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Uncovering Candidate Pathogenicity Genes in *Erwinia pyrifoliae* YKB12327 via Tn5-insertion Mutagenesis

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Erwinia pyrifoliae is a gram-negative bacterial pathogen that commonly causes black shoot blight in pear and apple tree. Although the pathogenicity of this bacterial species is very similar to *E. amylovora*, there is no specific explanation of its pathogenic genes and mechanisms. In this study, our investigation into *E. pyrifoliae* pathogenicity involved generating seven YKB12327 mutant strains using Tn5 transposon mutagenesis. Observations revealed weakened growth rate and loss of pathogenicity in these mutants. Whole-genome sequencing and alignment analysis identified transposon insertions within the coding sequences of five strains and in the intergenic region of two strains. Annotation analysis elucidated genes directly or indirectly associated with pathogenicity. Notably, mutant strain MT16 displayed a transposon insertion mutation in the cyclic-di-GMP phosphodiesterase (*pdeF*) gene, a key player in bacterial signaling, governing microbial behavior and adaptation to environmental changes. Our findings provide insights into the genetic regulation of *E. pyrifoliae* pathogenicity, suggesting potential avenues for further research aimed at understanding and controlling this bacterial pathogen by targeting *pdeF* to mitigate apple black shoot blight disease.

Keywords: Black shoot blight, *Erwinia pyrifoliae*, Mutagenesis, Pathogenicity, Tn-5 transposon

Introduction

Erwinia pyrifoliae is a gram-negative bacterium belonging to the family Erwiniaceae, which commonly cause 'Bacterial Shoot Blight' or 'Asian Pear Blight' disease (Kim et al., 2001; Lee et al., 2020; Thompson et al., 2019). Its entry into the plant occurs through wounds or natural orifices, initiating a disease called bacterial canker, which results in stunted plant growth, reduced fruit production, and potentially plant death (Kim et al., 2001; Rhim et al., 1999). Symptoms of *E. pyrifoliae* infection in trees include wilting, branch blackening, and the presence of exudate in infected tissue (Rhim et al., 1999).

In 1995, *E. pyrifoliae* disease was first discovered in diseased pear trees in South Korea (Kim et al., 1999). Subsequently, through whole-genome sequencing and gene prediction, the

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molecular characteristics and pathogenicity of this disease were identified. Previous researches had yielded nine assembled sequences of the *E. pyrifoliae* genome, including strains EpK1/15 (GCA_002952315) (Lee et al., 2018), Ep1/96 (GCA_000027265) (Kim et al., 1999), DSM12163 (GCA_000026985) (Smits et al., 2010), YKB12328 (GCA_020428355), CP201486 (GCA_025159015), CP201179 (GCA_025158995), CP20113301 (GCA_025402895), CP201264 (GCA_025656905), and CP20126903 (GCA_025153555) (https://www.ncbi.nlm.nih.gov/datasets/genome/?taxon=79967). Based on these genome sequences, genomic analysis has identified various genetic elements and key genes responsible for *E. pyrifoliae*'s pathogenicity and virulence, including the *hrp/hsv/dsp* cluster type III secretion system (T3SS), *Salmonella* SPI-1-like T3SS, effector and virulence-associated proteins, cell-influencing factors, and metabolic pathways (Kube et al., 2010). These genes enable the bacterium to invade plant tissues, suppress the host's defense mechanisms, and cause the characteristic symptoms of black shoot blight (Kube et al., 2010).

Comparative genomics studies have revealed numerous gene insertions/deletions, rearrangements, and inversions in the central regions of the chromosomes in the *E. pyrifoliae* genome compared to the related enterobacteriums, such as *E. amylovora*, *E. billingiae, E. tasmaniensis*, *Erwinia sp.* (Ejp617) and *Serratia proteamaculans* (Kamber et al., 2012; Smits et al., 2010; Thapa et al., 2013). Therefore, there is a pressing need for additional genomic information and gene annotation to enhance the identification of pathogenic genes in *E. pyrifoliae*.

Transposon mutagenesis is a vital tool for exploring gene function in organisms, especially when precise genome editing techniques like CRISPR-Cas9 are challenging or not yet fully established (Arroyo-Olarte et al., 2021; Ebrahimi and Hashemi, 2020; van Opijnen and Camilli, 2013). These genetic elements, known as transposons, can move or transpose within the genome, causing mutations by inserting themselves into various locations (Bourque et al., 2018; Dubin et al., 2018; Muñoz-López and García-Pérez, 2010). This disruption can lead to the loss of gene function or alterations in gene regulation (Dubin et al., 2018; Kawakami et al., 2017). By utilizing transposon mutagenesis, researchers can study how mutations affect an organism's phenotype, aiding in the understanding of specific gene functions or the discovery of new regulatory elements (Feddersen et al., 2019; Lin et al., 2014; Sanchez et al., 2019). Among the earliest composite bacterial transposons identified, the transposon Tn5 operates via a "cut-and-paste" mechanism (Penkov et al., 2023; Reznikoff, 2008). This mechanism allows Tn5 transposon mutagenesis to generate random mutations across the genome, enabling the investigation of broad genetic changes in a relatively unbiased manner (Reznikoff, 2008; Sato et al., 2019). Tn5 transposon mutagenesis has found widespread use in the study of various bacteria, including *Zymomonas mobilis, Phaeobacter inhibens, Pseudomonas stutzeri, Shewanella amazonensis, S. oneidensis*, *Acinetobacter baylyi*, *Escherichia coli*, and *Pseudomonas aeruginosa* (Naorem et al., 2018; Wetmore et al., 2015; Zhang et al., 2013). This technique offers researchers a powerful means to explore genetic mechanisms and address fundamental questions in biology.

In this study, we aimed to fill knowledge gaps regarding the pathogenicity of *E. pyrifoliae* by employing advanced genomic techniques. We generated seven distinct mutant lines of *E. pyrifoliae* strain YKB12327 to elucidate genes associated with pathogenesis and uncover new regulatory elements in this strain. Through re-sequencing analysis of these mutants, we precisely determined the transposon insertion sites and identified disrupted coding sequences (CDSs). Subsequent analysis of these disrupted CDSs led to the identification of candidate genes implicated in the pathogenicity of YKB12327. Our findings not only provide genomic insights into a newly identified pathogen but also lay the groundwork for discovering and functionally verifying its pathogenic genes. This study contributes valuable genetic information and aids in the identification and functional characterization of novel pathogenic genes or mechanisms using a discovery-driven approach.

Materials and Methods

Strain culture and DNA preparation. The *E. pyrifoliae* YKB12327 strain, originally isolated from a diseased apple branch in 2015, was obtained from the Crop Protection Department at the National Academy of Agricultural Science (NAAS), Rural Development Administration (RDA), in Korea (Park et al., 2018). Comprehensive characterization of this strain has been previously reported (Ham et al., 2022; Shin et al., 2018).

Total genomic DNA of YKB12327 was extracted using

a PureLink™ Genomic DNA Mini Kit (Cat. No. K182002; Thermo Fisher Scientific Inc., Waltham, MA, USA) following the manufacturer's instructions. Genomic DNA integrity was assessed via 1% agarose gel electrophoresis, while DNA purity was evaluated using a NanoDrop UV–Vis Spectrophotometer (Cat. No. ND-2000; Thermo Fisher Scientific Inc.).

Construction of the Tn5-insertion mutagenesis library. The Tn5 transposon mutant library was generated using an EZ-Tn5™ <R6Kγori/KAN-2> Tnp Transposome™ Kit (Cat. No. TSM08KR; Lucigen Co., Middleton, WI, USA) following the manufacturer's instructions. *In vitro* transposition reactions consisted of 2 µl 10× EZ-Tn5 reaction buffer, 1 µl of transposome complex, 2 µg of YKB12327 genomic DNA, and 15 µl of distilled deionized H_2O, was incubated for 4 hr at 37°C. The transposed DNA was then purified and repaired using a T4 DNA Polymerase Kit (Cat. No. M0203S; New England Biolabs GmbH, Buxtehude, Germany) according to the manufacturer's protocol. The resulting reaction mixture was utilized for the transformation following the natural transformation method described by Davis et al. (2008). Transformed colonies were selected on nutrient broth (BD Difco Inc., Franklin Lakes, NJ, USA) agar plates supplemented with 50 mg/l kanamycin. Selected mutants underwent DNA extraction and subsequent analysis and were stored at -80°C.

DNA sequencing and assembly. The same procedures outlined in the "Strain culture and DNA preparation" section were employed for DNA extraction and quality control. Genomic DNA paired-end libraries with 350-bp inserts were generated using the TruSeq Nano DNA High Throughput Preparation Kit (Cat. No. 20015965; Illumina Inc., San Diego, CA, USA). These paired-end libraries were sequenced at Macrogen Co. (Seoul, Korea) using Illumina Sequencing by Synthesis (SBS) Technology (Illumina Inc.). Short reads were assembled using CLC Genomics workbench v.23.0.4 (Qiagen) based on the genome sequence of YKB12327 obtained via Nanopore long-read sequencing in National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/; BioProject: PRJNA1044457). The Tn5 transposon was aligned with the mutagenized genome to determine insertion sites and connection sequences.

Characterization of mutant strains. The mutant bacterial cells were cultured on nutrient agar (BD Difco Inc.) at 27°C for 36 hr. A single colony was selected and transferred to 30 ml of nutrient broth (BD Difco Inc.) and incubated on a reciprocating shaker (110 rpm) at 27°C until reaching a concentration of 1×10^8 colony forming units/ml. Subsequently, cultured bacteria were centrifuged, and pellets were resuspended in equal volumes of 1× phosphate-buffered saline (Biosolution Co., Seoul, Korea). For pathogenicity examination, the mutant strains were inoculated into wounds by pricking apples (*Malus domestica* cv. Fuji) with scissors and then applying the inoculant.

Colony morphology was assessed by culturing 10 µl of bacterial suspension with an OD_{600} of 1.0 at the center of a semi-solid plate in a 28°C incubator for 48 hr. Bacterial colony populations were determined by preparing 10-fold serial dilutions (10^{-1} to 10^{-6}) of bacterial suspensions and enumerating the colonies on nutrient broth medium after incubation at 28°C for 6-48 hr.

Results

Construction and genome sequencing of transposon mutants. We constructed a comprehensive library of transposon mutants using Tn5 transposon mutagenesis, yielding over 100 candidate mutants identified through kanamycin resistance screening (Fig. 1A). We selected strains with normal growth and verified the authenticity of the mutation using primers (Kan-F and Kan-R) targeting the Kanamycin resistance (KmR) gene (Fig. 1B, Supplementary Table 1).

Subsequent short-read genome sequencing and assembly revealed insertion sites, yielding 129-156 contigs with N50 lengths ranging from 97,593 to 102,789 bp (Table 1, Supplementary Table 2). Ultimately, similar total contig sequences (ranging from 3,923,318 to 3,927,892 bp) were obtained across the seven mutants (MT3, MT4, MT5, MT6, MT12, MT13, and MT16).

Genetic information of transposon mutants. Tn5 transposon insertions resulted in the excision of small gene fragments, altering the original gene structure. Whole-genome alignment identified the insertion sites and sequence structures of Tn5 transposons in each mutation strain. Specifically, the MT3, MT4, MT5, MT12, and MT16 mutants exhibited Tn5

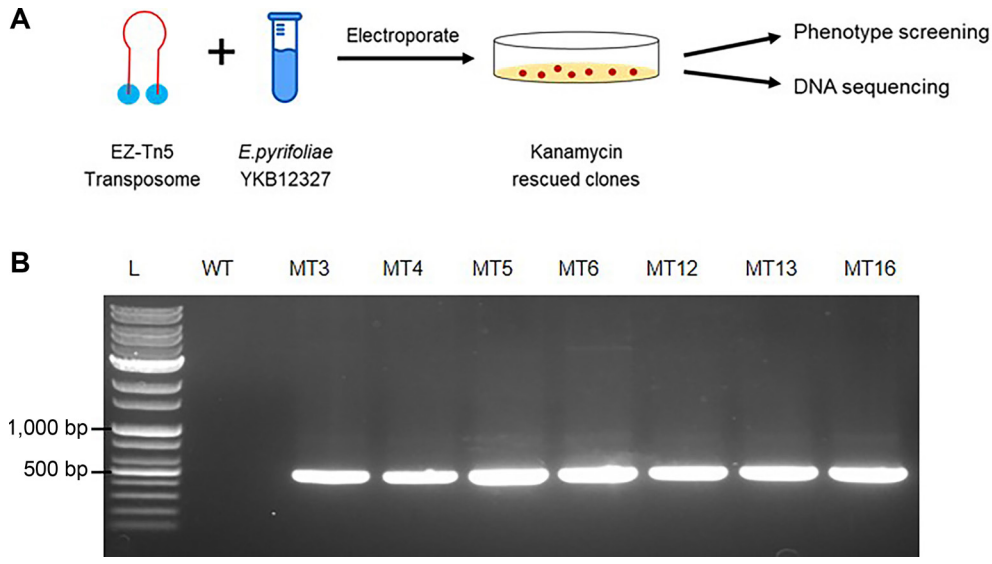

Fig. 1. Construction and sequence analysis of Tn5 transposon mutants. (A) Flow diagram illustrating the construction of Tn5 transposon mutants. (B) Gel electrophoresis of PCR products of *kmr* from kanamycin-resistant colonies. L, 1 kb DNA ladder; WT, wild-type; MT, mutant; PCR, polymerase chain reaction.

Table 1. General feature statistics of mutant strains

Sample	Raw reads	Trimmed reads	No. of contigs	Contig N50	Total contig length (bp)
MT3	7,207,132	7,207,086	156	97,593	3,927,092
MT4	7,373,354	7,373,309	137	101,840	3,927,892
MT5	7,779,926	7,779,869	129	98,454	3,923,528
MT6	7,448,434	7,448,335	139	101,815	3,923,634
MT12	8,401,766	8,401,725	145	98,580	3,923,318
MT13	8,423,666	8,423,633	136	101,804	3,926,334
MT16	7,015,454	7,015,405	141	102,789	3,923,077

MT, mutant.

transposon insertions in the chromosome (Utg1184), while the MT6 and MT13 mutants showed insertions in endogenous plasmids (Utg1110 and Utg1176, respectively) (Table 2).

Based on the assembled genome sequences, we examined the genome of each mutant and found that the Tn5 transposon inserted into the CDS in mutants MT4, MT5, MT6, MT13, and MT16, while inserting into the intergenic regions in MT3 and MT12 (Table 2). Mutant MT4 exhibited an insertion mutation in the CDS of L-ornithine N(5)-monooxygenase, a protein predicted to regulate the binding of flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide phosphate (NADP) cofactors. MT5 displayed an insertion mutation in a hypothetical protein. MT6 featured an insertion mutation in glycoside hydrolase, family 19 (*gh19*), predicted to control carbohydrate modification, decomposition, and assembly. MT13 showed an insertion mutation in methionine import system permease protein (*metP*), predicted to be important in the ABC transmembrane. Additionally, MT16 presented an insertion mutation in a cyclic-di-GMP phosphodiesterase (*pdeF*), predicted to regulate colony morphology and development. Thus, the construction and analysis of transposon mutants revealed diverse genetic disruptions, shedding light on potential gene functions and regulatory mechanisms in YKB12327.

Growth rate and pathogenicity assessment of mutant strains. The effect of genetic mutations on growth

Table 2. Annotation information of sequences containing transposon insertion sites

Mutants	Insertion site	Coverage sequence annotation					
		Type	Gene name	KEGG	COG	Description	Function
MT3	Utg1184: 2251173-2251186	Intergenic region					
MT4	Utg1184: 3124007-3124015	CDS	*pvdA1*	1.14.13.195	COG3486	L-ornithine N(5)-monooxygenase	Binding FAD and NADP cofactors
MT5	Utg1184: 2341038-2341052	CDS				Hypothetical protein	Unknown
MT6	Utg1110: 2430-2438	CDS	*gh19*	3.2. 1.14	-	Glycoside hydrolase, family 19	Control carbohydrate modification, decomposition, and assembly
MT12	Utg1184: 3629948-3629957	Intergenic region					
MT13	Utg1176: 31830-31838	CDS	*metP*		COG2011	Methionine import system permease protein MetP	ABC transmembrane
MT16	Utg1184: 2370130-2370138	CDS	*pdeF*	3.1.4.52		Cyclic-di-GMP phosphodiesterase	Regulating colony morphology and development

KEGG, Kyoto encyclopedia of genes and genomes; COG, Clusters of orthologous genes; MT, mutant; CDS, Coding sequence; FAD, Fflavin adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; *gh19*, glycoside hydrolase, family 19; *metP*, methionine import system permease protein; ABC, ATP binding cassette; *pdeF*, cyclic-di-GMP phosphodiesterase.

rate was determined by inoculating 10 µl of a bacterial suspension with an OD_{600} value of 1.0 at the center of a nutrient broth (BD Difco Inc.) plate and subsequently measuring the colony diameter. After incubation for 48 hr, all Tn5 mutants exhibited significantly reduced growth rate compared to the YKB12327 wild-type (WT) strain (Fig. 2A). Similar to the WT strain, the seven mutant strains displayed normal growth on nutrient broth agar plates, forming yellow smooth colonies, albeit significantly smaller. Specifically, while the colony diameter of the WT strain measured 13.17 mm, the colonies of MT3, MT4, MT5, MT6, MT12, MT13, and MT16 were reduced by 50.6%, 55.2%, 47.6%, 28.6%, 45.3%, 31.1%, and 43.5% respectively, compared to the WT strain (Fig. 2B). The mutant strain showed the same growth pattern as the WT in different culture media (Fig. 2C). Subsequently, the pathogenicity of the mutant strains was evaluated on apple fruits (*Malus domestica* cv. Fuji). Fruits infected by the WT strain turned black, while fruits infected by the negative control and the seven mutant strains showed no symptom changes (Fig. 2D).

Functional prediction of pathogenic genes. In our pathogenicity investigation, MT16 was chosen for functional analysis due to the Tn5 transposon insertion disrupting *pdeF* (Fig. 3A). PCR analysis confirmed this disruption, revealing a 2001-bp increase in gene length compared to the WT strain, as shown by *pdeF* gene-specific primers (Fig. 3B, Supplementary Table 1). We took the log value of colony forming unit per ml to compare the growth rate of the strains. In rich nutrient broth medium, MT16 exhibited delayed growth compared to the WT strain, evident between 24 and 36 hr post-inoculation (Fig. 3C), suggesting a potential link between the disrupted gene and pathogenicity loss.

Blast analysis indicated high homology between the original *pdeF* sequence and those of strains EpK1/15 and CP20113301, with 100% identities (Fig. 4, Supplementary Table 3). However, sequence differences were observed between the *E. amylovora* and *E. pyrifoliae* strains, with less than 91.77% identity. Previous studies have suggested a role for *pde* genes in regulating colony morphology and development (Hull et al., 2012). Taken together, our analysis highlights the critical role of the disrupted *pdeF* gene in pathogenicity, potentially influencing colony morphology and development.

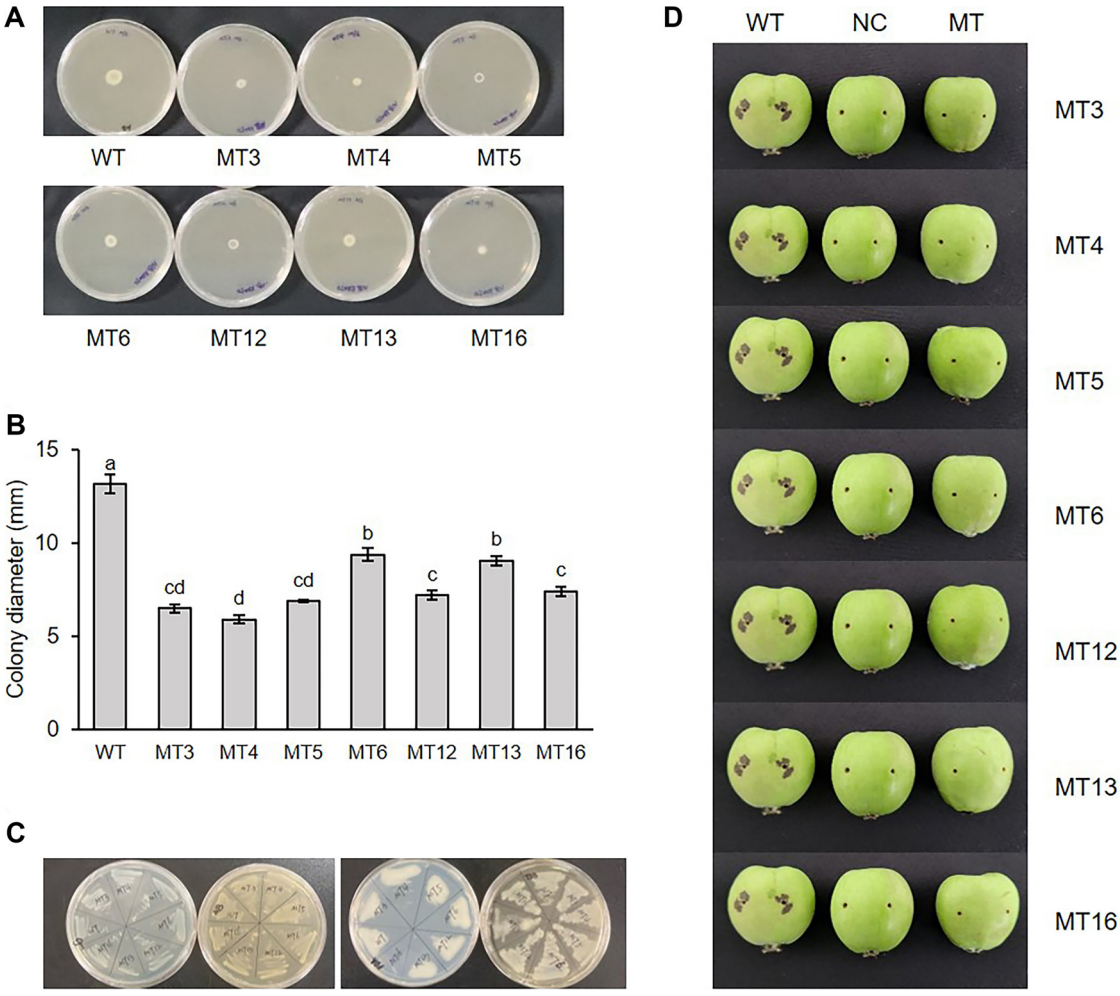

Fig. 2. Colonization and pathogenicity measurement of Tn5 transposon mutants. (A) Comparison of colony morphology between WT and Tn5 transposon mutants. (B) Measurement of colony diameter (mm) in WT and Tn5 transposon mutants. Values with the different letters are significantly different at $P<0.01$, determined by Tukey's honestly significant difference (HSD) test. (C) Colony morphology of WT and Tn5 transposon mutants. The medium are luria bertani, nutrient broth, potato dextrose, tryptic soy broth medium (start from the left). (D) Pathogenicity detection of mutants in apple fruit (*Malus domestica* cv. Fuji). WT, YKB12327 wild type; MT, Tn5 transposon mutant; NC, negative control (inoculation with water).

Discussion

The discovery of Tn5 transposon mutagenesis system was serendipitous and has become a valuable method for investigating unique genes in unknown genomes. The seven Tn5 transposon mutant strains developed here exhibited DNA sequence alterations and functional phenotypes. These strains displayed reduced pathogenicity, smaller colony diameters, and slower growth rate compared to the WT strain. This suggests that Tn5 transposon insertion influenced bacterial colonization, propagation, and pathogenicity. The genes originally present in a bacterial strain undergo mutation or disruption as a result of Tn5 transposon insertion, potentially leading to loss of gene function or alteration of gene regulation. However, linking specific mutations to particular phenotypic changes remains a significant challenge.

In our study, Tn5 insertion mutations were identified in the CDSs of MT4, MT5, MT6, MT13, and MT16, while mutations in non-coding regions were observed in MT3 and MT12 (Table 2). The mutant MT4 harbored a mutation in the CDS of the *pvdA1* gene, which encodes L-ornithine N(5)-monooxygenase, an enzyme involves in the biosynthesis of ferruginous siderophores (Campbell et al., 2020). This enzyme catalyzes the FAD- and NADPH-dependent hydroxylation of L-ornithine, a process

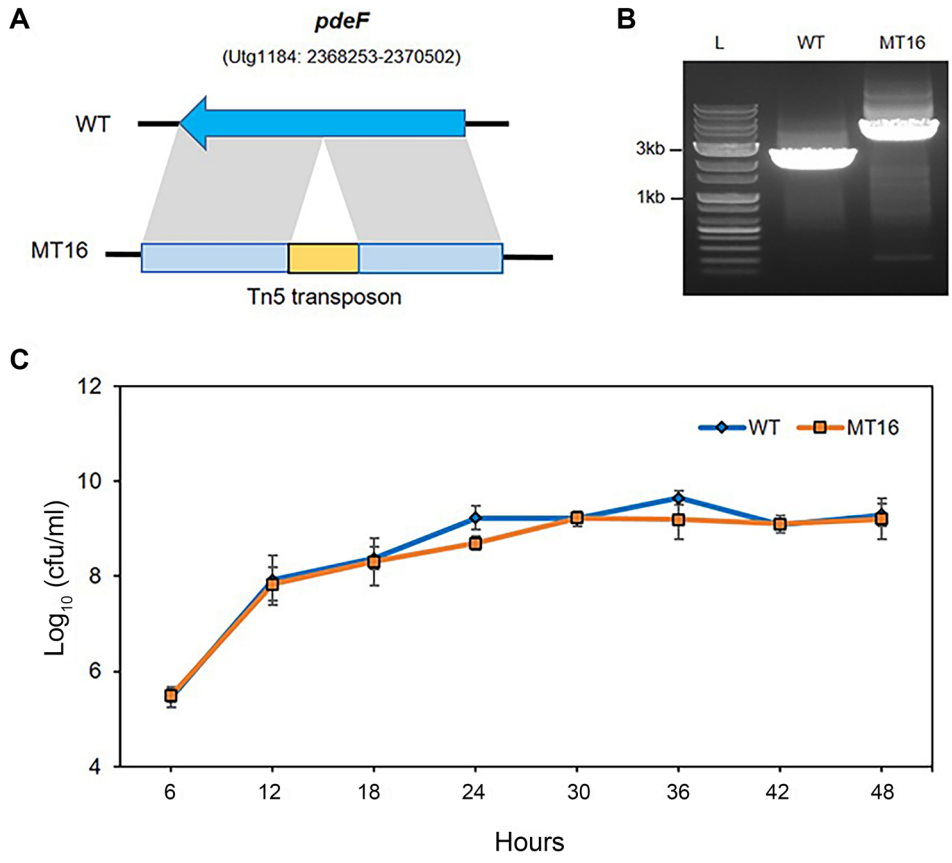

Fig. 3. Genome sequence variation and growth pattern of MT16. (A) The coding sequence of *pdeF* is disrupted by the insertion of the Tn5 transposon. (B) Gel electrophoresis of PCR products of *pdeF*. (C) Growth comparison of WT and MT16 in nutrient broth medium. Error bars represent standard deviation. L, 1-kp DNA ladder; WT, wild type; MT16, Tn5 transposon mutant line 16; PCR, polymerase chain reaction.

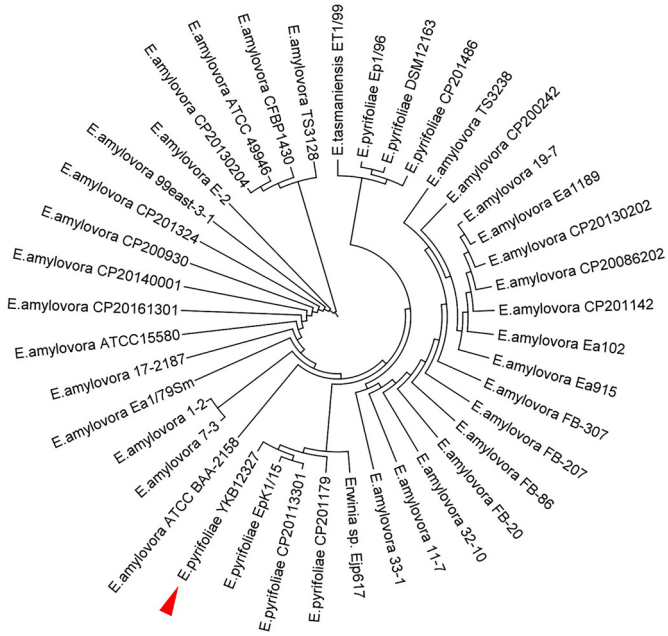

Fig. 4. Phylogenetic tree of *pdeF* in the *Erwinia* genus. The phylogenetic tree was generated using the neighbor-joining method with 1,000 bootstrap replicates based on CLC Genomics Workbench version 23.0.2.

crucial for the production of siderophores like triacetyl fusarium and desferriophore. Widely distributed in various eukaryotic organisms, including fungi, metazoans, protists, green plants, choanoflagellates, and ichthyosporeans (Franceschini et al., 2012), this enzyme is primarily produced under iron-limiting conditions, highlighting its significance in virulence (Campbell et al., 2020; Franceschini et al., 2012). The disruption of the CDS for this enzyme in MT4 may impair its ability to regulate siderophores, potentially contributing to the loss of virulence observed in this strain. The mutated CDS of MT5 encodes a hypothetical protein, warranting further investigation to elucidate its function. The mutant MT6 harbored a mutation in the CDS *gh19* gene, a bifunctional family of enzymes including chitinases and endolysins found across diverse bacterial taxa (Orlando et al., 2021). Pathogens infecting plants rely on hydrolytic enzymes to degrade complex polysaccharides in the plant cell wall to facilitate host invasion and disease development (Rafiei et al., 2021). Numerous studies have

identified cell wall-degrading enzymes within various glycoside hydrolase families (Blackman et al., 2014; Gao et al., 2020; Romero Victorica et al., 2020). The mutation in this gene likely hinders the synthesis of plant cell wall-degrading enzymes in this strain, potentially affecting plant infection. The mutated CDS of MT13 is annotated as a *metP,* critical for various cellular and biosynthetic processes, including protein synthesis initiation and S-adenosylmethionine-mediated methylation of proteins, RNA, and DNA. *De novo* methionine biosynthesis or high-affinity methionine transport is essential for sourcing methionine to support growth and virulence in pathogens such as *Salmonella typhimurium* during infection (Husna et al., 2018). Disruption of the *metP* gene could impair methionine biosynthesis, leading to severe *in vivo* growth attenuation of pathogens and weakening or eliminating the virulence of the mutant strain. The mutated genes in the aforementioned variants (MT4, MT6, and MT13) potentially disrupt the normal metabolic processes of the bacteria, serving as a possible indirect cause for the loss of bacterial independence and pathogenicity.

In contrast, the mutated CDS of MT16 was annotated as *pdeF*. Cyclic Diguanosine Monophosphate (c-di-GMP), composed of two guanosine monophosphate units linked cyclically, serves as a crucial signaling molecule in bacteria, influencing various cellular processes including biofilm formation, motility, virulence, cell cycle progression, and the transition between planktonic (free-swimming) and sessile (attached) lifestyles (Ha and O'Toole, 2015; Jenal et al., 2017; Krol et al., 2020; Valentini and Filloux, 2016). In *E. amylovora*, c-di-GMP positively regulates the secretion of amylovoran, the major exopolysaccharide for amylolytic degradation, leading to increased biofilm formation, and negatively regulates flagellar swimming motility, which plays a key role in the coordination of pathogenesis (Edmunds et al., 2013; Kharadi et al., 2022). Analysis of MT16's colony growth rate revealed significantly slower growth compared to the WT strain, indicating that disruption of *pdeF* inhibited the growth and pathogenicity of MT16. These results underscore the significance of *pdeF* in supporting bacterial colony growth.

These results indicate that many factors, both directly and indirectly, contribute to the pathogenicity of YKB12327, including non-coding sequences. The YKB12327 plasmids contain 49 CDSs, with Tn5 transposon insertion sites found in two CDSs (*gh19* and *metP*) in plasmids Utg1110 and Utg1176 of MT6 and MT13, respectively. This suggests that the plasmids carry genes related to pathogenicity factors in *E. pyrifoliae*, which are responsible for the bacterium's ability to infect and cause disease in pear and apple trees. Previous studies also describe plasmids as carriers of genes providing various advantages to the bacterium, such as antibiotic resistance or toxin production (Llop et al., 2012; Pal et al., 2015). Therefore, annotating plasmids of *E. pyrifoliae* is crucial for studying pathogenicity and developing strategies to manage and control diseases in pear trees caused by this bacterium.

In conclusion, our study represents an advancement in understanding the pathogenicity of *E. pyrifoliae*, particularly through the comprehensive analysis of strain YKB12327 using robust genomic techniques. By elucidating the genetic makeup of this novel strain and identifying key pathogenicity-related genetic elements, we have provided valuable insights into the complex mechanisms underlying the bacterium's virulence. The disruption of the cyclic-di-GMP phosphodiesterase gene *pdeF* in MT16 by Tn5 transposon insertion is noteworthy. This event highlights the crucial role of *pdeF* in regulating essential virulence factors. These findings suggest potential targets for future intervention strategies.

While our study offers substantial contributions to the field, it is essential to acknowledge its limitations, including potential biases and assumptions inherent in genomic analyses. Moving forward, complementary experiments, such as gene knockout studies and functional assays, will be crucial for validating the roles of identified genes in pathogenesis and further elucidating the intricate interplay between *E. pyrifoliae* and its host plants. Additionally, exploring the genetic variability of *E. pyrifoliae* strains and their interactions with host plants holds promise for refining disease management strategies and enhancing agricultural practices. By uncovering pathogenic genes and mechanisms, our findings not only advance our fundamental understanding of *E. pyrifoliae* pathogenicity but also offer practical implications for developing targeted interventions to mitigate its impact on pear and apple trees. Ultimately, this study contributes to the broader goal of promoting orchard health and enhancing fruit production in agricultural settings.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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Electronic Supplementary Material

Supplementary materials are available at Research in Plant Disease website (http://www.online-rpd.org/).

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