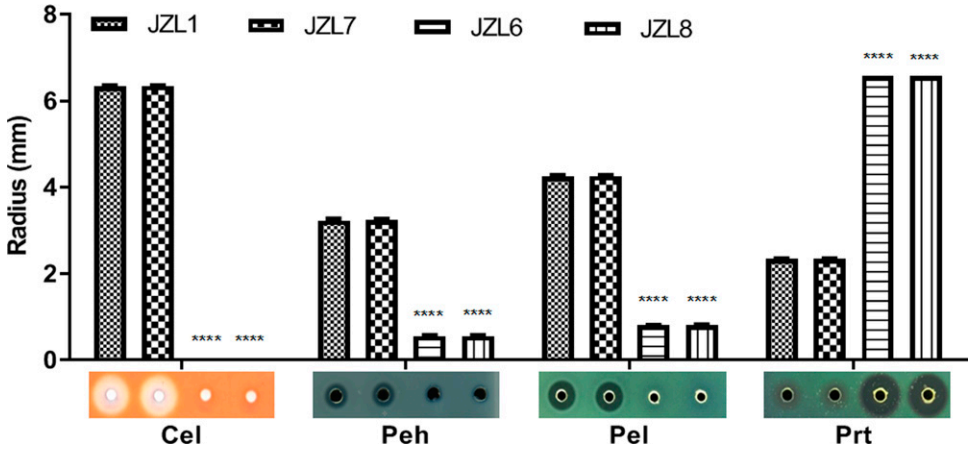

Fig. 3. Extracellular cell wall degrading enzymes produced by JZL strains. Samples of 40 µl bacterial cells (OD_{600} = 1.8) were added to the 5-mm-diameter assay plate wells and incubated at 28°C. The cellulase (Cel) assay plate was stained with 0.1% (wt/vol) Congo Red for 15 min after 14 h, and de-colorized with 1 M of NaCl twice. The pectate lyase (Pel) and polygalacturonase (Peh) assay plates were each treated with 4 M of HCl after 11 and 14 h, respectively. Photos of the protease (Prt) assay plate were then taken after 24 h without any further treatment. The experiment was repeated three times in duplicate. **** = $P < 0.0001$ (Student's *t* test).

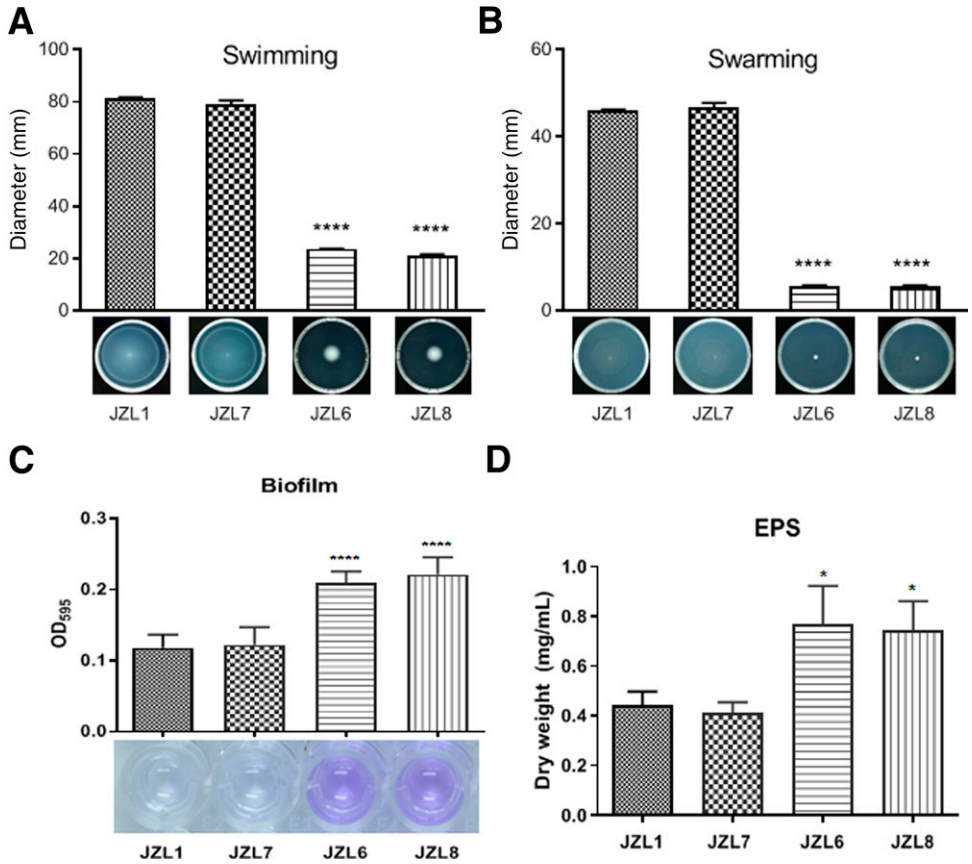

Fig. 4. Cell motility, biofilm formation, and exopolysaccharide (EPS) production of JZL strains. **A,** Swimming motility of JZL strains. **B,** Swarming motility of JZL strains. One microliter of bacterial culture (OD_{600} = 1.5 in lysogeny broth medium) was spotted onto the center of a plate containing ~15 ml of semisolid swimming or swarming medium, which was then incubated at 28°C for 20 h before measurement of the diameters of bacterial motility zone. **C,** Biofilm formation of JZL strains. Overnight bacterial culture was diluted 1:1,000 in super optimal broth medium, 3 ml of which was transferred to 14-ml glass tubes and incubated at 28°C for 24 h without shaking. Bacterial biofilm mass was stained with 2 ml of 0.1% crystal violet (wt/vol) for 15 min after pouring off the media and washing with water at least three times, and tubes were rinsed with water for three times until all unbound dye was removed. For measuring the biofilm mass, stained cells of each tube after dryness were de-colorized with 3 ml of 70% ethanol, and quantified under absorbance at 570 nanometers (nm). **D,** EPS production of JZL strains. One-milliliter samples of each of the three bacterial cultures (OD_{600} = 1.8) were applied into 100 ml of lysogeny broth medium and grown with shaking at 200 revolutions per min (rpm) for 12 h; these were centrifuged at 8,000 rpm for 40 min, and then at 4,000 rpm for 20 min, to obtain 180-ml supernatants. Double volumes of absolute ethanol were added to the supernatants, mixed thoroughly, stored at 4°C overnight for precipitation, and centrifuged at 8,000 rpm for 40 min. Finally, supernatants were discarded, and pellets were weighed after drying at 55°C overnight. All the assays were repeated three times in triplicate. * = $P < 0.05$; **** = $P < 0.0001$ (Student's *t* test).

S. maltophilia strains isolated from clinical sources (Brooke 2012). Because strains JZL6 and JZL8 have similar phenotypes associated with virulence, we used strain JZL8 for further characterization.

***S. maltophilia* JZL8 is multidrug-resistant.** According to Brooke (2012), *S. maltophilia* exhibits resistance to a broad range of antibiotics; this was suggested to have been acquired in natural non-human environments and is not solely from the use of antibiotics in medical/clinical settings. We then tested the sensitivity of strain JZL8 to eight different antibiotics. Results showed that strain JZL8 resisted all the tested antibiotics except for Polymyxin B, with >400 μg/ml of MIC to ampicillin, kanamycin, streptomycin, gentamicin, and rifampicin; 320 μg/ml of MIC to ciprofloxacin; and 40 μg/ml of MIC to tetracycline (Table 2). Our findings here are consistent with the report of Nicodemo and Paez (2007) on the resistance of *S. maltophilia* strains to most clinically used antibiotics, such as carbapenem, β-lactamases, aminoglycoside, and so on.

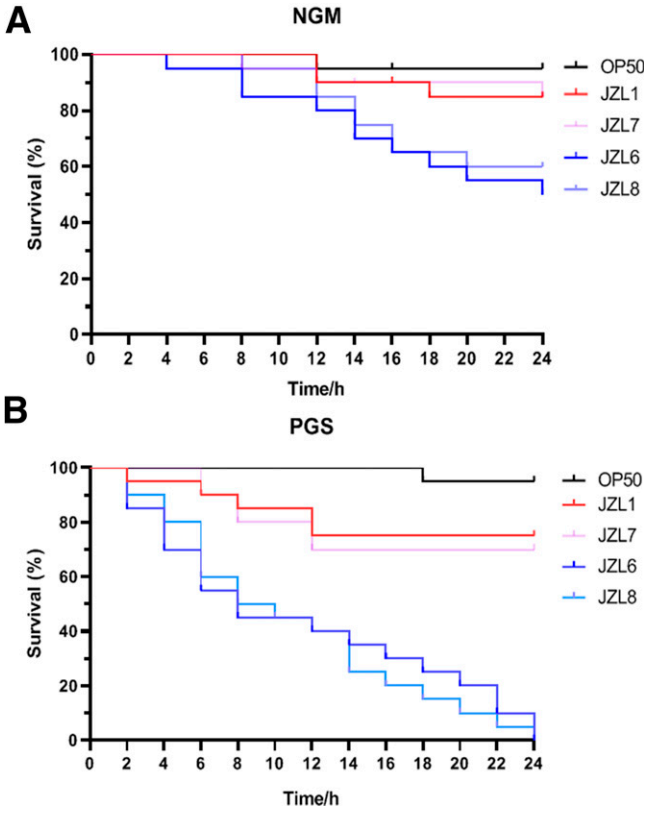

Fig. 5. Worm-killing ability of JZL strains toward *Caenorhabditis elegans*. **A,** Nematode growth medium (NGM)'s slow-killing ability of JZL strains. **B,** The peptone, glucose, and sorbitol (PGS) combination medium's fast-killing ability of JZL strains. JZL strains were grown in lysogeny broth medium at 28°C and the *C. elegans* food-source *Escherichia coli* OP50 (negative control) at 37°C overnight, and 50 μl of the liquid culture then spotted onto the center of NGM agar plates or PGS agar plates and allowed to dry thoroughly. In the slow-killing assay, 50 micromolar (μM) of floxuridine (Fudr, Sigma-Aldrich, St. Louis, MO) was added into NGM agar to inhibit hatching of nematode eggs. The plates containing bacteria were incubated overnight at 28°C and 37°C, respectively, then cooled for at least 2 h at room temperature before adding 20 L4 stage or adult hermaphrodite worms. The plates were kept at 20°C, and live worms were scored. Three replicates were performed for each treatment.

TABLE 2. The sensitivity of *Stenotrophomonas maltophilia* JZL8 to antibiotics

Antibiotics	Minimal inhibitory concentration (μg/ml)
Ampicillin	>400
Kanamycin	>400
Streptomycin	>400
Gentamicin	>400
Rifampicin	>400
Ciprofloxacin	320
Tetracycline	40
Polymyxin B	<5

Sequencing and analysis of the *S. maltophilia* JZL8 genome. To understand the pathogenic mechanism of strain JZL8 to plants at the genetic level, we sequenced the complete genome of JZL8 through high-throughput sequencing, resulting in 5,981,584 pairs of PE150 sequences with a total base number of 1,794,475,200 and coverage of 387×, and 175,144 PacBio sequences with a total base number of 1,434,803,371 and coverage of ∼310×. The strain JZL8 genome consists of a single, circular chromosome of 4,635,432 bp with a guanine-cytosine (GC) content of 66.32%; it contains 4,141 protein-coding genes (Fig. 6), as well as 13 rRNA, 73 tRNA, one ncRNA, one RNase P RNA, one SRP RNA, one tmRNA, and two active prophages. The positive and negative strands each contain two operons of ribosomal components, adjacent to each other, with 16S-23S-5S organization except for the second operon located on the positive strand, which has an unusual organization of 16S-23S-5S-5S (Fig. 6, red color). We have performed systematic functional annotation of the JZL8 genome, which provides insights to better understand the biology of JZL8. For instance, as mentioned above, we showed that *S. maltophilia* strains JZL6 and JZL8 could not produce cellulose, but had weak polygalacturonase and pectin lyase activity (Fig. 3). Our analysis of the JZL8 genome for this article identified dozens of glycoside hydrolases (Supplementary Table S2), thus explaining the weak pectin degradation enzymatic activity of the strains (Fig. 3). Moreover, we also identified several drug-resistant genes in the JZL8 genome, including *JZL8_001839*, *JZL8_002082*, *JZL8_002300*, *JZL8_003275*, and *JZL8_003919*, which have been predicted to convey resistance to kanamycin, aminoglycoside, carbapenem, β-lactam, and rifamycin, respectively. These predictions are well in line with our experimental results that JZL8 is resistant to all of these antibiotics (Table 2).

Comparative genomic analysis of JZL8 uncovers potential determinant genes of its pathogenicity on plants. We then performed pan-genome analysis to compare the JZL8 genome with high-quality genomes of 180 other *S. maltophilia* strains (genome integrity > 98% and contamination degree < 0.5%). Based on gene presence–absence patterns, we classified the 4,141 protein-coding genes of JZL8 into the following categories: nine proteins that were discarded for incompleteness or for possessing fewer than 34 amino acids, with the remaining proteins organized into 4,125 orthologous groups, comprising 1,080 core genes (99% ≤ strains ≤ 100%), 2,022 soft core genes (95% ≤ strains < 99%), 778 shell genes (15% ≤ strains < 95%), and 307 cloud genes (0% ≤ strains < 15%; Supplementary Fig. S3). JZL8 is the only strain that has been reported to infect plants among the *S. maltophilia* strains we analyzed; the vast majority of the other strains are either clinical isolates or animal pathogens. Therefore, we focused our investigation on genes that are specifically present or absent in JZL8, as they most likely contribute to the unique pathogenic characteristics of JZL8. Results showed that JZL8 does lack two genes that are present in >95% of the other 180 *S. maltophilia* strains – one to encode an RNA-splicing ligase RtcB and the other to encode an α-/β-fold hydrolase. Inversely, JZL8 has 50 unique genes that are absent in the other 180 *S. maltophilia* strains (Supplementary Table S1). Notably, seven of these JZL8-unique genes (ranging from *JZL8 003959* to *JZL8 003965*) are also absent in nearly all *S. maltophilia* genomes in the NCBI RefSeq database (Supplementary Table S1).

Proteins encoded by these seven genes include a hypothetical protein, two TonB-dependent receptors (TBDRs), a PepSY domain-containing protein, a superoxide dismutase (SOD), an α-/β-hydrolase, and a tyrosine-type recombinase/integrase. We searched the NCBI RefSeq database for homologs of these genes and found that this gene cluster as a unit is highly conserved in *Stenotrophomonas indicatrix* SC-N050 (amino acid identifies ranging from 88.89 to 96.76%;

Supplementary Table S1), the isolation source for which was unknown. In addition, interestingly, this cluster is also partially present in the genomes of several bacteria from plant sources, including *S. rhizophila* DSM14405 (isolated from the root of *Brassica napus*), *Stenotrophomonas* sp. 364 (isolated from pine tree), *Stenotrophomonas* sp. LM091 (isolated from *Citrus reticulata* × *Citrus sinensis*), and also *Xanthomonas oryzae* BB156-2 (isolated from rice; Fig. 7).

We also examined the best matches of these JZL8-specific genes outside *Stenotrophomonas*. The first TBDR shares 66.52 and 67.07% identities with that of *X. campestris* pv. *vesicatoria* 85-10 (pepper pathogen; Keshavarzi et al. 2004) and *X. perforans* NT1 (isolated from symptomatic lesions of *Solanum lycopersicum*), respectively. The PepSY domain-containing protein is 78.65% identical to that of *X. cucurbitae* ATCC 23378 isolated from *Cucurbita maxima*. The SOD gene is 88.89% identical to that of *X. floridensis* WHRI 8848 (isolated from watercress) and 81.82% to that of *X. oryzae* X8-1A (isolated from rice). The second TBDR gene is 82.16% identical, and the α-/β-hydrolase gene is 73.15% identical, respectively, to that of *X. nasturtii* WHRI 8853 isolated from watercress (Supplementary Table S1). Altogether, our results showed that the closest homologs of the seven JZL8-specific genes, at the levels of both gene cluster and individual genes, are mostly found in plant pathogens–strongly suggesting that these genes might be important for the pathogenicity of JZL8 on plants.

Characterization of virulence factors in JZL8. In the study of pathogenic microorganisms, it is of major interest to characterize their virulence factors and, particularly, the complex secretion systems that direct virulence factors either at the bacterial cell surface into the environmental extracellular milieu or into the host cell cytosol (Bleves et al. 2010; Costa et al. 2015). Therefore, we identified the genes encoding secretion systems in JZL8 and compared them with other *S. maltophilia* strains (Supplementary Fig. S2). Our analyses showed

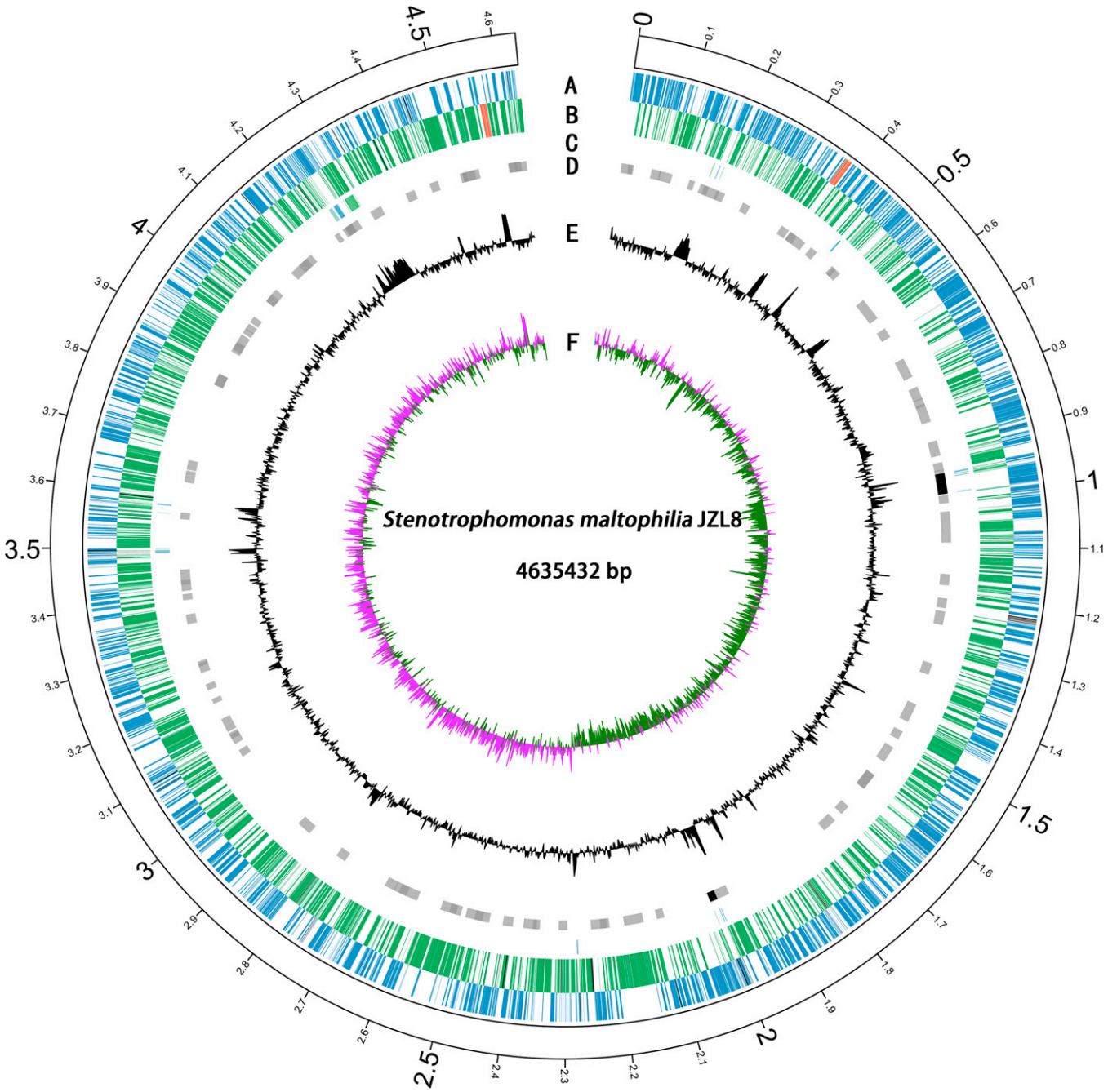

Fig. 6. Circular chromosome map of *Stenotrophomonas maltophilia* strain JZL8. The circles from outside to inside represent **A,** features of the positive strand, showing coding sequence (CDS) (blue), rRNA (red), and pseudogenes (black); **B,** features of the negative strand, showing CDS (green), rRNA (red), and pseudogenes (black); **C,** unique genes of strain JZL8; **D,** prophage sites, both active (black) and inactive (gray); **E,** guanine-cytosine (GC) content; and **F,** GC-skew.

that JZL8 and its closely related *S. maltophilia* strains all possess one conserved set of type-II and -IV secretion systems (T2SS and T4SS), but lack any T3SS or T6SS. They also have a variable number of T1SS; for instance, while strains MS_2008 and 22_SMAL do not have any T1SS, three copies of T1SS were found in JZL8 and two T1SS copies were present in the strains isolated from either hospital washroom sink samples or swine farm sewage (Fig. 8).

DISCUSSION

Plant diseases caused by more than one pathogen have been reported. Most of them have been proven to involve synergistic interactions in plants to enhance the severity of disease after co-infection (Lamichhane and Venturi 2015). In this study, we isolated *D. zeae* and *S. maltophilia* JZL strains from the same tissue of clivia soft rot, and both of them could result in soft rot symptoms with no synergistic relationship (Figs. 1A and 2). This is the first report that *S. maltophilia* could infect clivia. Interestingly, JZL strains shares high similarity to *S. maltophilia* Pb4-3 in the 16S rDNA sequences (Supplementary Fig. S1). That strain was isolated from the postharvest fruit rot of Lanzhou lily (Ling et al. 2019). All these reports should draw our attention to the risk of *S. maltophilia* as the causative agent of plant disease.

So far, the lack of a publicly available phytopathogen *S. maltophilia* complete genome sequence was limiting our understanding of its virulence mechanisms and pathogenicity determinants. In this study, we compared the genomes of strain JZL8 and another 180 strains obtained from plant, animal, environmental, and clinical habitats (Supplementary Fig. S3), and identified seven unique genes gathering in a gene cluster (Fig. 7), which are probably responsible for the pathogenicity on plants; one gene encodes a hypothetical protein; it is accompanied by two TBDR genes, a PepSY domain-containing protein gene, a SOD gene, an α-/β-hydrolase gene, and a tyrosine-type recombinase/integrase gene. TBDRs are outer member proteins actively transporting ferric ions, vitamin B12, nickel complexes, and carbohydrates (Noinaj et al. 2010). In *X. campestris* pv. *campestris*, TBDR transports sucrose with a very high affinity, acting with an amylosucrase and a regulator to utilize sucrose, required for full pathogenicity on *Arabidopsis*; this shows its importance for the adaptation to host plants (Blanvillain et al. 2007). This plant carbohydrate scavenging through TBDRs was further demonstrated as a feature shared by phytopathogenic and aquatic bacteria (Blanvillain et al. 2007). In *X. oryzae* pv. *oryzae* PXO99^{A}, there are 37 TBDRs in the genome, many of which are under the control of the PhoBR regulatory system (Zheng et al. 2018). Deletion of one of the TBDR coding genes, *tdrxoo*, affected bacterial growth in vitro; it reduced bacterial motility

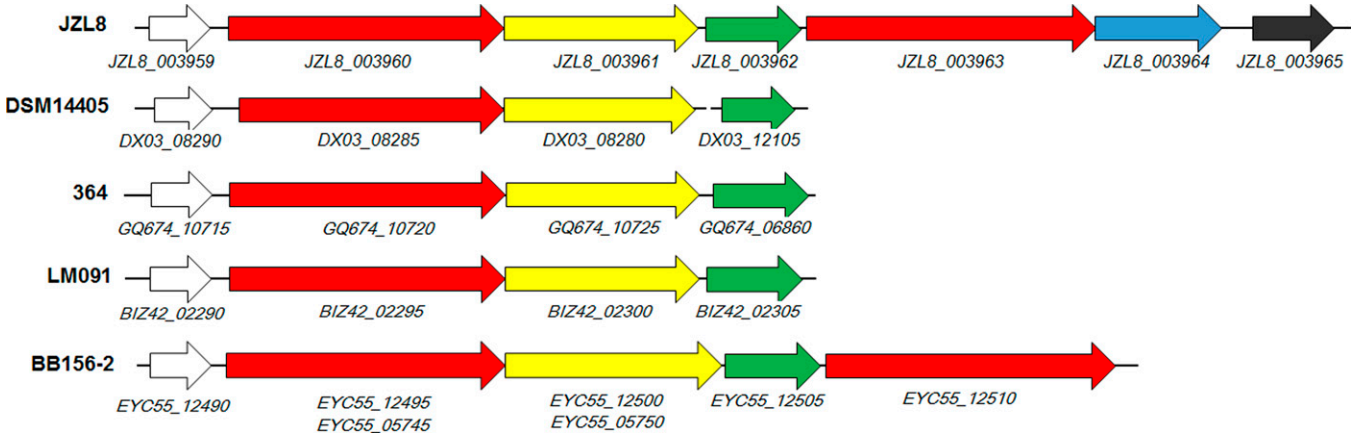

Fig. 7. A unique gene cluster present in the JZL8 genome but absent in the other *Stenotrophomonas maltophilia* genomes. Some of the genes are found in plant-associated strains, including *S. rhizophila* DSM14405 (CP007597.1), *Stenotrophomonas* sp. 364 (CP047135.1), *Stenotrophomonas* sp. LM091 (CP047135.1), and *Xanthomonas oryzae* BB156-2 (CP036254.1). Arrow colors represent proteins with different functions, including hypothetical protein (white), TBDT (red), PepSY domain-containing protein (yellow), superoxide dismutase (green), α-/β-hydrolase (blue), and tyrosine-type recombinase/integrase (black).

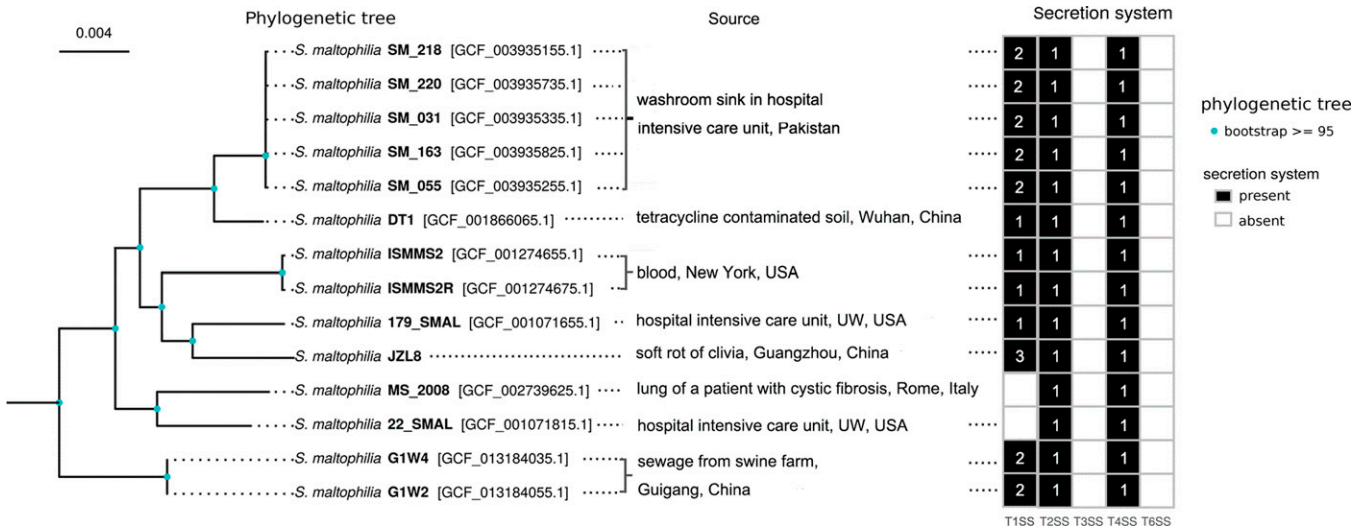

Fig. 8. Copy numbers of type-I to -VI secretion systems in JZL8, and its closely related *Stenotrophomonas maltophilia* strains. The tree displayed was a maximum-likelihood phylogeny constructed based on the 1,080 core genes identified in pan-genome analysis.

and production of cellulases and xylanases, and lost its ability to infect rice leaves (Xu et al. 2010). SODs are widespread and associated with pathogenesis as a counter measure against the plant defense response, catalyzing the degradation of the deleterious superoxide radical (Cabrejos et al. 2019). When grown in the pathogenicity-inducing XAM-M medium, *X. citri* subsp. *citri* SOD presents an increase in abundance, as compared with nutrient broth (Artier et al. 2018), implying its involvement in the disease process. Furthermore, a SOD (SodM) from *X. campestris* pv. *campestris* B100 has been shown to be an elicitor for oxidative burst in tobacco cell cultures (Watt et al. 2006). Although we think the 180 *S. maltophilia* strains isolated from other sources are non-pathogenic, the full pathogenicity potential of these 180 strains on plants was not experimentally tested. Thus, we could not definitively assign functions of genes discovered here to plant pathogenicity. Future works validating gene functions through genetic mutation and complementation will be needed to understand the mechanism(s) of virulence for the plant-pathogenic *S. maltophilia*.

On the other hand, while colonizing and infecting their hosts, pathogenic microorganisms have to face harsh and hostile environments. To cope with this, they have evolved a number of complex secretion systems to direct virulence factors into the extracellular environment or into the host cell (Bleves et al. 2010; Costa et al. 2015). T1SS usually transports proteins involved in pathogenesis and nutrient scavenging, such as extracellular toxins, proteases (Binet et al. 1997; Bleves et al. 2010), lipases, or the well-characterized iron scavenger protein HasA (Dalbey and Kuhn 2012; Kanonenberg et al. 2013). The much greater amounts of extracellular proteases produced by JZL8 than *D. zeae* strains JZL1 and JZL7 (Fig. 3) could be related to the extra copies of T1SS possessed by JZL8 (Fig. 8). Additionally, proteases were predicted in the genome of JZL8 (Supplementary Table S2), although none were found to be unique to it. T2SS is an important machinery through which Gram-negative pathogens secrete a large variety of proteins into the extracellular milieu and/or host organisms, including serine proteases, pectinases, cellulase, toxins, and other effectors, thus playing an important role in the transition of environmental bacteria to pathogens (Cianciotto and White 2017; Karaba et al. 2013; Korotkov et al. 2012). The T2SS we found in the JZL8 genome might explain its nematode-killing ability; the T2SS can transport diverse serine proteases against the survival of nematodes, similar to the serine protease activity possessed by *S. maltophilia* soil isolates against nematodes (Huang et al. 2009). Bacterial pathogens employ T4SSs for various purposes that deliver DNA and/or proteins (effectors) to aid in survival and proliferation in eukaryotic hosts, and this activity confers a selective advantage upon the invading pathogen in clinical settings through dissemination of antibiotic resistance genes and virulence traits (Gonzalez-Rivera et al. 2016; Grohmann et al. 2018). Furthermore, the finding that T4SS has a role in the interplay between *S. maltophilia* and mammalian hosts, as well as against other bacteria that inhabit the human host, has been a novel development in our understanding of *S. maltophilia* (Nas et al. 2019).

Moreover, high molecular mass glycopeptides were isolated from the pathogen, some of which were considered to cause plant cell wall disruption (Schuster and Coyne 1981). Our analysis of the JZL8 genome identified dozens of glycoside hydrolases (Supplementary Table S2). These glycoside hydrolases are responsible for the degradation of polysaccharides such as starch, cellulose, or chitin, and are also responsible for the de-glycosylation and re-glycosylation of glycoproteins during maturation and functionalization (Stütz and Wrodnigg 2011). Such characteristics would explain the weak pectin degradation enzymatic activity of the strains (Fig. 3).

Among bacterial plant pathogens, *S. maltophilia* is the least studied, with no information available from the virulence repertoires and pathogenicity determinants. In this study, we determined the host range of strain JZL8, which includes clivia, rice, Lanzhou lily, garlic, banana, and taro, but not dicotyledonous cucumber, Chinese cabbage, radish, potato, or carrot (Table 1; Supplementary Fig. S2). Furthermore, the virulence factors produced by *S. maltophilia* JZL6 and JZL8 were measured, which include large amounts of proteases, EPSs, and the formation of biofilms (Fig. 4). Whether they are the major virulence factors contributing to plant infection remains to be further proved. Another highlight of this study is the discovery of a gene cluster ranging from *JZL8_003959* to *JZL8_003965* (Fig. 7), which is likely to be the pathogenic determinant of *S. maltophilia* JZL8 on host plants. Because of the resistance of *S. maltophilia* JZL8 to many antibiotics (Table 2), it is impossible for us to study the specific functions of these seven genes by gene knockout.

In summary, the phylogenetic, genomic, and pathogenicity data obtained in this study provide new insight into the genetic diversity of *S. maltophilia*, which should arouse attention as a potential plant pathogen as it has acquired a plant-pathogenic gene island from heterologous plant pathogens.

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