International Journal of Science, Mathematics and Technology Learning
ISSN: 2327-7971 (Print) ISSN: 2327-915X (Online)
Volume 33 No. 1, 2025

Identification and Bioinformatic Characterization of Antimicrobial Resistance Genes in Pseudomonas syringae Phylogroup 2 Associated with Bacterial Stem Blight in Alfalfa (Medicago sativa)

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Abstract

Pseudomonas syringae Phylogroup 2 is a Gram-negative bacterial pathogen known to cause bacterial stem blight (BSB) in alfalfa (Medicago sativa), significantly affecting crop yield and quality. This study utilized whole-genome sequencing and comprehensive bioinformatic analysis to identify antimicrobial resistance (AMR) genes in P. syringae strain Susan2139. Annotated genome sequences were processed to generate a circular genome map, identify housekeeping genes, and construct a phylogenetic tree. Tools such as CARD, MLST, and PathogenFinder were used to predict AMR genes and pathogenicity potential. The strain demonstrated the presence of efflux pump genes (adeF, AbQ) associated with resistance to fluoroquinolones and tetracyclines, but lacked plasmid-mediated or acquired virulence genes. Phylogenetic analysis placed the strain closest to P. syringae pv. syringae B64 (98.1% ANI). Pathogenicity predictions classified the bacterium as non-pathogenic to humans. These results contribute valuable insights into AMR gene evolution in phytopathogens and offer potential avenues for disease control strategies in alfalfa cultivation.

Keywords

Pseudomonas Syringae, Alfalfa, Antimicrobial Resistance, Whole-Genome Sequencing, Phylogenomics, Efflux Pump, Bioinformatics.

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Acceptance: 05 June 2025, Publication: 18 June 2025
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1. Introduction

Alfalfa (*Medicago sativa*), a highly nutritious forage legume, plays a pivotal role in sustainable agriculture due to its nitrogen-fixing capacity and utility in livestock feed (Putnam et al. 2000). However, bacterial stem blight (BSB) caused by *Pseudomonas syringae* significantly compromises its productivity (Lipps et al. 2024). This Gram-negative pathogen infiltrates plants through wounds or stomata and spreads within the apoplast, forming lesions and oozes that impede plant growth and yield (Moya et al. 2023).

P. syringae possesses a Type III secretion system and produces various secondary metabolites that enhance its pathogenic potential. Of growing concern is its ability to develop antimicrobial resistance (AMR) genes, which are often encoded on mobile elements or within chromosomal loci, limiting the efficacy of conventional chemical treatments (Hwang et al. 2005). Despite this, data on the AMR gene composition and pathogenic potential of *P. syringae* strains affecting alfalfa remain limited. This study addresses this gap by characterizing the genome of strain Susan2139 using a suite of bioinformatic tools.

Materials and Methods

Genome Retrieval and Annotation

The genome of *P. syringae* strain Susan2139 (Accession: ASM1839437v1) was retrieved from NCBI. Annotation was performed using Prokka on the Galaxy platform to identify coding sequences (CDS) and generate GenBank (.gbk) files.

Genome Mapping and Strain Typing

The annotated genome was used to construct a circular map via PROKSEE, detailing tRNA, rRNA, and CDS locations. KmerFinder v3.2 (Centre for Genomic Epidemiology) was employed for bacterial strain identification.

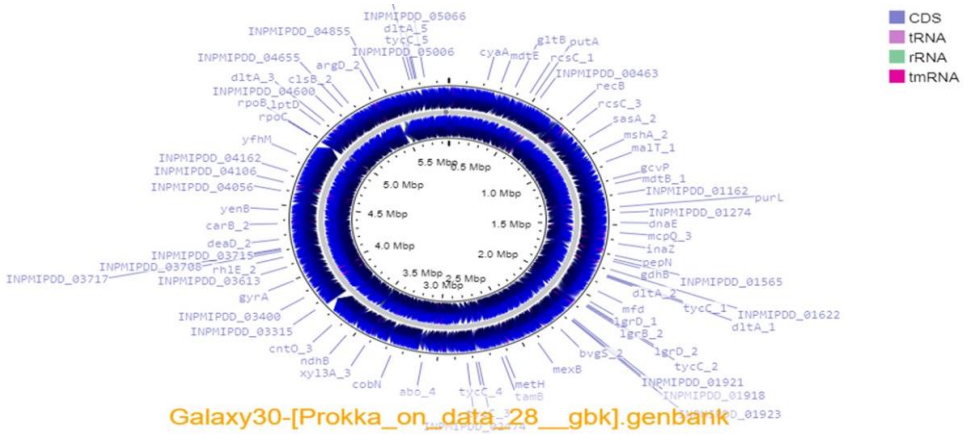

Figure 1: Circular Genomic Map of *P.syringae*.

Plasmid and Virulence Gene Detection

PlasmidFinder v2.11 and VirulenceFinder v2.0 were utilized to detect plasmid-borne and acquired virulence genes, respectively.

Table 1: Results from PlasmidFinder

Plasmid	Identity	Query/Template length	Contig	Position in Contig	Note	Accession no.
No Hit Found						

Table 2: Results from VirulenceFinder

Virulence Factor	Identity	Query/Template length	Contig	Position in Contig	Protein Function	Accession no.
No hit Found						
		Siga-toxin gene				
Virulence Factor	Identity	Query/Template length	Contig	Position in Contig	Protein Function	Accession no.
No hit Found						

Phylogenetic and Housekeeping Gene Analysis

Multilocus sequence typing (MLST) was conducted using autoMLST. Phylogenetic trees were constructed to assess strain relationships based on average nucleotide identity (ANI).

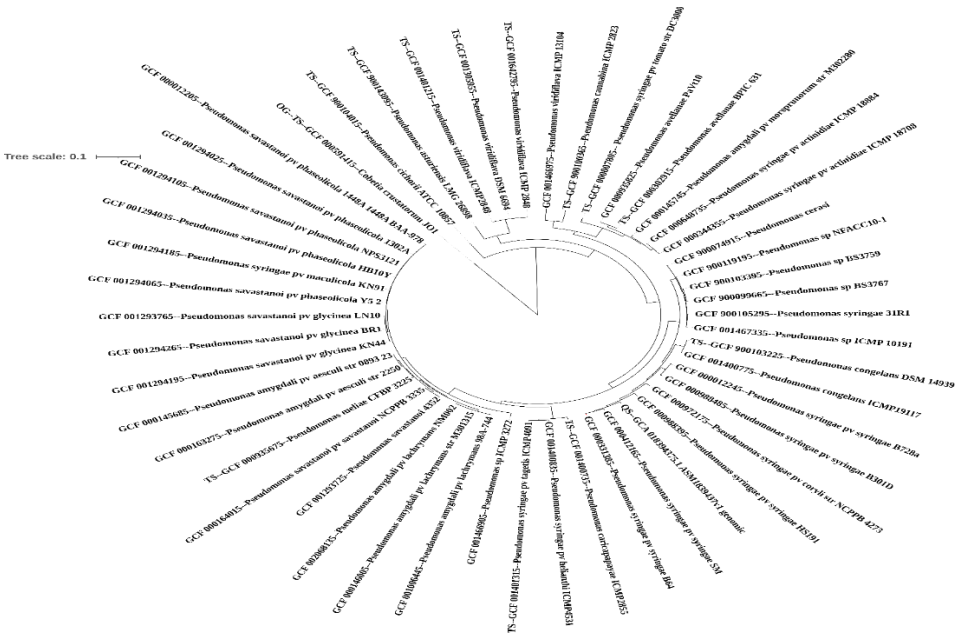

Figure 2: Phylogenetic Tree of *P.syringae* Strain Susan2139.

Table 3: MLST Results for Detection of Housekeeping Genes.

Query organism	Reference assembly ID	Reference name	Mash distance	Estimated ANI	Genus	Order	Type strain
QS… GCA_018394375.1_ASM1839437v1_genomic. fa	GCF_000331385	*Pseudomonas syringae* pv. *syringae* B64	0.0190	98.1%	Pseudomonas	Pseudomonadales	False
QS… GCA_018394375.1_ASM1839437v1_genomic. fa	GCF_000412165	*Pseudomonas syringae* pv. *syringae* SM	0.0190	98.0%	Pseudomonas	Pseudomonadales	False
QS… GCA_018394375.1_ASM1839437v1_genomic. fa	GCF_000988395	*Pseudomonas syringae* pv. *syringae* HS191	0.0190	97.4%	Pseudomonas	Pseudomonadales	False

AMR Gene Identification

CARD's Resistance Gene Identifier (RGI) was employed under strict and perfect match parameters to detect known AMR determinants.

Table 4: AMR Genes Detected and their Resistance Mechanisms.

RGI Criteria	ARO Term	SNP	Detection criteria	AMR Gene Family	Drug class	Resistance mechanism	% of of matchng region	% length of reference sequence
Strict	*Acinetobacter baumanii* AbQ		Protein homolog model	Major facilitator superfamily (MFS) antibiotic	Fluoroquinolone antibiotic	Antibiotic efflux	72.73	100.6

				efflux pump				
Strict	ArnT		Protein homolog model	Pmr phosphoethanolamine transferase	Peptide antibiotic	Antibiotic target alteration	43.56	99.6
Strict	adeF		Protein homolog model	Resistance nodulation cell division (RND) antibiotic efflux pump	Fluoroquinolone antibiotic tetracycline antibiotic	Antibiotic efflux	66.38	100.3
Strict	adeF		Protein homolog model	Resistance nodulation cell division (RND) antibiotic efflux pump	Fluoroquinolone antibiotic tetracycline antibiotic	Antibiotic efflux	42.41	99.5

Pathogenicity Prediction

PathogenFinder v1.1 assessed the likelihood of human pathogenicity based on whole genome content.

Table 5: PathogenFinder Results.

The input organism was predicted as non- human pathogen.

Probability of being a human pathogen - 0.2

Input proteome coverage (%) - 1.46

Matched Pathogenic Families - 0

Matched Not Pathogenic Families- 7.5

Sequences- 5121

Total bp- 1725746

Results

Strain Identification and Genome Overview

KmerFinder confirmed the strain as *P. syringae* Susan2139 with 99.31% query coverage. The genome consisted of 5,121 sequences totaling ~1.72 Mbp.

Genome Annotation and Mapping

Prokka annotated 2,090 coding regions. The PROKSEE map displayed the genomic arrangement of CDS, tRNAs, and rRNAs.

Plasmid and Virulence Factors

Neither PlasmidFinder nor VirulenceFinder detected any known plasmids or virulence genes in the genome.

Phylogenetic Analysis

MLST placed Susan2139 closest to *P. syringae* pv. syringae B64 (98.1% ANI), followed by strains SM and HS191.

AMR Gene Profile

CARD analysis identified four AMR genes including *adeF* (RND efflux pump), *AbQ* (MFS transporter), and *ArnT* (target modification protein). These conferred resistance primarily to fluoroquinolones and tetracyclines.

Human Pathogenicity

PathogenFinder predicted the strain as non-pathogenic to humans with a probability score of 0.2 and no matched pathogenic families.

Discussion

The study demonstrates that *P. syringae* strain Susan2139 carries intrinsic resistance mechanisms, particularly via efflux pumps, yet lacks plasmid-associated gene transfer vectors. The absence of virulence genes and human pathogenic markers supports its specialization as a plant pathogen. Phylogenetic alignment with known pathovars confirms its classification within Phylogroup 2.

The identification of AMR genes in a phytopathogen underscores the importance of genomic surveillance in agriculture. Efflux pumps such as *adeF* and *AbQ* likely evolved due to consistent antimicrobial exposure in agricultural ecosystems (Hwang et al. 2005). This adaptation could influence disease severity and persistence.

Conclusions

This study provides a comprehensive genomic profile of *P. syringae* strain Susan2139 infecting alfalfa. Bioinformatic analyses revealed intrinsic AMR genes and a lack of horizontal gene transfer or virulence potential. These findings support the development of

targeted strategies for managing bacterial stem blight and highlight the role of genomic tools in agricultural pathogen monitoring.

Acknowledgments

The authors gratefully acknowledge the Central University of Jharkhand for laboratory facilities. Special thanks to Prof. Manoj Kumar for supervision and guidance.

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