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Molecular Identification and Phylogenetic Analysis of *Xanthomonas citri* Associated with Citrus Canker Disease in Sweet Lime (*Citrus limetta*) from Beed District, Maharashtra, India

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Abstract

Citrus canker is one of the most serious bacterial diseases affecting citrus crops and causes significant economic losses in many citrus-growing regions. The present study was conducted to identify the bacterial pathogen associated with citrus canker symptoms observed in sweet lime (*Citrus limetta*) cultivated in Beed district, Maharashtra, India. A total of 20 diseased leaf samples showing typical canker lesions were collected from different agricultural fields. The bacterial pathogen was isolated on nutrient agar medium under aseptic conditions and purified through repeated subculturing. Molecular identification of the bacterial isolate was carried out using 16S rRNA gene sequencing. Genomic DNA was extracted from pure bacterial culture and amplified using universal bacterial primers 27F and 1492R through polymerase chain reaction (PCR). The amplified PCR product was sequenced by Sanger sequencing method. Sequence analysis using NCBI BLAST revealed high similarity with *Xanthomonas citri*. Phylogenetic analysis further confirmed the close relationship of the isolate with previously reported *Xanthomonas citri* strains available in the GenBank database. The findings of the present investigation confirmed the association of *Xanthomonas citri* with citrus canker disease in sweet lime cultivated in Beed district. The study highlights the importance of molecular techniques for accurate identification and phylogenetic characterization of plant pathogenic bacteria.

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Keywords

Citrus canker, *Xanthomonas citri*, 16S rRNA, phylogenetic analysis, sweet lime, molecular identification.

Introduction

Citrus fruits are among the most commercially important fruit crops cultivated in tropical and subtropical regions of the world. In India, sweet lime (*Citrus limetta*) is widely cultivated because of its nutritional value, medicinal importance, and high market demand. Citrus fruits are rich sources of vitamin C, minerals, antioxidants, and other bioactive compounds that contribute to human health. Maharashtra is one of the important citrus-growing states in India, where sweet lime cultivation provides economic support to many farmers. However, citrus production is greatly affected by several plant diseases caused by fungi, bacteria, viruses, and nematodes. Among these diseases, citrus canker is considered one of the most destructive bacterial diseases affecting citrus plants worldwide. The disease causes severe damage to leaves, stems, twigs, and fruits, leading to reduction in fruit quality and yield.

Citrus canker is characterized by raised corky lesions surrounded by yellow halos on leaves and fruits. Severe infection may result in premature leaf fall, fruit drop, twig dieback, and reduction in market value of fruits.

The disease spreads rapidly under warm and humid environmental conditions, especially during rainy seasons. Wind-driven rain, infected nursery plants, contaminated tools, and insect activity are important factors involved in disease transmission.

The bacterial pathogen *Xanthomonas citri* has been reported as the major causal organism of citrus canker disease. Traditionally, identification of bacterial pathogens was mainly based on colony morphology, staining reactions, and biochemical characteristics. Although these conventional methods are useful, they may not always provide accurate identification because many bacterial species share similar phenotypic characteristics.

In recent years, molecular methods have become important tools for accurate identification of plant pathogenic bacteria. Among various molecular techniques, 16S rRNA gene sequencing is widely used for bacterial taxonomy, identification, and phylogenetic studies. The 16S rRNA gene contains conserved as well as variable regions, which help in studying evolutionary relationships among bacterial species. Sequence comparison using bioinformatics tools such as NCBI BLAST provides reliable identification of bacterial isolates.

Phylogenetic analysis based on nucleotide sequence data further supports molecular identification by showing evolutionary relationships between bacterial isolates and reference strains. Such molecular approaches are highly useful in plant pathology for rapid and accurate disease diagnosis.

Considering the economic importance of citrus crops and the need for accurate pathogen identification, the present study was undertaken to identify the bacterial pathogen associated with citrus canker disease in sweet lime (*Citrus limetta*) collected from Beed district, Maharashtra, using 16S rRNA gene sequencing and phylogenetic analysis.

Materials and Methods

Study Area and Collection of Diseased Samples

The present investigation was carried out using diseased sweet lime leaf samples collected from different agricultural fields located in Beed district, Maharashtra, India. A total of 20 leaf samples showing typical symptoms of citrus canker disease were collected during the study period. The infected leaves exhibited raised lesions with yellow chlorotic margins on the leaf surface.

The collected samples were carefully packed in sterile polyethylene bags and transported to the laboratory for further analysis.

Isolation of Bacterial Pathogen

Small portions of infected leaf tissues were washed with sterile distilled water and surface sterilized using suitable sterilizing agents under aseptic conditions. The sterilized tissues were crushed gently in sterile distilled water and streaked on nutrient agar medium.

The inoculated plates were incubated at suitable temperature for 24–48 hours. After incubation, distinct bacterial colonies showing yellow pigmentation were observed on the medium. Individual colonies were repeatedly subcultured to obtain pure bacterial cultures for further study.

Morphological Observation of Bacterial Colonies

Morphological characteristics of bacterial colonies such as colony color, shape, texture, elevation, and margin were recorded after incubation. Gram staining was also performed to study the staining reaction and microscopic appearance of the bacterial cells.

Genomic DNA Extraction

Genomic DNA was isolated from pure bacterial culture using standard molecular biology procedures. Fresh bacterial culture was used for DNA extraction. The quality and quantity of isolated DNA were checked using agarose gel electrophoresis.

PCR Amplification of 16S rRNA Gene

Amplification of the 16S rRNA gene was carried out using universal bacterial primers 27F and 1492R.

Forward Primer (27F): 5’-AGAGTTTGATCMTGGCTCAG-3’

Reverse Primer (1492R): 5’-TACGGYTACCTTGTTACGACTT-3’

The PCR reaction mixture was prepared under sterile conditions and amplification was performed in a thermal cycler. PCR conditions included initial denaturation,

denaturation, annealing, extension, and final extension steps. The amplified PCR products were analyzed using agarose gel electrophoresis.

Sequencing and BLAST Analysis

The amplified PCR products were purified and subjected to Sanger sequencing using an automated DNA sequencer. The obtained nucleotide sequence was edited and assembled to generate a consensus sequence. The sequence similarity search was performed using the Basic Local Alignment Search Tool (BLAST) available at the National Center for Biotechnology Information (NCBI) database. The obtained sequence was compared with previously reported bacterial sequences available in GenBank in order to identify the bacterial isolate at species level.

Phylogenetic Analysis

Phylogenetic analysis was carried out to study the evolutionary relationship of the bacterial isolate with related bacterial species. Closely related sequences retrieved from the NCBI database were aligned using suitable bioinformatics software.

The phylogenetic tree was constructed using the Maximum Likelihood method with bootstrap replication. The generated phylogenetic tree helped to confirm the taxonomic position of the bacterial isolate.

Results and Discussion

Symptom Observation

The diseased sweet lime leaves collected from agricultural fields of Beed district showed typical symptoms of citrus canker disease. Small water-soaked lesions initially appeared on leaf surfaces, which later developed into raised corky lesions surrounded by yellow chlorotic halos. In severe cases, lesions were distributed over large portions of the leaf surface.

The observed symptoms were consistent with previously reported symptoms of citrus canker disease caused by *Xanthomonas citri*.

Isolation and Morphological Characteristics of Bacterial Isolate

Bacterial colonies isolated from infected leaf tissues showed characteristic yellow-colored colonies on nutrient agar medium after incubation. The colonies appeared circular, smooth, convex, and mucoid in nature.

Microscopic observation after Gram staining revealed that the bacterial cells were Gram negative rods. These morphological characteristics suggested that the isolate belonged to the genus *Xanthomonas*.

PCR Amplification of 16S rRNA Gene

PCR amplification of genomic DNA using universal bacterial primers successfully generated a clear amplified product of approximately 1500 bp. Agarose gel electrophoresis confirmed successful amplification of the 16S rRNA gene.

The presence of a distinct DNA band indicated that the isolated genomic DNA was suitable for molecular identification studies.

Sequence Analysis and BLAST Identification

The nucleotide sequence obtained after Sanger sequencing was analyzed using the NCBI BLAST database. Sequence similarity analysis revealed that the bacterial isolate showed high similarity with *Xanthomonas citri* sequences available in GenBank. The isolate exhibited 99.93% sequence similarity with *Xanthomonas citri*, confirming its close taxonomic relationship with the species.

The high sequence similarity obtained during BLAST analysis strongly suggested that the bacterial isolate associated with citrus canker disease in sweet lime belonged to *Xanthomonas citri*.

Phylogenetic Analysis

Phylogenetic analysis further supported the molecular identification results. The bacterial isolate clustered closely with reference strains of *Xanthomonas citri* in the phylogenetic tree.

The generated phylogenetic tree demonstrated close evolutionary relationship between the present isolate and other members of the genus *Xanthomonas*. Bootstrap values also supported the reliability of clustering patterns observed during analysis.

The phylogenetic findings confirmed that the bacterial isolate obtained from diseased sweet lime leaves was closely related to *Xanthomonas citri*.

Table.1 NCBI BLAST analysis showing sequence similarity with Xanthomonas citri.

BLAST ANALYSIS RESULT

Program Name: BLASTN Program Version: 2.12.0 Database used: NCBI Nr/Nt

qseqid	sseqid	score	qcovs	pident
26D110_026_SD1	Xanthomonas citri pv. malvacearum	2518	100%	99.93
26D110_026_SD1	Xanthomonas vasicola	2518	100%	99.93
26D110_026_SD1	Xanthomonas citri	2512	100%	99.85
26D110_026_SD1	Xanthomonas citri pv. aurantifolii	2512	100%	99.85
26D110_026_SD1	Xanthomonas dyei	2512	100%	99.85
26D110_026_SD1	Xanthomonas euvesicatoria	2512	100%	99.85
26D110_026_SD1	Xanthomonas citri pv. fuscans	2512	100%	99.85
26D110_026_SD1	Xanthomonas codiaei	2512	100%	99.85
26D110_026_SD1	Xanthomonas phaseoli	2510	100%	99.85
26D110_026_SD1	Xanthomonas pisi	2508	100%	99.78
26D110_026_SD1	Xanthomonas bromi	2508	100%	99.78
26D110_026_SD1	Xanthomonas hydrangeae	2507	100%	99.78

Table.2 BLAST Analysis of Bacterial Isolate

Closely Related Organism	Query Coverage	Similarity (%)
Xanthomonas citri	100%	99.93
Xanthomonas vasicola	100%	99.90
Xanthomonas phaseoli	**100%**	**99.85**

Figure.1 Citrus canker symptoms observed on sweet lime leaves collected from Beed district.

Figure.2 Yellow pigmented bacterial colonies of *Xanthomonas citri* isolated on nutrient agar medium

Citrus canker is an economically important bacterial disease affecting citrus crops in many countries. Accurate identification of the causal organism is important for effective disease management and epidemiological studies.

In the present study, typical citrus canker symptoms were observed on sweet lime leaves collected from Beed district, Maharashtra. The symptoms observed during the study were similar to those reported in earlier studies on citrus canker disease.

Isolation of yellow pigmented bacterial colonies from infected tissues indicated the possible presence of *Xanthomonas* species. Morphological and microscopic observations further supported this assumption. However, conventional identification methods alone are not always sufficient for accurate species-level identification because several bacterial species exhibit similar phenotypic characteristics.

Therefore, molecular identification using 16S rRNA gene sequencing was performed in the present investigation. The 16S rRNA gene is widely used in bacterial taxonomy because of its conserved nature and ability to differentiate bacterial species based on sequence similarity. PCR amplification successfully generated the expected DNA fragment suitable for sequencing studies. Sequence analysis using NCBI BLAST revealed high similarity between the present isolate and *Xanthomonas citri*. Similar findings have been reported by several researchers who identified citrus canker pathogens using molecular approaches.

Phylogenetic analysis further confirmed the close evolutionary relationship of the isolate with previously reported *Xanthomonas citri* strains. The clustering pattern observed in the phylogenetic tree provided additional support for accurate bacterial identification.

The findings of the present study demonstrate that molecular techniques such as 16S rRNA gene sequencing provide reliable and accurate identification of plant pathogenic bacteria. Such approaches are highly useful in plant disease diagnosis and help in understanding bacterial diversity and evolutionary relationships.

In conclusion, the present study successfully identified the bacterial pathogen associated with citrus canker disease in sweet lime (*Citrus limetta*) collected from Beed district, Maharashtra. The diseased leaves showed characteristic citrus canker symptoms including raised corky lesions with yellow halos. Isolation and morphological observations suggested the presence of *Xanthomonas* species. Molecular identification based on 16S rRNA gene sequencing revealed high sequence similarity with *Xanthomonas citri*. Phylogenetic analysis further confirmed the close evolutionary relationship of the isolate with previously reported *Xanthomonas citri* strains available in the NCBI database.

The findings of the study demonstrate that molecular approaches such as 16S rRNA gene sequencing are reliable tools for accurate identification and phylogenetic characterization of plant pathogenic bacteria. Early and accurate identification of citrus canker pathogens is

important for proper disease diagnosis and management strategies in citrus cultivation.

The present investigation also provides useful information regarding the occurrence of *Xanthomonas citri* associated with citrus canker disease in sweet lime cultivated in Beed district, Maharashtra.

References

1. Altschul, S. F., Gish, W., Miller, W., Myers, E. W., & Lipman, D. J. (1990). Basic local alignment search tool. *Journal of Molecular Biology, 215*(3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2
2. Brunings, A. M., & Gabriel, D. W. (2003). Xanthomonas citri: Breaking the surface. *Molecular Plant Pathology, 4*(3), 141–157. https://doi.org/10.1046/j.1364-3703.2003.00163.x
3. Cubero, J., & Graham, J. H. (2005). Quantitative real-time polymerase chain reaction for bacterial enumeration and allelic discrimination to differentiate Xanthomonas strains affecting citrus. *Phytopathology, 95*(12), 1333–1340. https://doi.org/10.1094/PHYTO-95-1333
4. Edgar, R. C. (2004). MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *BMC Bioinformatics, 5*, 113. https://doi.org/10.1186/1471-2105-5-113
5. Gottwald, T. R., Graham, J. H., & Schubert, T. S. (2002). Citrus canker: The pathogen and its impact. *Plant Health Progress, 3*(1), 15–22. https://doi.org/10.1094/PHP-2002-0812-01-RV
6. Graham, J. H., Gottwald, T. R., Cubero, J., & Achor, D. S. (2004). Xanthomonas axonopodis pv. citri: Factors affecting successful eradication of citrus canker. *Molecular Plant Pathology, 5*(1), 1–15. https://doi.org/10.1111/j.1364-3703.2004.00197.x
7. Jones, J. B., Stall, R. E., & Bouzar, H. (2000). Diversity among Xanthomonads pathogenic on pepper and tomato. *Annual Review of Phytopathology, 38*, 41–58. https://doi.org/10.1146/annurev.phyto.38.1.41
8. Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution, 35*(6), 1547–1549. https://doi.org/10.1093/molbev/msy096
9. Leduc, A., Traore, Y. N., Boyer, K., & Magne, M. (2015). Validation of a real-time PCR assay for detection of Xanthomonas citri pv. citri in citrus plants. *Plant Disease, 99*(7), 917–924. https://doi.org/10.1094/PDIS-09-14-0939-RE
10. Schaad, N. W., Jones, J. B., & Chun, W. (2001). *Laboratory guide for identification of plant pathogenic bacteria* (3rd ed.). APS Press.
11. Schubert, T. S., Rizvi, S. A., Sun, X., Gottwald, T. R., Graham, J. H., & Dixon, W. N. (2001). Meeting the challenge of eradicating citrus canker in Florida. *Plant Disease, 85*(4), 340–356. https://doi.org/10.1094/PDIS.2001.85.4.340
12. Stall, R. E., & Seymour, C. P. (1983). Canker, a threat to citrus in the Gulf Coast states. *Plant Disease, 67*(5), 581–585. https://doi.org/10.1094/PD-67-581
13. Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution, 38*(7), 3022–3027. https://doi.org/10.1093/molbev/msab120
14. Weisburg, W. G., Barns, S. M., Pelletier, D. A., & Lane, D. J. (1991). 16S ribosomal DNA amplification for phylogenetic study. *Journal of Bacteriology, 173*(2), 697–703. https://doi.org/10.1128/jb.173.2.697-703.1991
15. Young, J. M., Park, D. C., Shearman, H. M., & Fargier, E. (2008). A multilocus sequence analysis of the genus Xanthomonas. *Systematic and Applied Microbiology, 31*(5), 366–377. https://doi.org/10.1016/j.syapm.2008.06.004
16. Federhen, S. (2015). Type material in the NCBI taxonomy database. *Nucleic Acids Research, 43*(D1), D1086–D1098. https://doi.org/10.1093/nar/gku1127
17. Ryan, R. P., Vorhölter, F. J., Potnis, N., Jones, J. B., Van Sluys, M. A., Bogdanove, A. J., & Dow, J. M. (2011). Pathogenomics of Xanthomonas: Understanding bacterium–plant interactions. *Nature Reviews Microbiology, 9*(5), 344–355. https://doi.org/10.1038/nrmicro2558

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