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Good Thesis Awards

Biological Science Section

Mr. Punyaphat Bunroengsak

Master Degree Program

Major in Biotechnology

Whole-Genome Analysis of Priestia aryabhattai PTKU-123 for Its Potential in Inhibiting Plant Pathogenic Microorganisms in Economically Important Crops

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Abstracts

Phytophthora palmivora, Rhizoctonia solani, and *Fusarium moniliforme* are major plant pathogens causing severe losses in economically important Thai crops. To develop sustainable alternatives to chemical fungicides, the bacterium *Priestia aryabhattai* PTKU-123 isolated from durian orchard soil in Pattani province was characterized through integrated genomic, proteomic, and metabolomic analyses. Whole-genome sequencing revealed a 5.15 Mb circular chromosome with 38.2% G+C content and ~5,000 coding sequences, confirming species identity by 99.27% Average Nucleotide Identity (ANI) to *P. aryabhattai* LK20. Genome mining identified six biosynthetic gene clusters, including those associated with paeninodin (lassopeptide), a schizokinen-like siderophore, and surfactin-like lipopeptides. Proteomic and metabolomic profiling demonstrated active biosynthesis of plant growth-promoting compounds, notably indole-3-acetic acid (IAA) and salicylic acid. Antimicrobial assays revealed strong and selective inhibition of *Ph. palmivora* (82% with crude extract; 78% with cell-free supernatant), while no significant activity was observed against *R. solani* and *F. moniliforme*. This pathogen-specific antagonism highlights *P. aryabhattai* PTKU-123 as a promising eco-friendly biocontrol and plant growth-promoting agent, particularly effective against oomycete pathogens such as Ph. palmivora in durian and other tropical crops.

Introduction

Durian, rice, and sugarcane are major Thai export crops (~8.5, 5.8, and 4.4 billion USD respectively in 2023), yet all are threatened by pathogens: *Ph. palmivora* (durian root/stem rot), *R. solani* (rice sheath blight), and *F. moniliforme* (sugarcane red rot). Synthetic fungicides raise environmental and resistance concerns, motivating biological control using antagonistic microorganisms. *Priestia* species are known to produce antimicrobial lipopeptides (e.g., surfactin, iturin A) and phytohormones (IAA, salicylic acid), but the genetic basis of these traits in *P. aryabhattai* remains poorly characterized.

Objective

Identify bioactive compound-related genes and evaluate the antimicrobial activity of PTKU-123 crude extracts and cell-free supernatant against three plant pathogens in *vitro.*

Materials and Methods

1. Extracted PTKU-123 genomic DNA and confirmed its quality by gel electrophoresis.
2. Performed hybrid whole-genome sequencing using Illumina MiSeq (2 × 250 bp) and Oxford Nanopore MinION (R10.3), followed by assembly with Unicycler and quality assessment using QUAST.
3. Confirmed species identity using ANI (JSpeciesWS) and 16S rRNA phylogenetic analysis (MEGA11, neighbor-joining, 1,000 bootstrap replicates).
4. Annotated the genome using Prokka, eggNOG-mapper for functional COG classification, and antiSMASH for secondary metabolite biosynthetic gene clusters.
5. Cultured PTKU-123 in LB broth, extracted crude compounds by acid precipitation (pH 2) and 80% ethanol, and profiled them using LC-MS/MS shotgun proteomics and untargeted metabolomics.
6. Evaluated antimicrobial activity of the crude extract and cell-free supernatant using agar well diffusion against *Ph. palmivora*, *R. solani*, and *F. moniliforme.*

PROTEOMICS AND METABOLOMICS

Secretome Confirms Genomic Predictions

The cell-free supernatant proteome contained 900 expressed enzymes (55.7% of the 1,614 genome-predicted enzymes), dominated by transferases (31.1%), hydrolases (25.3%), and oxidoreductases (15.4%). Tryptophan-pathway enzymes (*TrpE*/*D*/*F*/*C*/*A*) and isochorismate synthase (*MenF*) were detected; downstream IAA enzymes (*TyrB, IpdC, DhaS*) and *PchB* were not consistent with their typically intracellular localization.

Bioactive Metabolites Detected

LC-MS/MS confirmed salicylic acid, tryptophan, and IAA in the cell-free supernatant and crude extract direct evidence that the predicted pathways are metabolically active. Compound superclasses: organic acids dominated (48.4%), followed by alkaloids (11%), nucleic acids (10.2%), fatty acyls (9.9%), benzenoids (8%), and organoheterocyclics (7.5%).

ANTIMICROBIAL ACTIVITY

Table 4. Antagonistic activity against *Ph. palmivora, R. solani, F. moniliforme* was evaluated using an agar well diffusion assay

Treatment	% Inhibition of *Ph. palmivora*	Effect on *R. solani* / *F. moniliforme*
Crude extract (5 mg/mL)	82.00 ± 1.05%	No inhibition observed
Cell-free supernatant	78.22 ± 2.83%	No inhibition observed
Sterile water (control)	0.00 ± 0%	No inhibition observed

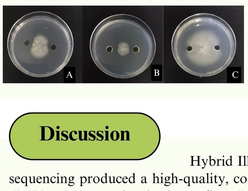


Figure 2. Representative antimicrobial assay plates demonstrating selective inhibition of *Ph. palmivora* by *P. aryabhattai* PTKU-123.
(A) Cell free Supernatant, (B) Crude extract , (C) Sterile water.

Results

GENOME FEATURES

Table 1. Average Nucleotide Identity (ANI) Values Between *Priestia aryabhattai* PTKU123 and Reference Genomes.

Reference genome	ANI (%)	Aligned (%)
P. aryabhattai LK20	99.27	93.93
P. aryabhattai B8W22	95.78	86.22
P. megaterium WSH-002	95.77	88
P. megaterium Riq5	95.24	86.52

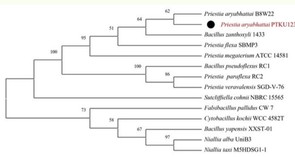


Figure 1. Phylogenetic relationships between *Priestia aryabhattai* PTKU-123 (based on the 16S rRNA gene).

Hybrid assembly yielded a 5,150,877 bp circular chromosome (38.2% G+C) with 5,086 CDSs, 113 tRNA, 35 rRNA, and 1 tmRNA gene. 99.27% ANI to *P. aryabhattai* LK20 exceeds the 95–96% species threshold, confirming identity; 16S rRNA phylogeny placed PTKU-123 within a well-supported Priestia clade (bootstrap 95–100%).

SECONDARY METABOLITE GENE CLUSTERS

Table 2. Secondary metabolite synthetic genetic cluster of PTKU-123.

Region	Type	Similar cluster	Similarity
1	Terpene	Carotenoid	50%
2	Type III PKS (T3PKS)	no close match	–
3	Terpene	no close match	–
4	Lassopeptide (RiPP)	Paeninodin	100%
5	NRPS	Surfactin	13%
6	NI-siderophore	Schizokinen	50%

PLANT GROWTH-PROMOTING AND ANTIMICROBIAL GENES

Table 3. Genetic Basis for Dual Function

Genes	Compound	Function
aroA, aroC, aroK	Chorismate	Biosynthesis precursor
trpE, trpD, trpF, trpC, trpA, trpB	Tryptophan	Biosynthesis precursor
tyrB, ipdC, dhaS	Indole-3-acetic acid (IAA)	Plant growth promotion
menF, pchB	Salicylic acid	Plant growth promotion / defense
aroH, bacA, bacB, bacD, bacE, bacF, bacG	Bacilysin	Antimicrobial activity

Discussion

Hybrid Illumina and Oxford Nanopore sequencing produced a high-quality, complete genome; ANI and 16S rRNA phylogenetics both confirm PTKU-123 as *P. aryabhattai*, Compared to *P. aryabhattai* B8W22, PTKU-123 shows a more specialized, peptide-oriented biosynthetic profile (paeninodin- and surfactin-like clusters plus a siderophore cluster) consistent with a targeted antagonistic and plant-associated ecological role. Genomic predictions for IAA, salicylic acid, and bacilysin biosynthesis were partly validated at the protein level and further confirmed by direct metabolite detection even where pathway enzymes were undetected in the secretome, likely due to their intracellular localization. The selective inhibition of *Ph. palmivora* but not *R. solani* or *F. moniliforme* points to a mechanism targeting oomycete-specific cell biology (cellulose-based walls) rather than the chitin-based walls of true fungi a selectivity that may help preserve beneficial fungal communities in the rhizosphere.

Conclusion

Integrative genomic, proteomic, and metabolomic analyses show that *P. aryabhattai* PTKU-123 produces a wide range of metabolites linked to plant growth promotion (IAA, salicylic acid) and antimicrobial defense (bacilysin, paeninodin-like and surfactin-like compounds), with selective antimicrobial activity against the oomycete pathogen *Ph. palmivora*. This target specificity is advantageous for developing environmentally compatible biocontrol formulations that minimize non-target effects on beneficial soil microorganisms.

Future work: compound purification, structural elucidation, and field validation translating these multi-omics insights into practical, bio-based tools for sustainable agriculture and integrated pest management.

Acknowledgements

Thesis Book File