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ISOLATION AND BIOCHEMICAL CHARACTERIZATION OF *PSEUDOMONAS VIRIDIFLAVA* ASSOCIATED WITH TOMATO PLANTS

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is a globally important vegetable crop, highly valued for its nutritional and economic significance. However, its productivity is often constrained by bacterial diseases, among which *Pseudomonas viridiflava* is an emerging phytopathogen responsible for leaf spots, blights, and fruit lesions. The present study aimed to isolate and characterize *Pseudomonas viridiflava* from diseased tomato plants grown in soil samples collected from Behror region of Rajasthan (India). Symptomatic plant parts, including leaves and fruits, were collected and subjected to bacterial isolation using Nutrient Agar (NA) medium. The isolated bacterial strain was purified and characterized based on morphological and biochemical tests, including Gram staining, catalase, oxidase, KOH, indole production, citrate utilization, cetrimide test, gelatin hydrolysis, arginine dihydrolase activity, and carbohydrate fermentation. The isolate was found to be Gram-negative, rod-shaped, catalase-positive, oxidase-negative, citrate-positive, indole-negative, and showed negative results for cetrimide, gelatin hydrolysis, arginine dihydrolase, and carbohydrate utilization tests. Based on these characteristics and comparison with standard taxonomic keys, the isolate was identified as *Pseudomonas viridiflava*. The study highlights the significance of biochemical characterization in the identification of plant pathogenic bacteria and provides baseline information for disease management strategies in tomato cultivation.

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INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated and economically important vegetable crops in the world. It is grown across diverse agro-climatic regions and plays a crucial role in human nutrition due to its richness in vitamins (A, C, and E), minerals, and bioactive compounds such as lycopene, which possess antioxidant properties. In India, tomato cultivation contributes significantly to the agricultural economy and supports the livelihood of millions of farmers. However, despite its high production potential, tomato yield and quality are severely affected by a range of biotic stresses, particularly plant diseases caused by fungal, bacterial, viral, and nematode pathogens. Among these, bacterial diseases represent a major constraint in tomato cultivation due to their rapid spread, difficulty in management, and ability to survive under diverse environmental conditions. Bacterial pathogens can infect various parts of the plant, including leaves, stems, flowers, and fruits, leading to symptoms such as leaf spots, blights, wilting, and fruit rot. These infections not only reduce yield but also deteriorate the market quality of produce, resulting in significant economic losses. The genus *Pseudomonas* comprises a diverse group of Gram-negative, rod-shaped, aerobic bacteria that are widely distributed in soil, water, and plant environments. Many species within this genus are known for their metabolic versatility and ecological adaptability. While some *Pseudomonas* species are beneficial and promote plant growth, others are pathogenic and cause diseases in a wide range of crops. Among the phytopathogenic species, *Pseudomonas viridiflava* has gained increasing attention due to its association with several plant diseases, including bacterial leaf spot, stem necrosis, and fruit lesions in tomato and other horticultural crops.

Pseudomonas viridiflava is considered an opportunistic plant pathogen that can survive epiphytically on plant surfaces as

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well as persist in soil and water. Its pathogenicity is influenced by environmental conditions such as temperature, humidity, and host susceptibility. The bacterium can enter plant tissues through natural openings or wounds and proliferate rapidly, leading to the development of disease symptoms. Its ability to adapt to different environmental niches makes it a challenging pathogen to manage in agricultural systems. Accurate identification of plant pathogenic bacteria is essential for understanding disease etiology, epidemiology, and for developing effective control strategies. Although molecular techniques such as PCR and sequencing are increasingly used for bacterial identification, conventional methods based on morphological and biochemical characterization remain widely used due to their simplicity, cost-effectiveness, and reliability, particularly in laboratories with limited resources. Biochemical tests such as Gram staining, catalase, oxidase, citrate utilization, and carbohydrate fermentation provide valuable information about the physiological and metabolic properties of bacterial isolates, aiding in their identification.

In this context, the present study was undertaken to isolate and characterize *Pseudomonas viridiflava* from diseased tomato plants grown in soil samples from the Behror region. The study focuses on the use of standard microbiological techniques to identify the pathogen based on its morphological and biochemical characteristics. Understanding the occurrence and characteristics of this pathogen will contribute to better disease diagnosis and can support the development of appropriate management strategies for sustainable tomato production.

MATERIALS AND METHODS

Study Area and Sample Collection

The present investigation was carried out using tomato plants grown in agricultural fields of the Behror region (Rajasthan, India). The area represents semi-arid agro-climatic conditions suitable for tomato cultivation. Plants exhibiting disease symptoms such as leaf spots, necrotic lesions, and fruit blemishes were selected for sampling. Symptomatic plant parts, including infected leaves and fruits, were collected randomly from different plants to ensure representative sampling. The samples were placed in sterile polythene bags, properly labeled, and transported to the laboratory under aseptic conditions. All samples were processed within 24 hours of collection to minimize contamination and deterioration.

Preparation and Sterilization of Culture Media

Nutrient Agar (NA) medium was prepared by dissolving peptone (5 g/L), beef extract (3 g/L), sodium chloride (5 g/L), and agar (15 g/L) in distilled water. The pH of the medium was adjusted to 6.9–7.2. The prepared medium was sterilized by autoclaving at 121°C under 15 lbs pressure for 15–20 minutes. Petri plates, glassware, and inoculation tools were sterilized simultaneously to maintain aseptic conditions.

Isolation of Bacterial Pathogen

Isolation of bacterial pathogens was carried out using the direct plating method. Small sections (2–3 mm) of infected plant tissues were excised using a sterile scalpel. The tissue pieces were surface sterilized with 0.1% sodium hypochlorite solution for 30–60 seconds and rinsed thoroughly with sterile distilled water. The sterilized tissue pieces were aseptically placed on solidified NA plates in triplicate and incubated at 37°C for 24 hours. After incubation, bacterial colonies appearing around the tissue segments were observed. Distinct colonies were selected based on their morphological characteristics and sub-cultured onto fresh NA plates to obtain pure cultures. The purified isolates were maintained on NA slants and stored at 4°C for further analysis.

Morphological Characterization

The purified bacterial isolate was examined for colony morphology, including shape, margin, elevation, texture, and pigmentation.

Gram Staining

Gram staining was performed to determine the Gram reaction and cellular morphology. A thin smear of bacterial culture was prepared on a clean glass slide, air-dried, and heat-fixed. The smear was stained with crystal violet for 30 seconds, followed by washing with distilled water. Gram's iodine was applied for 60 seconds, and the slide was rinsed again. Decolorization was carried out using 95% ethanol, followed by immediate washing. The smear was then counterstained with safranin for 30 seconds, washed, air-dried, and observed under a microscope using oil immersion.

Biochemical Characterization

The bacterial isolate was subjected to a series of biochemical tests to determine its physiological and metabolic characteristics.

Catalase Test

A small amount of bacterial culture was placed on a clean glass slide, and a few drops of 3% hydrogen peroxide were added. The reaction was observed immediately for the release of oxygen bubbles.

KOH Test

A loopful of bacterial culture was mixed with a drop of 3% potassium hydroxide solution on a clean glass slide and stirred for approximately 30–60 seconds. The mixture was observed for any change in consistency.

Oxidase Test

The oxidase test was performed using Kovács oxidase reagent. A portion of the bacterial culture was rubbed onto reagent-moistened filter paper and observed within 15–30 seconds for any color development.

Indole Production Test

Tryptone broth was prepared, sterilized, and inoculated with the bacterial culture. The inoculated tubes were incubated at 37°C for 48 hours. After incubation, Kovács reagent was added, and the tubes were observed for color development.

Citrate Utilization Test

Simmons' citrate agar was prepared, sterilized, and poured into tubes to form slants. The slants were inoculated with bacterial culture and incubated at 37°C for 48 hours. The tubes were examined for changes in color and growth.

Cetrimide Test

Cetrimide agar medium was prepared and sterilized. The medium was inoculated with the bacterial isolate and incubated

at 37°C for 48 hours. The presence or absence of growth and pigmentation was recorded.

Gelatin Hydrolysis Test

Gelatin medium was prepared, sterilized, and inoculated using stab culture. The tubes were incubated at 37°C for 4–7 days and subsequently refrigerated at 4°C for 30 minutes before observation.

Arginine Dihydrolase Test

Arginine dihydrolase broth was prepared, sterilized, and inoculated with the bacterial isolate. The inoculated tubes were incubated at 37°C for 48 hours and then observed for any color changes.

Carbohydrate Fermentation Test

Glucose fermentation broth containing bromocresol purple indicator was prepared, sterilized, and inoculated with bacterial culture. The tubes were incubated at 37°C for 24–48 hours and observed for any change in color.

Identification of Bacterial Isolate

The identification of the bacterial isolate was carried out based on its morphological and biochemical characteristics. The results obtained from various tests were compared with standard descriptions provided in *Bergey's Manual of Systematic Bacteriology* (Krieg and Holt, 1984) to confirm the identity of the isolate as *Pseudomonas viridiflava*.

RESULTS AND DISCUSSION

Disease Symptoms in Tomato Plants

Tomato plants collected from the Behror region exhibited characteristic symptoms of bacterial infection, primarily on leaves and fruits. The infected leaves showed small, water-soaked lesions that later turned necrotic, while fruits displayed superficial spots and blemishes. Such symptoms are commonly associated with bacterial leaf spot and blight diseases caused by species of the genus *Pseudomonas* (Hirano & Upper, 2000). The occurrence of these symptoms under field conditions indicates favorable environmental conditions for the survival and proliferation of bacterial pathogens. The manifestation of these symptoms suggests active colonization of plant tissues by bacterial pathogens capable of entering through natural openings or wounds. Similar symptomatology has been reported in tomato plants infected with *Pseudomonas viridiflava*, supporting its role as a potential causal organism in the present study (Young, 2010).

Isolation and Colony Characteristics

Bacterial colonies were successfully isolated from infected plant tissues using Nutrient Agar medium. The colonies appeared within 24 hours of incubation at 37°C. The isolate showed smooth, circular, and slightly raised colonies with a creamy to off-white appearance.

The uniformity in colony morphology after sub-culturing indicated the successful isolation of a pure bacterial culture. The absence of mixed colony types further confirmed that a dominant bacterial species was associated with the infected plant tissues. The colony characteristics observed in this study are consistent with those reported for *Pseudomonas viridiflava*, which typically forms smooth, non-pigmented colonies on general-purpose media (Schaad et al., 2001).

Morphological Characteristics

Microscopic examination following Gram staining revealed that the bacterial isolate was Gram-negative and rod-shaped (bacilli), appearing pink under the microscope. This observation is a defining characteristic of members of the genus *Pseudomonas* (Krieg & Holt, 1984).

The Gram-negative nature of the isolate indicates the presence of a thin peptidoglycan layer and an outer membrane rich in lipopolysaccharides. This structural feature contributes to the bacterium's adaptability and resistance to environmental stresses (Madigan et al., 2018). The rod-shaped morphology further supports its classification within *Pseudomonas*, as most species of this genus exhibit bacillary forms.

Biochemical Characterization

A series of biochemical tests were conducted to determine the physiological and metabolic properties of the bacterial isolate. The catalase test showed immediate formation of oxygen bubbles upon the addition of hydrogen peroxide, indicating the presence of catalase enzyme. This enzyme plays a crucial role in protecting bacterial cells from oxidative damage by breaking down hydrogen peroxide into water and oxygen (Facklam & Elliott, 1995). Catalase positivity is a common feature among aerobic bacteria, including *Pseudomonas* species.

The KOH test resulted in the formation of a viscous, stringy consistency, confirming the Gram-negative nature of the isolate. This result corroborates the findings of Gram staining and strengthens the reliability of the identification (Powers, 1995).

In the oxidase test, no development of purple coloration was observed, indicating a negative reaction. This result is particularly significant, as it helps differentiate *Pseudomonas viridiflava* from other oxidase-positive *Pseudomonas* species (Schaad et al., 2001). The absence of cytochrome c oxidase suggests variation in the electron transport system of the isolate.

The indole production test did not show the formation of a cherry-red ring after the addition of Kovács reagent, indicating that the isolate lacks the ability to degrade tryptophan into indole (MacFaddin, 2000). This characteristic further aligns with the known biochemical profile of *Pseudomonas viridiflava*.

The citrate utilization test showed a distinct color change of the medium from green to blue, indicating the ability of the isolate to utilize citrate as a sole carbon source. This reflects the metabolic versatility of the organism and its ability to survive in nutrient-limited environments such as soil (Madigan et al., 2018).

The cetrimide test showed no bacterial growth, indicating a negative response. This result is important in distinguishing the isolate from *Pseudomonas aeruginosa*, which typically grows on cetrimide agar (Brown & Lowbury, 1965).

Further confirmatory biochemical tests revealed that the isolate did not hydrolyze gelatin, as the medium remained solid after incubation and refrigeration. This indicates the absence of gelatinase enzyme and differentiates the isolate from gelatinase-positive bacteria (Lelliott & Stead, 1987).

Similarly, no color change was observed in the arginine dihydrolase test, indicating the inability of the isolate to hydrolyze arginine. This characteristic is consistent with the biochemical

profile of *Pseudomonas viridiflava* (Schaad et al., 2001).

In the carbohydrate fermentation test, no change in the color of the glucose broth was observed, indicating that the isolate does not ferment glucose. This non-fermentative nature is a typical feature of many *Pseudomonas* species, which rely on oxidative metabolism rather than fermentation (Hemraj et al., 2013).

lesions observed in the field (Young, 2010).

The ability of the bacterium to survive in soil and plant environments, combined with its metabolic adaptability, makes it a persistent and challenging pathogen. The findings of this study emphasize the importance of early detection and accurate identification of bacterial pathogens for effective disease

Table 1. Morphological and Biochemical Characteristics of *Pseudomonas viridiflava* Isolated from Tomato

Test Category	Test Parameter	Observation	Result	Scientific Interpretation
Morphological	Gram staining	Pink-colored rod-shaped cells	Negative	Gram-negative bacilli; typical of *Pseudomonas* spp.
	Cell morphology	Rod-shaped (bacilli)	—	Characteristic cellular structure
	Colony morphology	Smooth, circular, creamy-white colonies	—	Non-pigmented colonies on Nutrient Agar
Enzymatic	Catalase test	Immediate effervescence observed	Positive	Presence of catalase enzyme (aerobic metabolism)
	Oxidase test	No color change	Negative	Absence of cytochrome c oxidase (key differentiating trait)
	Gelatin hydrolysis	Medium remained solid	Negative	Lack of gelatinase activity
	Arginine dihydrolase	No color change	Negative	Inability to hydrolyze arginine
Cell Wall Test	KOH test	Viscous/stringy consistency observed	Positive	Confirms Gram-negative nature
Metabolic	Citrate utilization	Medium changed from green to blue	Positive	Ability to utilize citrate as sole carbon source
	Indole production	No red ring formation	Negative	Tryptophan not converted to indole
	Carbohydrate utilization	No color change (remains purple)	Negative	Non-fermentative metabolism (oxidative pathway)
Selective Media	Cetrimide test	No growth observed	Negative	Differentiates from *Pseudomonas aeruginosa*
Overall Diagnosis	—	—	—	Identified as *Pseudomonas viridiflava* based on biochemical profile

Identification of the Bacterial Isolate

The collective results of morphological and biochemical tests strongly indicate that the bacterial isolate belongs to the genus *Pseudomonas*. The Gram-negative rod shape, catalase positivity, citrate utilization, and non-fermentative metabolism are consistent with this classification (Krieg & Holt, 1984).

More importantly, the oxidase-negative reaction, absence of growth on cetrimide agar, and negative results for gelatin hydrolysis, arginine dihydrolase, and carbohydrate fermentation tests are characteristic features of *Pseudomonas viridiflava*. These distinguishing traits allow differentiation from closely related species such as *Pseudomonas aeruginosa* and *Pseudomonas syringae* (Schaad et al., 2001).

The identification of the isolate as *Pseudomonas viridiflava* was further confirmed by comparing the obtained results with standard descriptions provided in Bergey's Manual of Systematic Bacteriology (Krieg & Holt, 1984).

Significance of Findings

The isolation of *Pseudomonas viridiflava* from diseased tomato plants highlights its role as a potential pathogen in the studied region. Its presence in plant tissues suggests that it may contribute to the development of bacterial leaf spot and fruit management (Hirano & Upper, 2000).

Moreover, the study demonstrates the reliability of conventional biochemical methods in identifying plant pathogenic bacteria. Despite advancements in molecular diagnostics, these techniques remain valuable, particularly in resource-limited laboratories (Schaad et al., 2001).

CONCLUSION

The present study successfully achieved the isolation and biochemical characterization of *Pseudomonas viridiflava* from diseased tomato (*Solanum lycopersicum* L.) plants collected from the Behror region. The pathogen was consistently associated with characteristic disease symptoms such as leaf spots and fruit lesions, indicating its potential role in the development of bacterial disease in tomato under field conditions. Morphological observations revealed that the isolate was Gram-negative and rod-shaped, while biochemical characterization demonstrated a distinct profile, including catalase positivity, oxidase negativity, citrate utilization, and non-fermentative metabolism. The absence of growth on cetrimide agar, along with negative results for gelatin hydrolysis, arginine dihydrolase, and carbohydrate fermentation, further confirmed the identity of the isolate as *Pseudomonas viridiflava*. The results

were in close agreement with standard descriptions provided in Bergey's Manual of Systematic Bacteriology, validating the reliability of conventional identification methods. The study highlights the effectiveness of classical microbiological techniques in identifying plant pathogenic bacteria, particularly in laboratories where advanced molecular tools are not readily available. The detection of *Pseudomonas viridiflava* emphasizes its significance as an emerging phytopathogen in tomato cultivation and underscores the need for regular monitoring of bacterial diseases in agricultural systems. Furthermore, the findings provide a scientific basis for the development of targeted disease management strategies, including the use of resistant cultivars, improved cultural practices, and integrated disease management approaches. Future studies should focus on molecular confirmation, pathogenicity testing, and the evaluation of eco-friendly control measures to better understand the epidemiology and management of this pathogen.

In conclusion, the study contributes valuable insights into the occurrence and identification of *Pseudomonas viridiflava* in tomato, which can aid in improving disease diagnosis and ensuring sustainable crop production.

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