A Sec-Dependent Secretory Protein of the Huanglongbing-Associated Pathogen Suppresses Hypersensitive Cell Death in *Nicotiana benthamiana*

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“Candidatus Liberibacter asiaticus” (CLas) is a phloem-restricted Gram-negative bacterium that is the causal agent of citrus huanglongbing (HLB). In this study, we identified a CLas-encoded Sec-dependent secretory protein CLIBASIA_04405 that could contribute to the pathogenicity of this bacterium. The gene expression level of CLIBASIA_04405 was significantly higher in citrus than in psyllids. Transient overexpression of the mature CLIBASIA_04405 protein (m4405) in *Nicotiana benthamiana* leaves could suppress hypersensitive response (HR)-based cell death and 

INTRODUCTION

Citrus huanglongbing (HLB), also known as citrus greening, is the most devastating worldwide disease of citrus at the moment and greatly damages the citrus industry by shortening lifespan of trees and reducing fruit yield and quality (Bové, 2006; Gottwald, 2010). The disease is associated with “Candidatus Liberibacter asiaticus” species that are phloem-limited, fastidious alpha-proteobacteria (Bové and Ayres, 2007; Wang and Trivedi, 2013). To date, three *Ca. Liberibacter* asiaticus species have been identified to be the causal agent of HLB, including “Candidatus Liberibacter asiaticus” (CLas), “Ca. L. africanus” (CLaf), and “Ca. L. americanus” (CLam) (Jagoueix et al., 1996; Wang et al., 2017). Among them, CLas is transmitted among citrus by *Diaphorina citri* Kuwayama...
dependent secretory proteins, CLas also employs non-classically (Jain et al., 2019). These findings indicate that, apart from the Sec-identified to suppress oxylipin-mediated defense in citrus plants (Yang et al., 2019), two well-known hypersensitive response (HR)-based PCD inducers. In addition, we showed that transgenic Nicotiana benthamiana leaves of the 4-week-old N. benthamiana. The study revealed an effector of CLas that might contribute to the bacterial pathogenicity.

**Materials and Methods**

**In Silico Analysis**

The putative signal peptide (SP) of CLIBASIA_04405, namely, 4405SP, was predicted using SignalP (version 5.0) with default settings for Gram-negative bacteria.

**Alkaline Phosphatase (PhoA) Assay**

The coding sequence of 4405SP was amplified with the primers 4405F/4405R (Supplementary Table 1) and cloned into pET-mphoA (Liu et al., 2019) that was double digested with Nde I and Hin dIII. The resulting construct pET-4405SP-mphoA that harbored an in-frame gene fusion between 4405SP and mphoA was transformed into Escherichia coli BL21. To detect the alkaline phosphatase (PhoA) activities, the transformants were randomly selected and incubated on the indicator Luria–Bertani agar (90 µg/ml BCIP, 100 mM IPTG, and 75 mM Na2HPO4) at 37°C, as previously described (Liu et al., 2019). After 24 h of incubation, the transformants with a color change to blue were selected for PhoA activity.

**Detection of the Transcripts for CLIBASIA_04405 in CLas-Infected Citrus and Psyllids**

Total RNA was extracted from CLas-infected Valencia sweet orange (Citrus sinensis) seedlings and CLas-bearing psyllids (Liu et al., 2019). Using the specific primers of CLIBASIA_04405 (Supplementary Table 1), reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was conducted as previously described (Liu et al., 2019). Three biological replicates were carried out, using the CLas LasgyrA gene (GenBank No. CP001677.5) as an internal reference. The relative gene expression was determined using the 2−ΔΔCt method (Livak and Schmittgen, 2001), and statistical analyses were performed using the Student’s t-test.

**Subcellular Localization of m4405 in N. benthamiana**

The coding sequences of CLIBASIA_04405 and m4405 amplified with the specific primers (Supplementary Table 1) were inserted into pCAMBIA1300-35S-GFP digested with Kpn I and Xho I. The resulting p4405-GFP and pm4405-GFP were individually introduced into Agrobacterium tumefaciens EHA105, followed by co-infiltration with agrobacterial cells carrying a plasma membrane marker pm-rk CD3-1007 (Nelson et al., 2007) into leaves of the 4-week-old N. benthamiana, as previously described (Van der Hoorn et al., 2000). At 60 h post inoculation (hpi), the infiltrated patches were visualized for GFP fluorescence under a LSM700 confocal microscope (Zeiss, Germany).
**Agrobacterium-Mediated PVX Infection Assay**

The coding sequence of m4405 amplified with the primers m4405-F/m4405-R (Supplementary Table 1) was cloned into a Potato virus X (PVX)-based expression vector pGR107 (Jones et al., 1999) to generate PVX-m4405. Additionally, nine alanine-substituted mutants of m4405 (Supplementary Figure 1) were produced by PCR using the primers listed in Supplementary Table 1 and individually inserted into pGR107, resulting in pPVX-A9-10, pPVX-A24-25, pPVX-A39-40, pPVX-A45-47, pPVX-A52-54, pPVX-A59-60, pPVX-A64-65, and pPVX-A75-78, pPVX-A93-94.

The PCD suppression assay was carried out as described previously (Wang et al., 2011). Briefly, the A. tumefaciens GV3101 that harbored pPVX-m4405, pPVX-A9-10, pPVX-A24-25, pPVX-A39-40, pPVX-A45-47, pPVX-A52-54, pPVX-A59-60, pPVX-A64-65, pPVX-A75-78, or pPVX-A93-94 were first infiltrated into the expanded leaves of the 6-week-old N. benthamiana plants. The A. tumefaciens cells that harbored pPVX-GFP were included as a negative control. At 24 hpi, the infiltrated patches were further inoculated with the Agrobacterium cells that harbored pGR-BAX or pGR-INF1. At 2 days post second inoculation, one third of the infiltrated leaves were detached to perform histochemical staining with 3,3′-diaminobenzidine (DAB) as described (Vanacker et al., 2000). To record the cell death development, the rest leaves were continuously observed up to 5 days post second inoculation. The experiment was repeated three times, and each replicate included six to eight plants.

**Generation of Transgenic N. benthamiana**

The coding sequence of m4405 was inserted into a binary plasmid vector pBl121 (Chen et al., 2003), resulting in pBl-m4405 that was subsequently introduced into A. tumefaciens EHA105. The transgenic N. benthamiana lines expressing m4405 were generated using a leaf disk method (Gallois and Marinho, 1995). T1 generation seeds were screened on media containing 50 μg/ml of kanamycin (Kan), and the Kan-resistant seedlings were further confirmed by RT-PCR with the primers listed in Supplementary Table 1.

**Pseudomonas syringae Infection Assays**

The infection assay was performed as previously described (Wei et al., 2018). DC3000ΔhopQ1-1 (Wei et al., 2007), a knockout mutant of P. syringae pv. tomato strain DC3000 was cultured in King’s B (KB) broth with rifampicin (50 μg/ml) at 28°C overnight, and collected by centrifugation (2,500 × g, 5 min), followed by washing and resuspension in 10 mM MgCl₂ to a final concentration of 3 × 10⁸ CFU/ml. The leaves of 4-week-old N. benthamiana and transgenic m4405 lines were syringe infiltrated with the bacterial suspensions. At 6 days post inoculation (dpi), the infiltrated leaf tissues were collected and ground in distilled water. Serial 10-fold dilutions were spread on KB plates with rifampicin (50 μg/ml) and incubated at 28°C for 2 days. Bacterial counts were expressed as CFU/cm² leaf disc and represented the mean ± SD (standard deviation) of three biological replicates. Statistical analysis was determined using Student's t-test.

**Transcriptome Sequencing and Analysis of Differentially Expressed Genes**

Total RNA was extracted from leaves of the 4-week-old N. benthamiana and transgenic m4405 lines by using an RNeasy Mini kit (Qiagen, United States), followed by cDNA library preparation and in-depth sequencing using an Illumina HiSeq2000. The resulting clean reads were assembled into transcripts using the Trinity method (Grabherr et al., 2011). After removing redundancy, the unigenes were generated and subjected to a blast search against the public protein databases of NR (NCBI non-redundant protein sequences), Swiss-Prot, NT (NCBI nucleotide sequences), and Protein family (Pfam) by using NCBI blastX with a cutoff E-value of ≤1e-5. The clean reads from N. benthamiana and the transgenic m4405 lines were individually mapped to the assembled transcriptome using RSEM with no more than two nucleotide mismatches (Li and Dewey, 2011), and the resulting read count for each matched unigene was further normalized using the reads per kilo bases per million read (RPKM) method (Mortazavi et al., 2008). The differentially expressed genes (DEGs) were determined using DESeq (Wang et al., 2010) with threshold as "q-value < 0.005 and |log2 (foldchange)| > 1." Heatmap was created using the function heatmap.2 of gplots R package (Warnes et al., 2015). The nucleotide sequences of raw reads and the assembled unigenes were submitted to NCBI Sequence Read Archive (SRA) database under the accession number PRJNA663964.

**Detection of the Transcripts for sHSPs in the Transgenic m4405 N. benthamiana**

Total RNA from leaves of the 4-week-old N. benthamiana and transgenic m4405 lines was subjected to RT-qPCR analysis as previously described (Zhang et al., 2019). The gene-specific primers used are listed in Supplementary Table 1. The experiment was performed with three biological replicates, using the N. benthamiana actin (GenBank No. JQ256516.1) as a reference gene. The relative gene expression was determined using the 2⁻∆ΔCt method (Livak and Schmittgen, 2001), and statistical analysis was conducted using Student's t-test.

**RESULTS**

**CLIBASIA_04405 Was a Sec-Dependent Secretory Protein That Exhibited Higher Transcriptional Level in Citrus Than in Psyllids**

The CLIBASIA_04405 of CLas is composed of 121 amino acids (aa) (Duan et al., 2009). A BLAST search of the GenBank database revealed that this protein was 100% conserved among the CLas strains with the full genome available (Supplementary Figure 2), but was absent from both CLaf and CLam. An in silico analysis with SignalP indicated that CLIBASIA_04405
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had 4405SP, a 20-aa SP at its N-terminus (Figure 1A). To functionally validate 4405SP, the E. coli-based phoA fusion assay was performed based on the chromogenic reaction of the secreted PhoA protein on LB medium containing BCIP substrate (Liu et al., 2019). After 24 h of incubation, the BL21 cells that expressed mPhoA lacking its native SP, which served as a negative control, remained white, whereas the bacteria that expressed 4405SP-mPhoA turned dark blue (Figures 1B,C) after 6 h of incubation, indicating that 4405SP successfully directed the extracellular secretion of the mPhoA moiety. The in silico prediction combined with the experimental validation supported the concept that CLIBASIA_04405 had an SP that could direct the protein secretion through the Sec-dependent pathway.

We next detected the transcripts for CLIBASIA_04405 in host plants and insect vectors. RT-qPCR analysis was performed on total RNA extracted from the CLas-infected sweet orange and ACPs, and the result showed that the gene expression level of CLIBASIA_04405 in citrus was significantly higher (∼50-fold) than that in psyllids (Figures 2A,B).

**m4405 Was Localized in Plasma Membrane of N. benthamiana Cells**

Since CLIBASIA_04405 was a Sec-dependent secretory protein, we investigated the subcellular localization of its mature protein m4405 in plants. The m4405 coding sequence was fused with the GFP gene (Figure 3A) and expressed in N. benthamiana via Agrobacterium-mediated transient expression. The N-terminal GFP-tagged m4405 was detected under confocal fluorescence microscopy. As shown in Figure 3B, m4405-GFP was predominately colocalized with the plasma membranal marker pm-rk CD3-1007 (Nelson et al., 2007), indicating the plasma membrane localization of m4405 in plants. Using the same strategy, we observed that CLIBASIA_04405 shared a similar subcellular localization in N. benthamiana cells with m4405 (Figure 3B). This suggested that the SP of CLIBASIA_04405 had little or no effect on plasma membrane localization of the protein in plants.

**m4405 Suppressed BAX- and INF1-Triggered Hypersensitive Cell Death in N. benthamiana**

On the basis of a plant virus expression vector pGR107 (Jones et al., 1999), the mouse BAX and the P. infestans INF1 have been widely utilized to identify the PCD suppressor of phytopathogens (Bos et al., 2006; Li et al., 2015; Xiang et al., 2016; Fang et al., 2019). We employed this strategy to evaluate the ability of m4405...
in PCD suppression. As shown in Figure 4A, m4405 completely suppressed both BAX- and INF1-triggered HR-based cell death in N. benthamiana, whereas cell death suppression had not been observed for GFP and the infiltration buffer.

To further understand the role of m4405 that leads to cell death suppression, the accumulation of H$_2$O$_2$, a reactive oxygen species (ROS) critical in plant cell death (Petrov and Van Breusegem, 2012), was analyzed in the N. benthamiana leaves co-expressing m4405 plus either BAX or INF1. Using a DAB staining method (Vanacker et al., 2000), we detected that m4405 completely inhibited both BAX- or INF1-stimulated H$_2$O$_2$ burst (Figure 4B). By contrast, neither GFP nor the infiltration buffer interfered with H$_2$O$_2$ accumulation.

To identify the key domain(s) of the m4405 involved in PCD suppression, nine clusters of charged amino acids (ED$^{9-10}$, KE$^{24-25}$, ER$^{39-40}$, EKR$^{45-47}$, EER$^{52-54}$, RK$^{59-60}$, DE$^{64-65}$, DEDK$^{75-78}$, and KK$^{93-94}$) of m4405 were individually selected for alanine-substitution mutagenesis (Supplementary Figure 1). The resulting mutants were expressed through pGR107 to test their abilities of PCD suppression. As results, there were two mutants (A45-47 and A64-65), whose abilities to suppress PCD induced by INF1 and BAX were both greatly reduced or even lost completely (Table 1), indicating that EKR$^{45-47}$ and DE$^{64-65}$ are necessary for m4405 ability to suppress cell death.

**The Transgenic N. benthamiana Expressing m4405 Exhibited Improved Resistance to P. syringae**

To gain further insight into the impact of m4405 on plant immunity, we generated transgenic N. benthamiana that stably expressed m4405 under the control of Cauliflower mosaic virus (CaMV) 35S promoter. A total of six independent transgenic lines were produced, and each of them exhibited phenotypes of dwarfism and leaf malformation. The lines m4405-18 and m4405-25 (Figure 5A) were then randomly selected for the

![Figure 3](image-url)
following experiments. In the first experiment, RT-qPCR analysis was performed on total RNA of transgenic *N. benthamiana* and disclosed that the expression level of m4405 positively correlated with the phenotypic changes of the transgenic lines (Figures 5A,B). Second, using *N. benthamiana* as a control, the transgenic plants were challenged with DC3000ΔhopQ1-1, a *P. syringae* DC3000 mutant that lacks the type III effector HopQ1-1 and thus is able to grow in *N. benthamiana* instead of inducing an HR reaction (Wei et al., 2007, 2013). Measurement of growth of DC3000ΔhopQ1-1 in the inoculated leaf tissues at 6 dpi showed that the transgenic m4405 plants supported significantly less bacterial proliferation than WT (Figure 5C). Indicating that the transgenic plants exhibited improved resistance to DC3000ΔhopQ1-1.

To address the underlying mechanism of resistance to DC3000ΔhopQ1-1 in the transgenic m4405 lines, we compared the transcriptome profiles of m4405-18 and m4405-25 with that of WT and identified an array of DEGs (Supplementary Table 2). Mining the DEGs revealed that a subset of genes encoding small heat shock proteins (sHSPs), including sHSP17.7, sHSP17.3, sHSP18.1a, sHSP18.1b, sHSP21, and sHSP22, were significantly upregulated in the transgenic lines compared with WT (Figure 6A). RT-qPCR analysis confirmed that the mRNAs of the six sHSPs were significantly accumulated in the transgenic lines (Figure 6B). The sHSPs are known to be a group of proteins with a molecular mass of 15 to 42 kDa that act as molecular chaperones to protect the plants against various biotic and abiotic stress conditions (Haslbeck and Vierling, 2015). Taking these results together, we inferred that the upregulated sHSPs in the transgenic m4405 *N. benthamiana* could contribute to the increased resistance to DC3000ΔhopQ1-1.

**DISCUSSION**

Although the molecular basis of the CLas pathogenicity remains largely unknown, much progress has been made toward elucidating its mechanisms of pathogenesis, particularly through the identification and characterization of CLas-encoded effectors, such as the Sec-dependent secretory proteins (Pitino et al., 2016, 2018; Clark et al., 2018; Liu et al., 2019; Shi et al., 2019; Ying et al., 2019; Zhang et al., 2019; Li et al., 2020). In a previous study, a genome-wide *in silico* analysis on CLas psy62 strain predicted a total of 166 proteins containing SPSs that may target them out of the cytoplasm via the Sec-dependent pathway (Prasad et al., 2016). However, 51 of these proteins, including CLIBASIA_04405, were determined to be false-positive predictions (Prasad et al., 2016) when experimentally validated using an early developed phoA fusion system (Mdluli et al., 1995). In this study, we revisited the putative SP of CLIBASIA_04405 with an updated phoA system (Liu et al., 2019) and provided evidence that CLIBASIA_04405 was instead a Sec-dependent secretory protein of CLas. Furthermore, we found that the expression level of CLIBASIA_04405 was significantly higher in a citrus host than in insect vectors. It is worthy to

**TABLE 1 | Identification of the key amino acids of m4405 involved in PCD suppression.**

| Mutant | PCD Inducer |
|--------|-------------|
| BAX    | INF1        |
| m4405  | 0/135       |
| GFP    | 135/135     |
| A9-10  | 1/45        |
| A24-25 | 1/45        |
| A39-40 | 1/45        |
| A45-47 | 45/45       |
| A52-54 | 0/45        |
| A59-60 | 0/45        |
| A64-65 | 22/45       |
| A75-78 | 6/45        |
| A83-94 | 10/45       |

a The m4405 and the alanine-substituted m4405 mutants were transiently expressed in *Nicotiana benthamiana* via Agroinfiltration. b The mouse BAX and the Phytophthora infestans INF1 were used as PCD inducers. The development of cell death was recorded at 5 days post infiltration of BAX or INF1. The PCD suppression of m4405 and its related mutants are shown as “lesion number/infiltration number.”
FIGURE 5 | The characteristics of the transgenic Nicotiana benthamiana expressing m4405. (A) Phenotypes of the transgenic N. benthamiana. The m4405-18 and m4405-25 represent the transgenic lines with typical phenotypes of dwarfism and leaf deformation. Scale bars represent 3 cm. (B) Transcriptional levels of m4405 in the transgenic N. benthamiana. RT-qPCR was conducted on total RNA prepared from the leaves of m4405-18 and m4405-25. Bars represent standard error of the means. (C) Bacterial growth in leaves of the transgenic m4405 lines and N. benthamiana. Pseudomonas syringae DC3000 hopQ1-1 was infiltrated at $10^4$ CFU ml$^{-1}$, and populations were measured from leaf discs at 6 days after inoculation. Bars represent standard error of the mean, and asterisks indicate the significant differences (Student's t-test, **p < 0.01).

FIGURE 6 | The sHSP genes were significantly upregulated in the transgenic N. benthamiana expressing m4405. (A) Heatmap representation of the normalized gene expression values of six small heat shock proteins (sHSPs) in N. benthamiana and the transgenic m4405 lines. (B) Validation of the expression of six sHSP genes by RT-qPCR. The transcript abundance of the indicated gene was normalized against the expression of Nbactin. Bars represent the standard error, and asterisks indicate the significant differences (Student’s t-test, **p < 0.01).
emphasize that a similar expression pattern was also observed in several CLas genes, such as CLIBASIA_00255, SC2_gp095, CLIBASIA_RS00445, CLIBASIA_00460, and CLIBASIA_03875, which have been suggested to function in the context of the CLas infection on the host plants (Jain et al., 2015, 2018; Li et al., 2017; Liu et al., 2019; Zhang et al., 2019). In addition, we show that m4405, the mature form of CLIBASIA_4405, completely suppresses PCD triggered by both BAX and INF1 in N. benthamiana. The data collectively indicates that CLIBASIA_4405 served as a virulence factor and was implicated in CLas pathogenesis in plant.

Programmed cell death plays roles in multiple cellular processes of plants, including immunity (Huysmans et al., 2017; Locato and De Gara, 2018). Upon pathogen infection, the plants may prime a form of PCD termed HR, a strong immune response that is accompanied by localized cell death to prevent the spread of the pathogen (Coll et al., 2011; Stael et al., 2015). In contrast, the pathogens, including bacteria, fungi, oomycetes, and nematodes, often secrete an arsenal of effectors that contribute to cell death suppression to enable their successful infection (da Cunha et al., 2007; Dou and Zhou, 2012; Goverse and Smant, 2014; Lo Presti et al., 2015). In CLas, the mature forms of two Sec-dependent secretory proteins CLIBASIA_03875 and CLIBASIA_04025, termed m3875 and SDE15, have been determined as PCD suppressors (Zhang et al., 2019). Herein, we demonstrated that m4405, the mature form of the Sec-dependent secretory protein CLIBASIA_04405, also inhibited hypersensitive cell death. Together, the findings highlight that, despite lacking the conserved secretion machineries such as T3SS (Duan et al., 2009), CLas employs the Sec-dependent secretory proteins to overcome HR of the citrus host.

To inhibit the development of hypersensitive cell death, pathogen-encoded PCD suppressors may interact with the host immune receptors (Bos et al., 2010; Wilton et al., 2010; Chen et al., 2012), or interfere with ROS accumulation that accompanies cell death (Dong et al., 2011; Hemetsberger et al., 2012; Zhang et al., 2015). It has been shown that the CLas PCD suppressor m3875 regulates the gene expression of cell death regulators, cyclic nucleotide-gated channels (CNGC), Bax-inhibitor 1 (BI-1), and WRKY9 (Zhang et al., 2019), while another CLas PCD suppressor SDE15 directly interacts with a citrus protein ACCELERATED CELL DEATH 2 (ACD2), a negative regulator of PCD (Pang et al., 2020). In this study, how m4405 suppressed hypersensitive cell death remains elusive. However, alanine-substitution mutagenesis of m4405 showed that two amino acid clusters (EK45−47 and DE64−65) were crucial for suppressing both BAX- and INF1-triggered cell death. Further study on the two loss-of-function mutants (A45-47 and A64-65) as well as identification of the potential target protein(s) in plants could be helpful to elucidate the mechanism by which m4405 suppressed PCD.

A number of pathogen effectors have been shown to mediate disease resistance when expressed transgenically (Kawamura et al., 2009; Rajput et al., 2015; Zhang et al., 2015b; Wang et al., 2019). Likewise, the study here displayed that the transgenic N. benthamiana expressing m4405 exhibited improved resistance to the bacterial pathogen DC3000ΔhopQI-1. Transcriptome profiling and RT-qPCR revealed that the gene expression of six sHSPs (sHSP17.7, sHSP17.3, sHSP18.1a, sHSP18.1b, sHSP21, and sHSP22) were significantly upregulated in the transgenic lines. The sHSPs are evolutionarily conserved proteins in almost all organisms, ranging from bacteria and fungi to plants and animals (Waters and Vierling, 1999; Haslbeck and Vierling, 2015). The plant sHSPs are usually below detection limits in vegetative tissues under normal growth conditions but instead are induced by environmental stresses and developmental stimuli (Jacob et al., 2017; Carra et al., 2019). So far, there is emerging evidence that sHSPs serve as defense regulators and function in diverse defense mechanisms (Ul Haq et al., 2019). For example, the N. tabacum sHSP17 is required for basic immune responses other than HR-based resistance in plants (Maimbo et al., 2007). In contrast, sHSP20s plays a positive role in the hypersensitive defense response, and silencing of HSP20s in N. benthamiana can compromise the HR induced by the tomato resistance (R) genes I-2 and Mi-1 (Van Ooijen et al., 2010). In addition, a subset of the Arabidopsis sHSPs, including sHSP22, has been identified to be downregulated upon Pst DC3000 infection (Bricchi et al., 2012), implying their functions in response to the P. syringae infection. Remarkably, sHSP22 was one of the six sHSPs that were transcriptionally activated in the transgenic m4405 N. benthamiana. The potential of sHSP22, as well as those of the other five sHSPs, in P. syringae resistance merits further investigation.

In conclusion, the study identified a Sec-dependent secretory protein of CLas and showed that its mature form m4405 could suppress hypersensitive cell death in N. benthamiana. Transgenic overexpression of m4405 in N. benthamiana interfered with the plant growth and development and, in particular, upregulated the transcription of a group of sHSP genes. The data collectively indicated the potential role of m4405 in CLas infection and might shed new light on the pathogenicity of CLas.

**DATA AVAILABILITY STATEMENT**

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

**AUTHOR CONTRIBUTIONS**

WL, XW, and CZ designed the experiments. PD, HY, CZ, and ZZ performed the experiments. WL and CZ analyzed the data. WL, CZ, and XW wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.594669/full#supplementary-material

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