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First whole genome report of *Mangrovibacter phragmitis* PSU-3885–11 isolated from a patient in Thailand

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ABSTRACT

Mangrovibacter phragmitis is a Gram-negative bacterium typically found in plant roots that supports nitrogen fixation in nutrient-poor environments such as mangrove ecosystems. Although primarily found in environmental niches, an unusual case in Thailand of *M. phragmitis* strain PSU-3885–11 isolated from the sputum of a 29-year-old female patient with spinal tuberculosis. This isolate was initially misidentified as part of the *Enterobacter cloacae* complex (ECC) by MALDI-TOF. However, WGS subsequently confirmed its correct identity as *M. phragmitis*. The genome contains 4,651 coding sequences, along with 72 tRNA genes and 1 tmRNA. Moreover, comparative genomic analysis showed 99.32 % average nucleotide identity (ANI) similar to *M. phragmitis* MP23, and several antibiotic resistance genes (ARGs) and mobile genetic elements (MGEs) were identified in the PSU-3885–11 genome which may contribute to its ability to survive in diverse environments, including human hosts. The PSU-3885–11 displayed resistance to beta-lactam antibiotics such as ampicillin and cefotaxime, while remaining sensitive to a wide range of other antibiotics. Key virulence genes including *ompA, hcp/tssD,* and *rpoS*, were identified which may play a role in its persistence in human hosts as an opportunistic pathogen. The presence of ribosomally synthesized and post-translationally modified peptides (RiPPs) and bacteriocins indicates the antimicrobial properties that may provide a competitive advantage in both environmental and clinical settings of this strain. Therefore, this study provides valuable insights into the genomic features, antibiotic resistance, and potential pathogenicity of *M. phragmitis* PSU-3885–11. The findings also emphasize the importance of continued surveillance and genomic analysis of environmental bacteria that may emerge as opportunistic pathogens in human infections.

1. Introduction

The genus *Mangrovibacter* is a Gram-negative, rod-shaped, and facultatively anaerobic bacteria that belongs to the Enterobacteriaceae family. *Mangrovibacter* sp. is recognized for its connection to plant roots, where it typically contributes to enhancing plant growth and health such as nitrogen fixation (Rameshkumar et al., 2010). This process converts atmospheric nitrogen into a form usable by plants, such as ammonium. It is especially beneficial in nutrient-poor environments, such as mangrove ecosystems, where it greatly enhances nitrogen availability for host plants (Alfaro-Espinoza and Ullrich, 2015). Only three known species of *Mangrovibacter* were identified and their nucleotides were deposited in the National Center for Biotechnology Information (NCBI) database including *M. plantisponsor, M. yixingensis*, and *M. phragmitis*. Among these species, *M. plantisponsor* was first identified during a study investigating plant-microbe interactions in mangrove ecosystems. It was isolated from the roots of *Porteresia coarctata*, a wild rice species naturally adapted to saline and waterlogged environments along India's

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coasts. This halophytic plant is renowned for its exceptional salt tolerance and ability to thrive in challenging mangrove habitats (Rameshkumar et al., 2010). Similarly, *M. yixingensis* was isolated in 2015 from farmland soil in Yixing, Jiangsu Province, China. This species was identified through 16S rRNA gene sequencing, revealing a close relationship to the *M. plantisponsor* (Zhang et al., 2015). In contrast, *M. phragmitis* was recovered from the roots of *Phragmites karka* (tall reed) in Odisha, India. This bacterium exhibits significant plant growth-promoting traits, including nitrogen fixation and phosphate solubilization, making it a promising candidate for agricultural applications (Behera et al., 2017). Furthermore, its ability to adapt to saline environments highlights its potential for biotechnological use in managing saline soils. Their genomes also exhibit adaptive features for survival in specific environments, such as salt tolerance mechanisms in *M. plantisponsor* and *M. phragmitis* (Behera et al., 2017; Rameshkumar et al., 2010). These species are primarily non-pathogenic and lack significant virulence factors, aligning with their roles as beneficial plant symbionts rather than pathogens. Genetic analyses reveal evidence of horizontal gene transfer, suggesting adaptability to varying environmental conditions. Transmission occurs naturally through soil and water, facilitating the colonization of host plants. Their plant growth-promoting traits and environmental adaptability position them as promising candidates for agricultural applications, including enhancing crop productivity in nutrient-poor or saline soils, while also holding potential for broader biotechnological use.

The draft genome sequence of *M. phragmitis* provides functional characteristics of oxidative stress, uptake of nutrients, and nitrogen fixation to produce a range of enzymes and secondary metabolites that may have applications in biotechnology and agriculture (Behera et al., 2016). Overall, *M. phragmitis* is typically found in environmental areas and is rarely associated with human infections. The presence of *M. phragmitis* in common is uncommon in humans is uncommon and could either be an incidental observation or, in rare cases, indicate the emergence of an opportunistic pathogen, especially in individuals with underlying health conditions or compromised immune systems. In our study, this bacterium was unexpectedly isolated from a 29-year-old female patient presenting with symptoms consistent with spinal tuberculosis. Her clinical signs included a gibbous deformity, cold abscess, paradiscal lesion, anterior vertebral loss, narrowed disc space, and paravertebral shadows. Moreover, she exhibited tuberculosis-related symptoms such as loss of appetite, weight loss, and malnutrition, with a body mass index (BMI) below 18.5, which is a significant risk factor for TB infection.

During our one-year surveillance of *Enterobacter cloacae* complex (ECC) from patients in Songklanagarind Hospital, we obtained this PSU-3885–11 strain from the sputum of the patient that was first misidentified as ECC by MALDI-TOF analysis. The whole-genome sequencing (WGS) is an advanced and powerful tool with accuracy in current microbiological research that allows for a comprehensive analysis of the bacterial genome including its gene content, regulatory elements, and non-coding regions (Quainoo et al., 2017). This approach offers specific genes responsible for its adaptation to human hosts, potential virulence factors, and antibiotic resistance profile which are crucial information on the rarity of this strain in clinical settings and provide valuable insights into its possible role as an opportunistic pathogen (Sornchuer et al., 2024; Zaghloul and El Halfawy, 2022). Therefore, our study focuses on the genomic characterization of *M. phragmitis* PSU-3885–11 isolated from a patient in Thailand to obtain a comprehensive understanding of its genetic makeup, potential pathogenic traits, and antibiotic resistance profile. The insight information gained from this study is crucial for enhancing public health strategies of environmental bacteria, guiding investigations into how these bacteria may adapt to human hosts and transition into pathogenic roles.

2. Materials and methods

2.1. Patient sample and bacterial isolation

Mangrovibacter phragmitis PSU-3885–11 was isolated from the sputum of a 29-year-old female patient presenting with symptoms consistent with spinal tuberculosis at Songklanagarind Hospital. A bone tissue sample was obtained from the spine and the patient was diagnosed with tuberculosis using Xpert® MTB/RIF Ultra. The bacterial isolate was obtained using the BD BACTEC™ MGIT™ culture system. Bacterial isolate was then identified by All the procedures involving human participants were carried out following the rules of the Declaration of Helsinki and approved by the Human Research Ethics Committee (HREC) of Prince of Songkla University (protocol code: 64–284–14–1, date of approval: 9 June 2021).

2.2. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing (AST) of *M. phragmitis* PSU-3885–11 was conducted using disk diffusion assay. Antibiotic drugs were used in this study including amikacin (AK) 10 μg, ampicillin (AM) 10 μg, cefotaxime (CTX) 30 μg, cefoxitin (FOX) 30 μg, ceftazidime (CAZ) 30 μg, ceftriaxone (CRO) 30 μg, cefuroxime (CXM) 30 μg, ciprofloxacin (CIP) 5 μg, trimethoprim-sulfamethoxazole (Co-trimozole; STX) 1.25/23.75 μg, gentamicin (GM) 10 μg, imipenem (IPM) 10 μg, meropenem (MEM) 10 μg, piperacillin-tazobactam (TZP) 100/10 μg, ertapenem (ETP) 10 μg, and chloramphenicol (CL) 30 μg. The results of the AST were interpreted according to the Clinical & Laboratory Standards Institute (CLSI) standard (CLSI, 2015).

2.3. Genomic dna extraction and whole-genome sequencing

M. phragmitis PSU-3885–11 was cultured on Luria Bertani (LB) agar (Himedia, Mumbai, India) and then incubated at 37 °C for 24 h A single colony was then subcultured in Luria Bertani (LB) broth (Himedia, Mumbai, India) under shaking conditions at 37 °C for 4 h. The culture was centrifuged, and the cell pellet was washed twice with phosphate-buffered saline (PBS). Genomic DNA (gDNA) was subsequently extracted from the cell pellet using the ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Irvine, CA, USA) according to the manufacturer's protocol. The quality of the gDNA was assessed using 1 % agarose gel electrophoresis. The DNA samples were sent to the Beijing Genomics Institute (BGI) for short-read whole genome sequencing (WGS) using the MGISEQ-2000 platform.

2.4. Genome assembly, annotation and visualization

For genome assembly, the quality of raw reads was initially assessed using FASTQC v0.10.0. Subsequently, the reads were quality-filtered with Trimmomatic v0.32 (Bolger et al., 2014). The high-quality filtered reads were then de novo assembled using SPAdes v4.0.0 (Bankevich et al., 2012). The annotation was then performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al., 2016), genome visualization was generated with Proksee v1.0.0a6 (Grant et al., 2023) and functional prediction was conducted using Rapid Annotations using Subsystems Technology (RAST) (Aziz et al., 2008).

2.5. Mobile genetic element (MGE) prediction

To predict mobile genetic elements (MGEs) in the *M. phragmitis* PSU-3885–11 genome, mobileOG-db was utilized to identify MGEs and their associated orthologous gene families (Brown et al., 2022).

2.6. Species identification

To identify the bacterial species, FastANI v1.34 (Jain et al., 2018) was used to perform a pairwise comparison of *M. phragmitis* PSU-3885–11 against the reference genome *M. phragmitis* MP23 (GCF_001655675.1).

2.7. Sequence analysis

The restriction-modification (R-M) sites were detected using Restriction-ModificationFinder v1.1 (Roer et al., 2016), applying thresholds of 95 % minimum identity and 80 % minimum coverage. Additionally, Ribosomally synthesized and Post translationally modified Peptides (RiPPs) and bacteriocins were predicted by the Bagel4 server (van Heel et al., 2018).

2.8. Antimicrobial resistance profile among Mangrovibacter sp.

Antimicrobial resistance genes (ARGs) presented in *M. phragmitis* PSU-3885–11 were identified using the Comprehensive Antibiotic Resistance Database (CARD) with a cutoff of 80 % identity and an E-value threshold of 1e-5 (McArthur et al., 2013). The virulence-associated genes were detected by applying an 80 % identity cutoff and an E-value threshold of 1e-30 against the Virulence Factor Database (VFDB) (Chen et al., 2012).

3. Results and discussion

3.1. Bacterial identification

The biochemical test results of isolate PSU3885–11 are presented in Table S1. The results suggest the isolate belongs to the *Enterobacter* genus based on its metabolic profile, including positive results for VP, malonate utilization, gas production, and the fermentation of sucrose, mannitol, arabinose, and sorbitol. These traits align with typical *Enterobacter* characteristics, such as the ability to ferment a wide range of sugars and produce gas during fermentation (Rogers, 2024). The negative tests for indole production, H_2S production, and citrate utilization further support this classification. However, the MALDI-TOF analysis identified the isolate as *Klebsiella oxytoca* with a low confidence score of 1.7 (Table S1), which is below the standard threshold for reliable species-level identification (typically $\geq$2.0) (Panda et al., 2014). This discrepancy is not unexpected due to the MALDI-TOF relying on spectral matching with reference databases which may lack sufficient or accurate entries for rare, novel, or less-studied bacteria (Rychert, 2019). Hence, high-resolution methods, such as whole genome sequencing provide greater precision and reliability compared to biochemical tests and MALDI-TOF. This approach would help clarify whether the isolate belongs to *Enterobacter* sp., *K. oxytoca*, or another closely related species. Moreover, the use of genomic analysis can offer more precise identification and provide insights into antimicrobial resistance genes, virulence factors, and metabolic pathways, enabling clinicians to tailor antibiotic treatment more effectively.

3.2. Antimicrobial susceptibility profiles

The antibiotic susceptibility pattern of *M. phragmitis* PSU-3885–11 against the tested antibiotics is shown in Table 1. The strain PSU-3885–11 exhibited sensitivity to a range of antibiotics, indicating its susceptibility to various treatments. However, it showed resistance to certain antibiotics from Group I, specifically beta-lactams including ampicillin and cefotaxime.

The antibiotic susceptibility results of *M. phragmitis* PSU-3885–11 reveal a selective resistance profile, particularly within the beta-lactam group suggesting the presence of resistance mechanisms such as beta-lactamases that degrade these antibiotics (Mora-Ochomogo and Lohans, 2021). The resistance to ampicillin and cefoxitin while susceptibility to other antibiotics, highlights the importance of conducting detailed antibiotic susceptibility testing to guide treatment in clinical settings.

Table 1
Antibiotic susceptibility of *Mangrovibacter phragmitis* PSU-3885–11.

Group numbers	Antibiotics	Antibiotic susceptibility
Group I: Cell wall synthesis inhibitors	AM (10 μg)	R
	TZP (100/10 μg)	S
	CTX (30 μg)	S
	FOX (30 μg)	R
	CAZ (30 μg)	S
	CRO (30 μg)	S
	CXM (30 μg)	S
	IPM (10 μg)	S
	MEM (10 μg)	S
	ETP (10 μg)	S
Group II: Aminoglycosides	AK (10 μg)	S
	GM (10 μg)	S
Group III: Fluoroquinolones	CIP (5 μg)	S
Group IV: Folate synthesis inhibitors	SXT (1.25/23.75 μg)	S
Group VI: Chloramphenicol	CL (30 μg)	S

Susceptibility is presented as resistant (R) and sensitive (S).

3.3. Genome features of M. phragmitis PSU-3885–11

The genome of *M. phragmitis* PSU-3885–11 consists of 5035,050 bp with a GC content of 50 %. The sequence includes 4651 coding sequences (CDS), 72 tRNAs, and 1 tmRNA. The average nucleotide identity (ANI) of the PSU-3885–11 strain compared to the reference strain M. phragmitis MP23 (GCF_001655675.1 from the National Center for Biotechnology Information (NCBI) database) is 99.32 %. ANI analysis, which compares the genomic similarity between two isolates, is a robust and widely accepted method for confirming taxonomic relationships. This result suggested that the limitations of MALDI-TOF could provide misidentification data that delay appropriate treatment or lead to ineffective therapy, potentially exacerbating patient outcomes (Feucherolles et al., 2019). The comprehensive features of the assembled genome are presented in Table 2, and the circular representation of the genome is depicted in Fig. 1A. Notably, a comparative analysis of the PSU-3885–11 genome with four other *Mangrovibacter* strains including *M. phragmitis* MP23, *M. yixingensis* SaN21–3, *M. plantisponsor* DSM19579, and *Mangrovibacter* sp. MFB 070 from the NCBI database revealed that PSU-3885–11 shares a common core genome with other *Mangrovibacter* strains. It has acquired unique genetic elements, particularly MGEs, that may enhance its adaptability and potential virulence in human hosts. Specifically, the presence of several transferred regions in the PSU-3885–11 strain suggests that it has acquired genetic material from other bacterial species (Fig. 1B). This result suggests a significant role of horizontal gene transfer (HGT) in its evolutionary history. These transferred regions likely contain genes that confer advantageous traits, such

Table 2
Characteristics of *M. phragmitis* PSU-3885–11 genome.

Features	*M. phragmitis* PSU-3885–11
Size (bp)	5035,050
Number of Contigs	52
GC Content (%)	50.0
Number of Coding Sequences (CDS)	4651
N50	207,029
L50	7
tRNA	72
tmRNA	1
RAST subsystem	360
ANI value with *M. phragmitis* MP23 from NCBI (%)	99.32

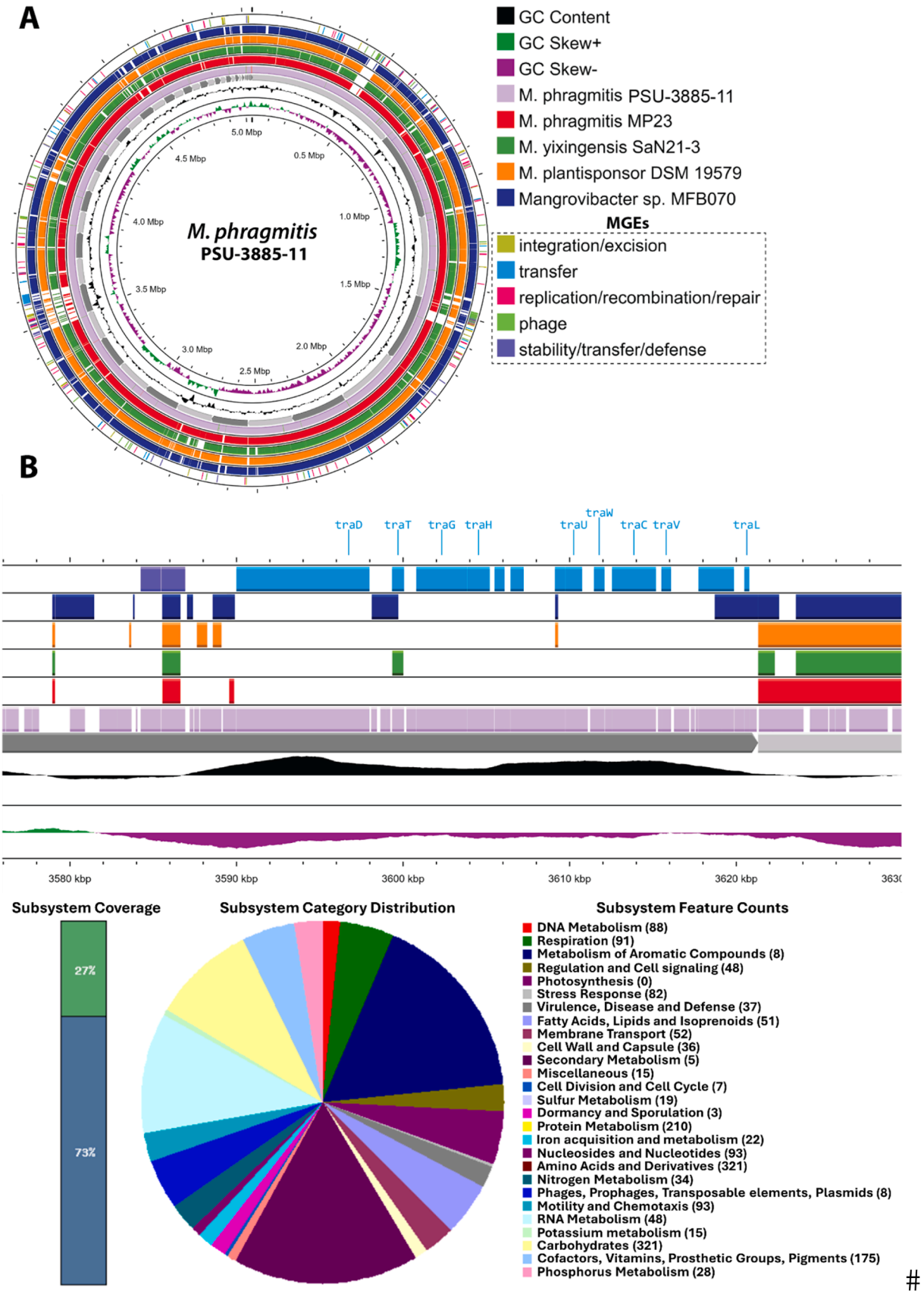

Fig. 1. Circular genome map of *Mangrovibacter phragmitis* PSU-3885–11 (A) and comparison of mobile genetic elements (MGEs) in transfer regions (highlighted in blue) with four other *Mangrovibacter* strains: *M. phragmitis* MP23, *M. yixingensis* SaN21–3, *M. plantisponsor* DSM 19,579, and *Mangrovibacter* sp. MFB 070. The genomes of the comparative strains were obtained from the NCBI database (B). Subsystem coverage of the *M. phragmitis* PSU-3885–11 genome as annotated by Rapid Annotations using Subsystems Technology (RAST) (C).

as antibiotic resistance, virulence factors, or metabolic capabilities that allow this strain to adapt to new environments, including human hosts (Hall et al., 2017; Panda et al., 2018).

Moreover, the core subsystem structure of the genome annotated by Rapid Annotations using Subsystems Technology (RAST) includes genes associated with carbohydrates (321), protein metabolism (210), virulence, disease, and defense (37), stress response (82), and other functional categories (Fig. 1C). The annotation of *M. phragmitis* PSU-3885–11 with genes associated with carbohydrate and protein metabolisms reveals key metabolic capabilities, indicating its adaptability to diverse nutrient sources. The annotation of virulence, disease, and defense-related genes suggests that, although *M. phragmitis* is primarily an environmental bacterium, it possesses the genetic arsenal necessary for pathogenic interactions (Beceiro et al., 2013; Strateva and Mitov, 2011). This includes genes that may enhance its ability to evade host immune defenses, adhere to host cells, and cause infections in immunocompromised individuals. These virulence factors, in combination with its metabolic capabilities, may allow *M. phragmitis* to transition from an environmental organism to an opportunistic pathogen in certain conditions. The identification of stress response genes suggests robust survival mechanisms in hostile environments, including human hosts (Fang et al., 2016). Additionally, the presence of genes linked to secondary metabolism, iron acquisition, and phosphate metabolism points to its capacity for producing secondary metabolites and efficiently managing essential nutrients, which could enhance its competitiveness and pathogenicity (Chevrette et al., 2022; Klebba et al., 2021). This comprehensive genomic profile underscores the versatility of the strain and its possible role as an emerging pathogen in immunocompromised individuals.

3.4. Mobile genetic element (MGE) identification

The MGEs within the genome of *M. phragmitis* PSU-3885–11 were identified using the mobileOG-db, a specialized database for categorizing and understanding mobile genetic components. The distribution and diversity of these MGEs are depicted in the outermost rings of the circular genome map shown in Fig. 1A. The result found that the PSU-3885–11 genome processes various MGE regions including integration and excision (IE; yellow), transfer (T; light blue), replication, recombination, and repair (RRR; dark pink), phage (P; light green), and stability, transfer, or defense (STD; purple). Moreover, the 329 MGEs identified in *M. phragmitis* PSU-3885–11 were categorized into the groups illustrated in Fig. 2. The identification of diverse MGEs further in *M. phragmitis* PSU-3885–11 provides significant insights into its genomic plasticity and adaptability. The large number of MGEs, particularly RRR (107) and IE (60), suggests that PSU-3885–11 has a high potential for acquiring foreign DNA, including antibiotic resistance and virulence genes through HGT (Jeon et al., 2023; Michaelis and Grohmann, 2023). Moreover, the significant presence of phage-related genes also underscores the role of bacteriophages in facilitating genetic diversity and may contribute to the evolutionary success of the strain (Pfeifer et al., 2022). The genomic flexibility may play a critical role in the ability of bacteria to adapt in different environments, especially human hosts, and poses a concern for the horizontal transfer of ARGs to other pathogens in clinical environments (Emamalipour et al., 2020; Wang et al., 2024). These findings suggest the importance of monitoring environmental bacteria of *M. phragmitis* PSU-3885–11 for their potential to become emerging pathogens through the acquisition of novel genetic elements.

3.5. Detection of restriction-modification (R-M) sites

The restriction-modification (RM) systems within the *M. phragmitis* PSU-3885–11 genome were thoroughly analyzed. The study identified three key genes: EcoEI, M.Ecl93I, and M.Ecl884AI, with sequence identities of 88.29 %, 88.59 %, and 90.97 %, respectively. The EcoEI gene, encoding a restriction enzyme, is located between 101,254 and

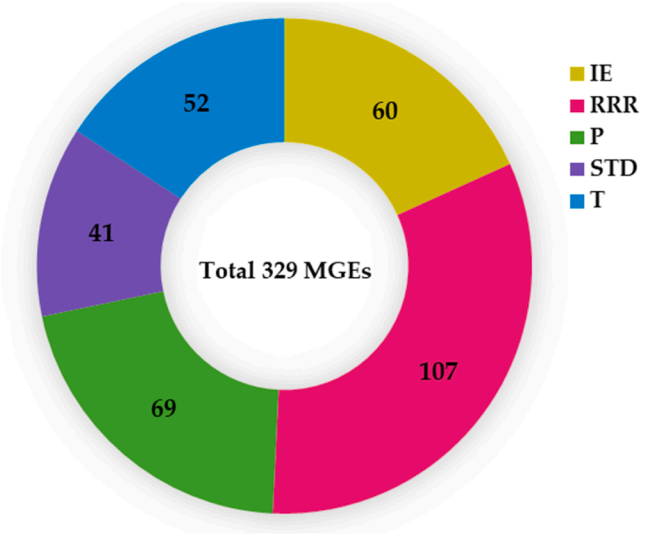

Fig. 2. Distribution of MGE features in *M. phragmitis* PSU-3885–11. Genes are categorized into five functional groups, each color-coded: Integration and excision (IE; yellow), transfer (T; light blue), replication, recombination, and repair (RRR; dark pink), phage (P; light green), and stability, transfer, or defense (STD; purple).

103,695 bp, while the M.Ecl93I and M.Ecl884AI genes, both responsible for encoding methyltransferases, are situated between 122,784 to 124,290 bp and 99,709 to 101,181 bp, respectively, as detailed in Table 3. The presence of these R-M systems indicates a mechanism for defense against foreign DNA, which might provide a selective advantage by protecting the bacterium from phage infections and other genomic threats in diverse environments (Rusinov et al., 2018; Vasu and Nagaraja, 2013).

3.6. Ribosomally synthesized and post translationally modified peptides (RiPPs) and bacteriocins

Four regions in *M. phragmitis* PSU-3885–11 were identified as containing RiPPs and bacteriocins, as predicted by the BAGEL4 server. These include bottromycin, sactipeptides, carocin D, and colicin E9 as shown in Table 4. The identification of RiPPs and bacteriocins, including bottromycin, sactipeptides, carocin D, and colicin E9, provides another layer of complexity to the potential role of *M. phragmitis* PSU-3885–11 in human infections. These antimicrobial peptides may confer a competitive advantage by inhibiting the growth of other bacteria in both environmental and host-associated settings by inhibiting the growth of competing bacteria (Nawrocki et al., 2014; Simons et al., 2020). This result could support the survival and persistence of *M. phragmitis* PSU-3885–11.

3.7. Antimicrobial resistance gene (ARG) profiles among Mangrovibacter sp.

The identification of ARGs in *Mangrovibacter* sp. was performed using the Comprehensive Antibiotic Resistance Database (CARD), as illustrated in Fig. 3. The heatmap displays the ARG profiles for *M. phragmitis* PSU-3885–11 alongside data from other *Mangrovibacter* strains available in the NCBI database, including *Mangrovibacter* sp. MFB070, *M. phragmitis* MP23, *M. plantisponsor* DSM 19,579, and *M. yixingensis* SaN21–3. The ARGs are categorized based on their roles in resistance mechanisms including antibiotic efflux, target modification, and inactivation. Specifically, genes involved in antibiotic efflux include *acrA, acrAB-TolC, CRP, qacG, emrR, emrB, rsmA,* and *msbA*. Genes associated with target alteration, such as *H—NS, arnT, PBP3, EF-Tu*, and *lmoG*, modify the antibiotic target sites, while only fosA8 is implicated in

Table 3
Restriction-Modification sites identified in *M. phragmitis* PSU-3885–11.

Gene	%Identity	Query length	Contig	Position	Type	Function	Accession No.
EcoEI	88.29	2442 / 2442	3885–11_00008	101,254……103,695	Type I	restriction enzyme	NEBM62
M.*Ecl93I*	88.59	1507 / 1512	3885–11_00013	122,784…124,290	Type I	methyltransferase	CP027604
M.*Ecl884AI*	90.97	1473 / 1473	3885–11_00008	99,709……101,181	Type I	methyltransferase	CP022532

Table 4
Bacteriocins identified *M. phragmitis* PSU-3885–11.

Contig	Start	End	Class	E-value
Contig 1	686,638	697,258	Colicin E9	9.56e-26
Contig 5	135,050	155,050	Bottromycin	1.96e-18
Contig 14	54,623	76,219	Sactipeptides	1.05e-27
Contig 14	89,681	110,053	Carocin D	8.44e-178

antibiotic inactivation. For *M. phragmitis* PSU-3885–11, the ARG profile indicates a range of resistance mechanisms, showing moderate to high identity in several antibiotic efflux genes such as *acrA, acrAB-tolC, CRP,* and *rsmA*. Moreover, only the PSU-3885–11 carries *msbA* which can provide this strain resistance to the nitroimidazole antibiotic class. The antimicrobial susceptibility pattern of PSU-3885–11 indicates resistance to beta-lactam antibiotics, such as ampicillin and cefotaxime, while retaining susceptibility to a broad range of other antibiotics. This resistance is likely mediated by specific ARGs identified in the genome, including those associated with efflux pumps, such as *acrA, acrAB-TolC, CRP*, and *rsmA*. These genes are known to contribute to multidrug resistance by actively expelling antibiotics from bacterial cells, thereby lowering intracellular drug concentrations and diminishing their effectiveness (Gaurav et al., 2023; Weston et al., 2018). Moreover, the presence of the *msb*A which is associated with resistance to nitroimidazole antibiotics is unique in *M. phragmitis* PSU-3885–11 among the studied *Mangrovibacter* strains (Alfaray et al., 2023). The result suggests that this strain may possess a broader resistance profile than others. Therefore, the antimicrobial susceptibility profile suggests the presence of intrinsic or acquired resistance mechanisms that enable PSU-3885–11 to survive specific antibiotic treatments, especially beta-lactams, which are commonly used to treat bacterial infections.

3.8. Virulence factor identification in Mangrovibacter sp. among available strains

In this study, the presence and identity of key virulence factor genes were assessed in various strains of *Mangrovibacter* sp. The heatmap in Fig. 4 illustrates the conservation of selected virulence genes across four strains. Among the genes analyzed, *ompA* and *hcp/tssD* exhibited near-complete conservation with 100 % identity across all strains, indicating a high degree of functional preservation. The gene *rpoS* also showed high conservation, suggesting its importance in adapting to hostile environments. In contrast, the genes *fur* and *rcsB* showed variable identity across *Mangrovibacter* strains and exhibited lower conservation of these genes.

The detection of genes associated with virulence, stress response, and host adaptation, such as *ompA, hcp/tssD*, and *rpoS*, suggests that PSU-3885–11 possesses a genetic repertoire capable of survival in hostile environments, including the human host. The *ompA,* encoded for outer membrane protein A, plays a critical role in adhesion to host cells, promoting colonization and invasion. This adhesion ability is critical for the establishment of infections, as it allows bacteria to interact intimately with host tissues, a key step in the pathogenesis process. Moreover, *ompA* has been extensively reported as a major virulence factor across a wide range of human opportunistic pathogens, including *Acinetobacter baumannii, Acinetobacter nosocomialis, Pseudomonas*

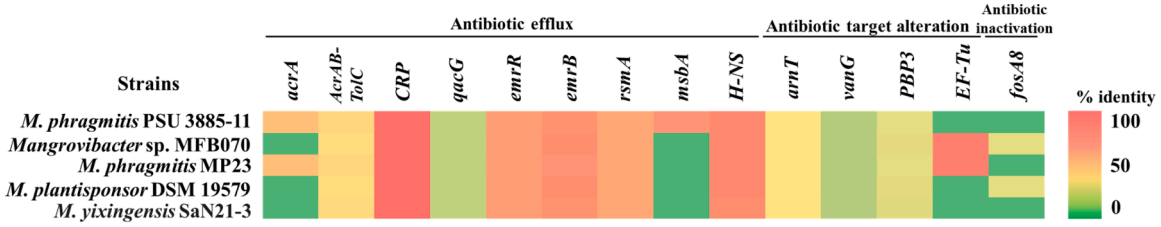

Fig. 3. Antimicrobial resistance gene (AMR) profile of *Mangrovibacter* sp. across available strains from the NCBI database. The percentage identifications of the gene are represented by colors.

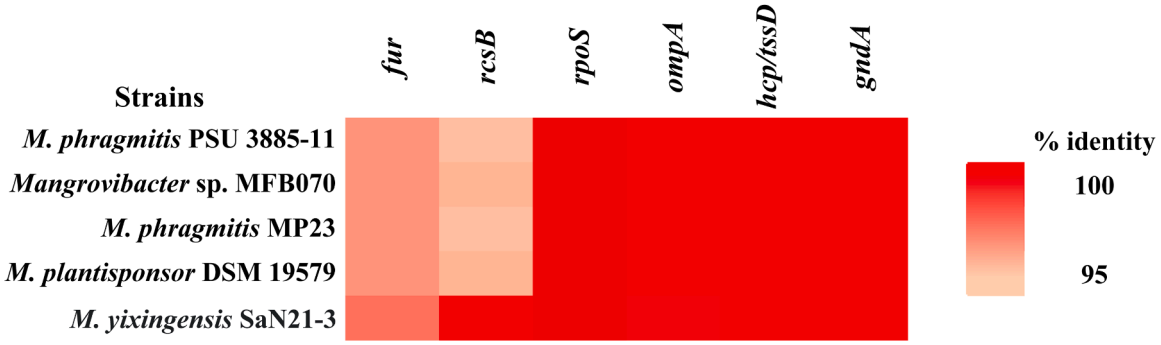

Fig. 4. Heatmap of virulence factor genes identified in *Mangrovibacter* sp. The heatmap displays the percentage of virulence-related genes across one strain in this study and the other four isolates obtained from the NCBI database. The color gradient represents the percentage identity, ranging from 95 % to 100 %, with darker red indicating higher identity.

aeruginosa, Escherichia coli, Cronobacter sakazakii, and *Salmonella* Typhimurium (Gao et al., 2021; Kim et al., 2016; Paulsson et al., 2021; Roy Chowdhury et al., 2022). In these pathogens, *ompA* contributes not only to adhesion but also to biofilm formation, immune evasion, and intracellular survival, which are essential for persistence and infection progression. Similarly, *hcp/tssD*, a key component of the Type VI secretion system (T6SS), enables the transport of effector proteins between neighboring cells. The T6SS functions by injecting toxic effector proteins directly into competing bacterial cells, providing a competitive edge and possibly facilitating interactions with host cells to enhance infection (Hernandez et al., 2020; Ma et al., 2017). Previous studies have extensively highlighted the dual role of the T6SS in both microbial competition and host-pathogen interactions (Gallegos-Monterrosa and Coulthurst, 2021; Yin et al., 2024). For example, T6SS-mediated delivery of antibacterial toxins, such as cell wall-degrading enzymes, has been shown to provide a significant advantage in polymicrobial environments by eliminating competing bacteria (Alcoforado Diniz et al., 2015). In *P. aeruginosa*, T6SS has been shown to deliver effector proteins into prokaryotic and eukaryotic cells to enhance the survival of the donor cell (Wood et al., 2019). The near-complete conservation of *ompA* and *hcp/tssD* across various *Mangrovibacter* strains highlights their essential roles in bacterial survival and pathogenicity by maintaining cell envelope integrity, facilitating bacterial interactions, and helping bacteria evade the immune system by interacting with host complement proteins (Confer and Ayalew, 2013; Hersch et al., 2020). Furthermore, *rpoS* acts as a global regulator of stress response, activating genes that enable bacterial survival under harsh conditions such as oxidative stress, nutrient deprivation, acid stress, osmotic stress, exposure to antimicrobial agents, and immune attacks. By enabling bacterial adaptation to such challenges, *rpoS* plays a pivotal role in ensuring long-term persistence, particularly in chronic infections (da Cruz Nizer et al., 2021; Zhu et al., 2024). In *E. coli, rpoS* has been shown to enhance resistance to oxidative bursts by activating the expression of *katE*, a catalase gene crucial for neutralizing hydrogen peroxide (Fasnacht and Polacek, 2021). Moreover, the role of *rpoS* extends beyond stress tolerance to broader survival strategies, including biofilm formation, quorum sensing regulation, and virulence factor expression in pathogens (Zhang et al., 2021). The high conservation of *rpoS* suggests its vital role in the stress response, which could be advantageous for survival under unfavorable conditions, such as in a human host with a compromised immune system. Interestingly, the variable conservation of genes such as *fur* and *rcsB*, which are associated with iron regulation and biofilm formation, respectively, across different *Mangrovibacter* strains, suggests strain-specific adaptations or reduced reliance on these pathways for virulence (Yuan et al., 2020). The *fur* is encoded for a regulator of iron homeostasis which might have evolved alternative strategies to acquire iron or may inhabit niches where iron is more readily available, reducing the need for strict iron regulation (Latorre et al., 2018). Previous studies have shown that *fur* regulates the abundance of key virulence factors in *Staphylococcus aureus*, coordinating their expression during pathogenesis. In *S. aureus* pneumonia, *fur* adapts gene expression to iron-limited conditions, facilitating immune evasion and infection establishment (Torres Victor et al., 2010). Likewise, *rcsB* encodes the response regulator of the *rcs* (regulator of capsule synthesis) two-component regulatory system, a critical pathway involved in biofilm formation and other surface-associated phenotypes in bacteria. Biofilm is a key factor in chronic infections and environmental persistence in bacteria (Mikkelsen et al., 2013). In chronic infections, biofilm formation regulated by *rcsB* contributes significantly to bacterial survival and other critical virulence factors in pathogens (Fei et al., 2021; Li et al., 2023; Meng et al., 2021). Overall, the identified virulence factors may enable *M. phragmitis* PSU-3885–11 to adapt, survive, and effectively interact with its host. For example, genes associated with adhesion, biofilm formation, or secretion systems could enable the bacteria to colonize host tissues, evade immune responses, or acquire essential nutrients. While *Mangrovibacter* species are primarily environmental bacteria, the presence of such virulence-related genes raises the possibility of opportunistic infections, particularly in immunocompromised individuals or under specific environmental triggers. Therefore, recognizing the functional roles of these factors in human infections is essential for evaluating the potential risks these bacteria may pose and their ability to adapt to host environments, emphasizing the importance of monitoring their virulence gene profiles.

By comprehensively characterizing the genome of *M. phragmitis* PSU-3885–11, isolated from a patient in Thailand, we gained valuable insights into its genetic features, potential pathogenicity, and antibiotic resistance profile. Moreover, the transition from an environmental niche to a clinical setting not only underscores its survival mechanisms but also provides a deeper understanding of how environmental bacteria may adapt to colonize human hosts. This study emphasizes the critical need for ongoing surveillance and genomic analysis of environmental bacteria that may emerge as opportunistic pathogens in clinical environments. The presence of virulence factors, ARGs, and MGEs in *M. phragmitis* PSU-3885–11 points out the need for further investigation into its clinical significance and potential impact on public health.

4. Conclusions

This study offers significant insights into the genomic characteristics, antibiotic resistance, and potential pathogenicity of *M. phragmitis* PSU-3885–11, a bacterium generally associated with plant roots but isolated from a human patient with spinal tuberculosis. The whole genome sequencing confirmed its identity and revealed antibiotic resistance genes, mobile genetic elements, and virulence factors, which may facilitate its persistence in human hosts as an opportunistic pathogen. The resistance to beta-lactam antibiotics, alongside the presence of antimicrobial peptides and bacteriocins of this strain, suggests its adaptability in both environmental and clinical settings. These findings emphasize the importance of ongoing surveillance of environmental bacteria, which may transition to human pathogens, particularly in immunocompromised individuals.

Author contributions

Conceptualization, N.C., K.S. (Komwit Surachat) and K.S. (Kamonnut Singkhamanan); methodology, N.C., T.Y., S.S., M.Y., N.T., and M.W.; software, N.C., T.Y., S.S. and K.S. (Komwit Surachat); validation, K.S. (Komwit Surachat), N.C., T.Y. and R.P.; formal analysis, N.C. and K.S. (Komwit Surachat); investigation, N.C. and K.S. (Komwit Surachat); resources, M.Y., S.C. and R.P.; data curation, N.C., T.Y. and K.S. (Komwit Surachat); writing—original draft preparation, N.C., T.Y., K.S. (Komwit Surachat) and K.S. (Kamonnut Singkhamanan); writing—review and editing, N.C., T.Y., K.S. (Komwit Surachat) and K.S. (Kamonnut Singkhamanan); visualization, N.C., T.Y. and K.S. (Komwit Surachat); supervision, K.S. (Komwit Surachat) and K.S. (Kamonnut Singkhamanan); project administration, K.S. (Komwit Surachat); funding acquisition, K.S. (Komwit Surachat). All authors have read and agreed to the published version of the manuscript.

Institutional review board statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the Human Research Ethics Committee (HREC) of Prince of Songkla University (protocol code: 64–284–14–1, date of approval: 9 June 2021).

Informed consent statement

According to retrospective reviews, the ethical committee allowed the waiver of consent forms.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.crmicr.2025.100350.

Data availability

Data will be made available on request.

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