Access this article online
Quick Response Code:

Website: https://eesrd.us

Manuscript ID:
IJRSEAS-2024-010201

DOI: 10.5281/zenodo.14621234

DOI Link:
https://zenodo.org/records/14621234

Volume: 1

Issue: 2

Month: December

Year: 2024

E-ISSN: 3066-0637

Submitted: 16- Oct -2024

Revised: 30- Oct-2024

Accepted: 15-Nov-2024

Published: 31-Dec-2024

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How to cite this article:
Hanmant, K. S., Ahiwale, S. S., & Shinde, M. S. (2024). Sphingomonas jeddahensis: A Newly Identified Causal Organism of Bacterial Blight Disease in Pomegranate from the Solapur District, India. International Journal of Research Studies on Environment, Earth, and Allied Sciences, 1(2), 1–8.
https://doi.org/10.5281/zenodo.14621234

Sphingomonas jeddahensis: A Newly Identified Causal Organism of Bacterial Blight Disease in Pomegranate from the Solapur District, India

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Abstract:

Pomegranate is a medicinally and economically important fruit crop cultivated in India. However, production of pomegranate has been severely reduced due to bacterial blight disease. According to recent studies, bacterial blight disease is recognized as one of the most devasting diseases affecting this crop, particularly in Tamil Nadu, Maharashtra, Haryana, and Punjab. The primary causal organism of blight disease is Xanthomonas axonopodis pv. punicae, which has been reported to reduce crop yields by up to 50% in India. To date, existing disease management strategies have proven ineffective in controlling this disease. In recent years, Pseudomonas spp. and other bacterial strains have also been implicated as causative agents of bacterial blight in pomegranates.

In this study, research focused on the isolation of pathogens responsible for the bacterial blight disease in pomegranates. Samples, especially fruits, and leaves were used for the isolation from various zones of the Solapur district, Maharashtra. The ooze test was performed, and macerated samples of fruits and leaves were streaked onto YDCA medium. Well-isolated, yellow-pigmented bacterial colonies with acid hydrolysis activity were observed within 48 hrs. at 28°C. Further characterization and isolates were performed using cultural, morphological, biochemical, and molecular techniques, including 16S rRNA gene sequencing (up to 1200 bp). The isolates were identified as belonging to Sphingomonas jeddahensis. Pathogenicity was confirmed using an in vitro detached leaf assay, supporting the identification.

According to the outcomes of this study, Sphingomonas jeddahensis is reported as a newly identified strain responsible for the cause of bacterial blight disease in pomegranate in the Solapur district, Maharashtra, in addition to Xanthomonas axonopodis pv. punicae and Pseudomonas spp.

Keywords: Pomegranate, bacterial blight, Xanthomonas axonopodis pv. punicae, Pseudomonas spp., Sphingomonas jeddahensis, 16S rRNA gene sequencing.

Introduction

The pomegranate, family *Punicaceae* is highly important nutritional medicinal fruit (Raghuvanshi et al., 2013) and the crop generally grown in subtropical and tropical regions in India (Doddaraju et al., 2019). Several abiotic factors, in addition to biotic stresses, contribute to the reduction of pomegranate crop yield, including crop variety, climate, soil nutrient availability, and cultivation practices employed by farmers (Kumar, 2018). The production has been significantly impacted by blight disease caused by *Xanthomonas axonopodis* pv. *punicae* (Petersen et al., 2010). The pathogen is responsible for reducing both the nutritional quality and productivity of pomegranate crops by as much as 80–90% in cases of severe infestation (NP et al., 2017). This disease was initially observed in India (Hingorani and Mehta, 1952). Subsequently, Hingorani and Singh (1959) declared the disease from various regions of India and identified *Xanthomonas axonopodis* pv. *punicae* it as a causal organism (K.K. Sharma et al., 2011). Currently, available disease management practices have proven ineffective in controlling bacterial blight disease, which has become one of the most significant challenges for pomegranate farmers, of Maharashtra, and Telangana (Kumar et al., 2010). Additionally, the causal organism of a novel blight disease, *Pseudomonas sp.* has been isolated and reported (Jagdale et al., 2018). In a recent study, 15 infected samples of pomegranate leaves and fruits were collected for the isolation of the causative agent. Isolated bacterial colonies were characterized using morphological, cultural, and biochemical methods.

Molecular characterization was performed using the 16S rRNA sequencing method and the phylogenetic tree developed with the MEGA 11 version (Tamura et al., 2021). Phylogenetic analysis identified the causative agent as *Sphingomonas jeddahensis*, which was further confirmed through Koch's postulates. This represents a new report of blight disease of pomegranate. Another strain characterized during the study was identified as an unusual yellow-pigmented bacterial strain which was observed on pomegranate fruits and leaves. Further strains were confirmed through biochemical and molecular analyses as *Pseudomonas* sp. (Jagdale et al., 2018).

Materials and Methods: -

Preparation of Samples for Isolation

Samples of pomegranate leaves and fruits (infected) were used from various regions of the Solapur district, particularly from Sangola (21 samples), Mangalvedha (20 samples), Mohol (18 samples), Barshi (11 samples), and North Solapur (10 samples).

The infected pomegranate fruits and leaves were sterilized using a sodium hypochlorite (2%), and further washed the residues by sterile distilled water, Disease lesions on infected plant parts were sterilized using a mercuric chloride (HgCl2) (0.1%) for 60–90 seconds and subsequently washed three times using sterile distilled water (Gargade & Kadam, 2015). A few drops of sterile saline solution were applied to the infected lesions' surfaces, where the formation of saline turbidity indicated the presence of etiological agents in the form of bacterial ooze (K.K. Sharma et al., 2011).

Sterilized infected plant tissues (fruits, leaves, and stems) were macerated, and a suspension was prepared in sterile saline. This suspension was serially diluted and streaked onto sterile YDCA (Yeast Dextrose Calcium Carbonate Agar) plates containing yeast extract (10.0 g), dextrose (20.0 g), calcium carbonate (20.0 g), agar (20.0 g), and adjusted to pH 7.0 in 1,000 mL of distilled water (Jagdale et al., 2018a).

Identification of Pathogens

The isolated bacterial colonies were analyzed for morphological and biochemical characteristics (Kumar, 2018). Identification of isolates was conducted concerning Bergey's *Manual of Systematic Bacteriology* (Shali et al., 2018). Molecular characterization was performed using 16S rRNA sequencing (Sambrook J et al., 1989). The phylogenetic analysis was performed by using MEGA software version 11.

Pathogenicity testing

The pathogenic nature of isolates was confirmed using a detached leaf inoculation technique (P. S. Randhawa et al., 1985). For this method, freshly detached middle-aged leaves of pomegranate were collected, washed with tap water, wiped with 70% ethyl alcohol, and air-dried. The prepared leaves were inoculated with bacterial suspensions at 10^8 CFU/mL concentrations by spraying (Breitschwerd et al., 2013). The leaves were kept for incubation in a sterilized Petri dish (containing moist filter paper) at 30°C for 4–5 days. Post-incubation and re-isolation of the pathogen confirmed its identity by comparison with the original culture.

Pathogenicity was further validated through in vivo assays on healthy six-month-old pomegranate plants (Jagdale et al., 2018). The selected plants were disinfected with 2% sodium hypochlorite and further treated with sterile distilled water. Bacterial suspensions (10^8 CFU/mL) were applied to the plants, which were then covered with, transparent polythene cloth to maintain a controlled environment (Figure 7) Test and control plants were observed for 7–8 days under normal light and room temperature. The appearance and progression of disease symptoms on leaves were documented, and the pathogen was reisolated for comparison (Chavan et al., 2016).

Additional studies have refined these techniques, emphasizing the importance of maintaining optimal humidity for pathogen proliferation during detached leaf assays, a factor critical for successful inoculation.

Results and Discussion

Results

Isolation, Morphological, and Biochemical Characterization

After 48–72 hours of incubation on YDCA agar, isolates displayed typical yellow-pigmented, circular, mucoid colonies measuring 1–2 mm in diameter (Figure 1). All isolates were gram-negative and motile, with positive KOH string tests. Morphological and cultural characteristics (Table 1) confirmed consistency among isolates collected from bacterial blight-infected pomegranate fruits and leaf lesions from the Solapur district. A total of four isolates were successfully identified.

Biochemical tests (Table 2) showed that all isolates utilized glucose and lactose as sole carbon sources and were positive for catalase and oxidase production, gelatin and starch hydrolysis, phenylalanine deamination, and hydrogen sulfide production. Negative results were observed for IMViC tests, nitrate reduction, urease production, asparagine medium, and lysine utilization. These biochemical characteristics align with the descriptions of *Sphingomonas spp.* in *Bergey's Manual of Systematic Bacteriology*.

Molecular Characterization

Isolates were characterized by 16S rRNA sequencing identified the isolates as *Sphingomonas jeddahensis*, with a 98.95% similarity to *Sphingomonas jeddahensis* G39(T). The sequences (Figure 2-5) were submitted to GenBank and assigned accession number OP457176.1. Phylogenetic analysis using MEGA 11 software revealed a close evolutionary relationship between *Sphingomonas jeddahensis* and *Xanthomonas axonopodis* pv. *punicae*, as shown in the phylogenetic tree (Figure 6).

Pathogenicity Testing

Pathogenicity assays, including detached leaf inoculation and in vivo testing, confirmed that *Sphingomonas jeddahensis* caused characteristic bacterial blight symptoms, such as water-soaked lesions that gradually turn brown to dark with a diffuse water-soaked margin with a yellow halo on pomegranate leaves within 7–8 days post-inoculation. Control plants remained asymptomatic. Reisolated pathogens matched the original isolates, fulfilling Koch's postulates and confirming their role as causal agents. In vivo, and In vitro pathogenicity testing results are shown here (Figure 8, 9,10)

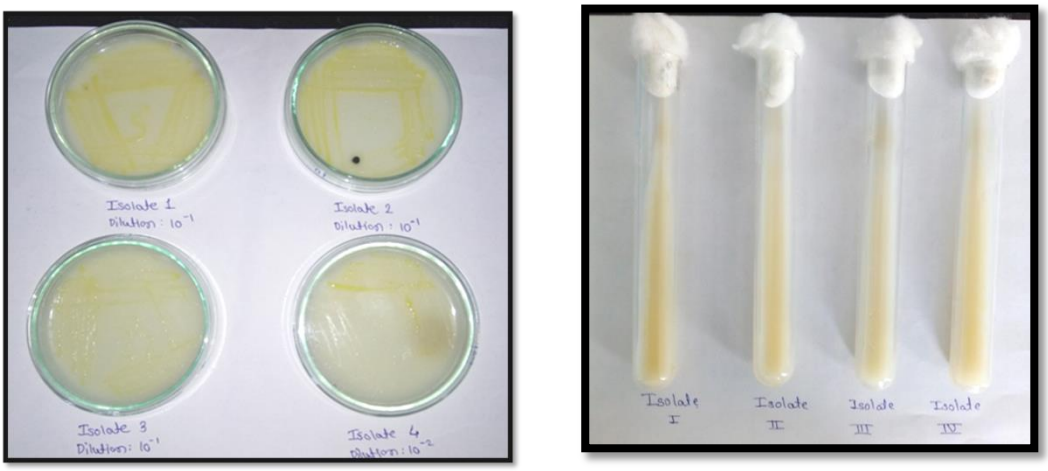
Table 1: Morphological characters

Name of isolate	Size	Shape	Color	Margin	Elevation	Opacity	Consistency	Gram nature	Motility
SHK 1	**1 mm**	**Circular**	**Yellow**	**Entire**	**Raised**	**Opaque**	**Mucoid**	**Gram -ve**	**Motile**
SHK 2	**2 mm**	**Circular**	**Yellow**	**Entire**	**Raised**	**Opaque**	**Mucoid**	**Gram -ve**	**Motile**
SHK 3	**1mm**	**Circular**	**Yellow**	**Entire**	**Raised**	**Opaque**	**Mucoid**	**Gram -ve**	**Motile**
SHK 4	**1 mm**	**Circular**	**Yellow**	**Entire**	**Raised**	**Opaque**	**Mucoid**	**Gram -ve**	**Motile**

Table 2: Biochemical characterization of the isolates

Biochemical Test	Results
Indole	-
Methyl Red	-
Voges Proskauer	-
Citrate	-
Glucose utilization	+
Lactose Utilization	+
Asparagine Medium	-
Lysine utilization test	-
H2S Production	+
Nitrate reduction test	-
Urease Production	-
Gelatin Hydrolysis	+
Oxidase	+
Catalase	+
Starch Hydrolysis	+
Phenylalanine deamination	+

'+' Positive Test , '-' : Negative Test



a) b)

Fig. 1 a) Bacterial isolates on YDCA agar plates showing yellow colored, mucoid colonies, b) Isolates on YDCA slant.

- >B_MAY_22_128
  GATCCTGGCTCAGAACGAACGCTGGCGGCATGCCTAACACATGCAAGTCGAACGAAGGCTTCGGCC
  TTAGTGGCGCACGGGTGCGTAACGCGTGGGAATCTGCCCCTTGGTTCGGAATAACAGTTAGAAATG
  ACTGCTAATACCGGATGACGACGTTAAGTCCAAAGATTTATCGCCGAGGGATGAGCCCGCGTAGGA
  TTAGCTAGTTGGTGTGGTAAAGGCGCACCAAGGCGACGATCCTTAGCTGGTCTGAGAGGATGATCA
  GCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAA
  TGGGCGAAAGCCTGATCCAGCAATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTTT
  ACCCGGGATGATAATGACAGTACCGGGAGAATAAGCTCCGGCTAACTCCGTGCCAGCAGCCGCGGT
  AATACGGAGGGAGCTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGCTTTGTAAG
  TTAGAGGTGAAAGCCTGGAGCTCAACTCCAGAATTGCCTTTAAGACTGCATCGCTCGAATCCAGGA
  GAGGTCAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAA
  GGCGGCTGACTGGACTGGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATA
  CCCTGGTAGTCCACGCCGTAAACGATGATAACTAGCTGTCCGGGGACTTGGTCCTTGGGTGGCGCA
  GCTAACGCATTAAGTTATCCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGG
  GGGCCTGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCGTTT
  GACATGGTAGGACGGCTCCGAGAGATCGGTTCCTTCCCTTCGGGGACCTACACACAGGTGCTGCAT
  GGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCTTTA
  GTTACCATCATTTAGTTGGGTACTCTAAAGGAACCGCCGGTGATAAGCCGGAGGAAGGTGGGGATG
  ACGTCAAGTCCTCATGGCCCTTACGCGCTGGGCTACACACGTGCTACAATGGCAACTACAGTGGGC
  AGCAATCCCGCGAGGGTGAGCTAATCTCCAAAAGTTGTCTCAGTTCGGATTGTTCTCTGCAACTCGA
  GAGCATGAAGGCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCAGGCCT
  TGCACACACCGCCC

Fig.2 Isolate-SHK-I

- >B_MAY_22_129
  GTTTGATCCTGGCTCAGAACGAACGCTGGCGGCATGCCTAACACATGCAAGTCGAACGAAGGCTTC
  GGCCTTAGTGGCGCACGGGTGCGTAACGCGTGGGAATCTGCCCCTTGGTTCGGAATAACAGTTAGA
  AATGACTGCTAATACCGGATGACGACGTTAAGTCCAAAGATTTATCGCCGAGGGATGAGCCCGCGT
  AGGATTAGCTAGTTGGTGTGGTAAAGGCGCACCAAGGCGACGATCCTTAGCTGGTCTGAGAGGATG
  ATCAGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGG
  ACAATGGGCGAAAGCCTGATCCAGCAATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTC
  TTTTACCCGGGATGATAATGACAGTACCGGGAGAATAAGCTCCGGCTAACTCCGTGCCAGCAGCCG
  CGGTAATACGGAGGGAGCTAGCGTTGTTTGGAATTACTGGGCGTAAAGCGCACGTAGGCGGCTTTG
  TAAGTTAGAGGTGAAAGCCTGGAGCTCAACTCCAGAATTGCCTTTAAGACTGCATCGCTCGAATCC
  AGGAGAGGTCAGTGGAATTCCGAGTGTAGAGGTGAAATTTGTAGATATTTGGAAGAACACCAGTGG
  CGAAGGCGGCTGACTGGACTGGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTA
  GATACCCTGGTAGTCCACGCCGTAAACGATGATAACTAGCTGTCCGGGGACTTGGTCCTTGGGTGG
  CGCAGCTAACGCATTAAGTTATCCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTG
  ACGGGGGCCTGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGC
  GTTTGACATGGTAGGACGGCTCCGAGAGATCGGTTCCTTCCCTTCGGGGACCTACACACAGGTGCT
  GCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGC
  CTTTAGTTACCATCATTTAGTTGGGTACTCTAAAGGAACCGCCGGTGATAAGCCGGAGGAAGGTGG
  GGATGACGTCAAGTCCTCATGGCCCTTACGCGCTGGGCTACACACGTGCTACAATGGCAACTACAG
  TGGGCAGCAATCCCGCGAGGGTGAGCTAATCTCCAAAAGTTGTCTCAGTTCGGATTGTTCTCTGCAA
  CTCGAGAGCATGAAGGCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCA
  GGCCTTGCACACACCGCCCGTCA

Fig. 3 Isolate SHK -II

- >B_MAY_22_130
  ATCCTGGCTCAGAACGAACGCTGGCGGCATGCCTAACACATGCAAGTCGAACGAAGGCTTCGGCCT
  TAGTGGCGCACGGGTGCGTAACGCGTGGGAATCTGCCCCTTGGTTCGGAATAACAGTTAGAAATGA
  CTGCTAATACCGGATGACGACGTTAAGTCCAAAGATTTATCGCCGAGGGATGAGCCCGCGTAGGAT
  TAGCTAGTTGGTGTGGTAAAGGCGCACCAAGGCGACGATCCTTAGCTGGTCTGAGAGGATGATCAG
  CCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAAT
  GGGCGAAAGCCTGATCCAGCAATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTTTA
  CCCGGGATGATAATGACAGTACCGGGAGAATAAGCTCCGGCTAACTCCGTGCCAGCAGCCGCGGTA
  ATACGGAGGGAGCTAGCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGCTTTGTAAGT
  TAGAGGTGAAAGCCTGGAGCTCAACTCCAGAATTGCCTTTAAGACTGCATCGCTCGAATCCAGGAG
  AGGTCAGTGGAATTCCGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAAG
  GCGGCTGACTGGACTGGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATAC
  CCTGGTAGTCCACGCCGTAAACGATGATAACTAGCTGTCCGGGGACTTGGTCCTTGGGTGGCGCAG
  CTAACGCATTAAGTTATCCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGG
  GGCCTGCACAAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCGTTTG

ACATGGTAGGACGGCTCCGAGAGATCGGTTCCTTCCCTTCGGGGACCTACACACAGGTGCTGCATG
GCTGTCGTCAGCTCGTGTCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCTTTAG
TTACCATCATTTAGTTGGGTACTCTAAAGGAACCGCCGGTGATAAGCCGGAGGAAGGTGGGGATGA
CGTCAAGTCCTCATGGCCCTTACGCGCTGGGCTACACACGTGCTACAATGGCAACTACAGTGGGCA
GCAATCCCGCGAGGGTGAGCTAATCTCCAAAAGTTGTCTCAGTTCGGATTGTTCTCTGCAACTCGAG
AGCATGAAGGCGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCAGGCCTT
GCACACACCGCCCGT

Fig. 4 Isolate SHK -III

- >B_MAY_22_131
AACGCTGGCGGCATGCCTAACACATGCAAGTCGAACGAAGGCTTCGGCCTTAGTGGCGCACGGGTG
CGTAACGCGTGGGAATCTGCCCCTTGGTTCGGAATAACAGTTAGAAATGACTGCTAATACCGGATG
ACGACGTTAAGTCCAAAGATTTATCGCCGAGGGATGAGCCCGCGTAGGATTAGCTAGTTGGTGTGG
TAAAGGCGCACCAAGGCGACGATCCTTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAG
ACACGGCCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGGACAATGGGCGAAAGCCTGATC
CAGCAATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTCTTTTACCCGGGATGATAATGA
CAGTACCGGGAGAATAAGCTCCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGAGCTA
GCGTTGTTCGGAATTACTGGGCGTAAAGCGCACGTAGGCGGCTTTGTAAGTTAGAGGTGAAAGCCT
GGAGCTCAACTCCAGAATTGCCTTTAAGACTGCATCGCTCGAATCCAGGAGAGGTCAGTGGAATTC
CGAGTGTAGAGGTGAAATTCGTAGATATTCGGAAGAACACCAGTGGCGAAGGCGGCTGACTGGACT
GGTATTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCC
GTAAACGATGATAACTAGCTGTCCGGGGACTTGGTCCTTGGGTGGCGCAGCTAACGCATTAAGTTA
TCCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGGGCCTGCACAAGCGGT
GGAGCATGTGGTTTAATTCGAAGCAACGCGCAGAACCTTACCAGCGTTTGACATGGTAGGACGGCT
CCGAGAGATCGGTTCCTTCCCTTCGGGGACCTACACACAGGTGCTGCATGGCTGTCGTCAGCTCGTG
TCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGCCTTTAGTTACCATCATTTAGTTG
GGTACTCTAAAGGAACCGCCGGTGATAAGCCGGAGGAAGGTGGGGATGACGTCAAGTCCTCATGG
CCCTTACGCGCTGGGCTACACACGTGCTACAATGGCAACTACAGTGGGCAGCAATCCCGCGAGGGT
GAGCTAATCTCCAAAAGTTGTCTCAGTTCGGATTGTTCTCTGCAACTCGAGAGCATGAAGGCGGAAT
CGCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCAGGCCTTGCACACACCGCCCGTC
A

Fig. 5 Isolate SHK -IV

Table 3: Molecular Characterization of Isolates

Isolates	Sampling part	NCMR Accession Number	GenBank accession Number	Taxonomic designation
SHK -1	**Pomegranate fruit**	**B_MAY_22_128**	**OP457176**	***Sphingomonas jeddahensis G39(T)***
SHK-2	**Pomegranate fruit**	**B_MAY_22_129**	**OP457176**	***Sphingomonas jeddahensis G39(T)***
SHK-3	**Pomegranate fruit**	**B_MAY_22_130**	**OP457176**	***Sphingomonas jeddahensis G39(T)***
SHK-4	**Pomegranate fruit**	**B_ MAY_22 131**	**OP457176**	***Sphingomonas jeddahensis G39(T)***

Molecular characterization using 16S rRNA sequencing confirmed that all isolates belong to *Sphingomonas jeddahensis*. To further explore the evolutionary relationship among potential bacterial blight causative agents, including *Xanthomonas axonopodis* pv. *punicae*, phylogenetic analysis was conducted. The 16S rRNA sequencing of the isolates was performed at the National Center for Microbial Resources (NCMR), Pune, India. BLAST analysis of the nucleotide sequences against the NCBI rRNA database revealed 98.95% similarity with *Sphingomonas jeddahensis* strain G39. No other strain demonstrated 100% sequence identity, indicating the novelty of the strain, which was designated as *Sphingomonas sp. strain SHK*.

Upon sequence release, alignment was performed using the NCBI non-redundant database and the BLASTn program. Homologous sequences were selected for phylogenetic analysis, which included *Sphingomonas jeddahensis* strain G39, *Xanthomonas axonopodis* strain IDSG-BT-06-2022, and *Pseudomonas aeruginosa* strain 57.

Studied the evolutionary relationship by phylogenetic analysis and developed a tree by using MEGA (Version 11, Arizona, USA) with the Clustal W alignment method and the Neighbor-Joining algorithm (Tamura et al., 2021). Phylogenetic analyses revealed a significant evolutionary relationship between *Sphingomonas sp. strain SHK* and *Xanthomonas axonopodis* strain IDSG-BT-06-2022, supported by high sequence similarity in the 16S rRNA region.) The analysis indicates that the novel *Sphingomonas sp. strain SHK* shares a significant evolutionary relationship with *Xanthomonas axonopodis* and has the potential to cause bacterial blight in pomegranate. This type of

phylogenic analysis was earlier reported by Surwase and Jadhav (2011), underscoring the pathogenic role of *Sphingomonas sp. strain SHK*.

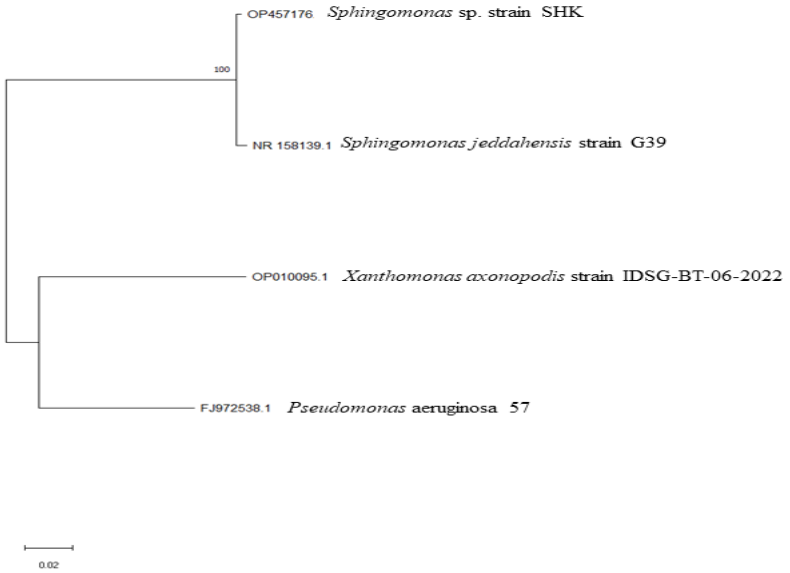


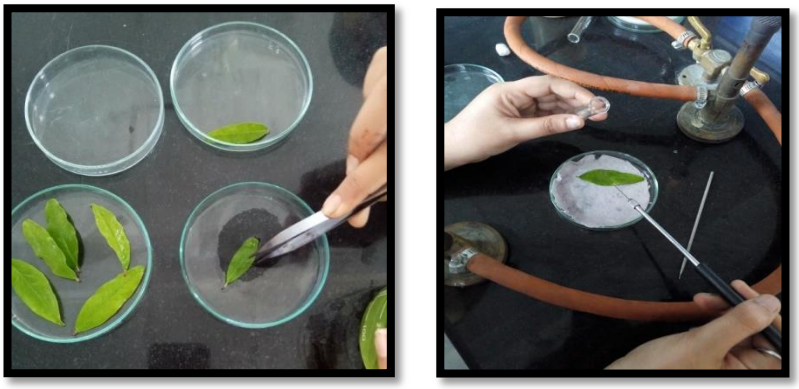
Fig 6. Phylogenetic Analysis

Fig. 7 In-vivo Pathogenicity Testing (Plants were covered with, transparent polythene cloth to maintain a controlled environment)

a) b)

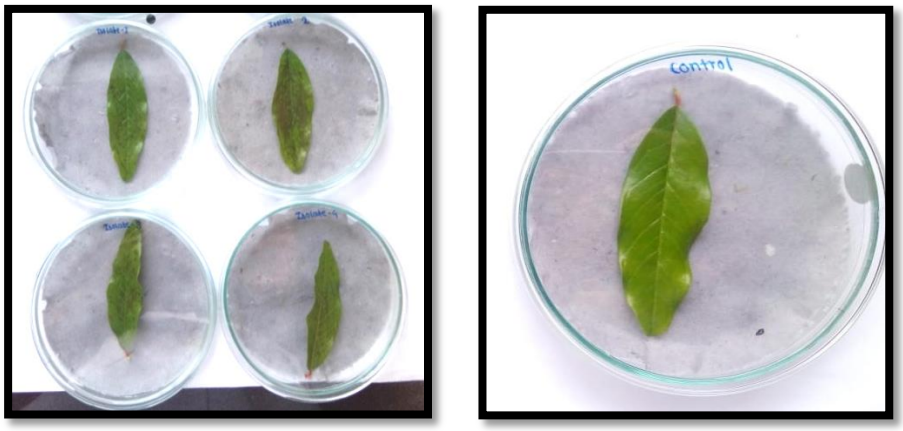
c)

Fig. 8. Pathogenicity Testing: -
a) Control plant b) Test, c) Test (b and c plants showing water-soaked margin with a yellow halo on pomegranate leaves)



a) b)

Fig 9. a) Treating the pomegranate leaves in 2% Sodium Hypochlorite b) Spreading the bacterial suspension by using the wire loop



a) b)

Fig 10 a) Inoculated pomegranate leaf showed the symptoms b) Control leaf

Discussion: -

In the present study, *Sphingomonas strains* are actual causal organism of bacterial blight disease in pomegranate which was confirmed by the molecular characterization, and phylogenetic analysis. According to the phylogenetic analysis, *Sphingomonas* Strains are closely related to the *Xanthomonas sp*. The bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae* (Hingorani and Mehta 1952) and *Pseudomonas sp.* (Jagdale et. Al. 2018) are the reported strains. The symptoms showed on leaves that were studied by detached leaf assay as well as In vivo assay and molecular characterization with phylogenetic analysis confirmed that the isolated strains can cause the

bacterial blight of pomegranate in addition to the *Xanthomonas axonopodis* pv. punicae (Hingorani and Mehta 1952) and *Pseudomonas sp.* (Jagdale et. Al. 2018)

Acknowledgments
I am Mr. Kamble Sainath Hanmant, and I am thankful to Principal Prof. Dr. B. H. Damji Sir, D.B.F. Dayanand College of Arts and Science, Solapur, for granting permission to carry out the work.

Financial Support and Sponsorship
Nil.

Conflicts of Interest
There are no conflicts of interest.

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