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Isolation and biochemical characterization of Xanthomonas campestris PV. Ricini from castor bean plant

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Abstract

Xanthomonas campestris pv. ricini is a significant bacterial pathogen affecting castor bean (*Ricinus communis*), leading to considerable yield losses. Accurate identification and characterization of the pathogen are essential for effective disease management. This study aimed to isolate and biochemically characterize *X. campestris pv. ricini* from infected castor bean plants. The bacterium was isolated from symptomatic leaf samples and subjected to a series of biochemical tests, including Gram staining, catalase test, potassium hydroxide (KOH) test, and methyl red test. Gram staining confirmed the bacterium as Gram-negative and rod-shaped. The KOH solubility test further supported its Gram-negative nature through the formation of a viscous thread. The catalase test showed a positive reaction, indicating the presence of the catalase enzyme, while the methyl red test confirmed its ability to produce stable acid end-products. These biochemical characterizations provide fundamental insights into the identity of *X. campestris pv. ricini*, laying the groundwork for future studies on its pathogenicity and control strategies.

Keywords: *Xanthomonas campestris pv. ricini*; Castor bean; Gram-negative bacteria; Leaf spot; Biochemical characterization

1. Introduction

Xanthomonas campestris pv. ricini is a significant plant pathogen that poses a serious threat to agricultural productivity, particularly affecting castor bean (*Ricinus communis*), an economically vital crop known for its diverse applications in biodiesel production, pharmaceuticals, and industrial uses. Castor bean is a hardy, drought-resistant perennial shrub that thrives in tropical and subtropical regions, making it an essential crop for marginal lands with low fertility. It yields castor oil, which is increasingly in demand due to its biodegradable nature and utility in manufacturing soaps, lubricants, biopolymers, cosmetics, and even pharmaceuticals, with a global market growth of 3-5% annually (Kaur et al., 2020). Additionally, castor oil is rich in ricinoleic acid, a unique fatty acid that imparts exceptional chemical properties, further increasing its industrial value.

However, the cultivation of castor bean is severely impacted by bacterial leaf spot disease caused by *X. campestris*, which is characterized by dark, water-soaked lesions on leaves that later turn necrotic, leading to defoliation and significant yield losses (Sood & Chauhan, 2018). The pathogen spreads rapidly through contaminated seeds, water, and agricultural tools, exacerbating the challenges faced by farmers, particularly in regions with high humidity and frequent rainfall, which favour bacterial proliferation (Gahukar, 2017). Conventional management strategies, such as crop rotation and the use of resistant cultivars, have shown limited success due to the pathogen adaptability and persistence in the environment. Addressing these biotic stresses through genetic engineering and biotechnological advancements is crucial for enhancing disease resistance and ensuring the sustainability of castor bean cultivation. Recent advancements in plant biotechnology, including CRISPR-Cas9 genome editing and transgenic approaches, offer promising solutions for developing disease-resistant castor varieties. Additionally, the integration of nanotechnology-based antimicrobial

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treatments and bio-control agents, such as beneficial rhizobacteria and bacteriophages, is being explored as an eco-friendly alternative to chemical bactericides. Strengthening disease management strategies will not only help mitigate yield losses but also secure the long-term viability of castor bean in the global market, reinforcing its role as a sustainable resource for multiple industries (Maroto & Alonso, 2018).

Xanthomonas campestris pv. *campestris* (Xcc) significantly impacts castor bean cultivation by causing black rot, a disease prevalent in various cruciferous crops. This bacterial pathogen leads to severe economic losses by reducing seed quality and overall yield, particularly in regions with high humidity and fluctuating temperatures that favor its spread. The disease manifests through characteristic symptoms such as V-shaped chlorotic lesions, vein blackening, and eventual necrosis, severely affecting plant health and productivity. Effective isolation and characterization methods are crucial for managing this pathogen, as traditional techniques are often labor-intensive and time-consuming (Laala et al., 2015). Classical microbiological approaches, including culturing on semi-selective media and biochemical assays, have been the standard for pathogen identification. However, these methods require significant expertise and extended incubation periods, making them less efficient for large-scale disease surveillance (Giri et al., 2011)

Recent studies have employed molecular techniques, such as PCR and rep-PCR, to enhance the accuracy and speed of *Xcc* identification, revealing high genetic diversity among isolates (Rathaur et al., 2016). These molecular tools have facilitated the differentiation of strains, allowing for precise epidemiological studies and targeted breeding programs for resistance. For instance, Jaccard's similarity coefficients indicated substantial variability, with some studies identifying multiple races of *Xcc* across different regions, emphasizing the pathogen's adaptability and the need for region-specific management strategies. The identification of distinct genetic lineages within *Xcc* populations has also provided insights into disease outbreaks, helping researchers develop resistant cultivars through marker-assisted selection. Additionally, innovative methods like seed-qPCR have been developed to detect viable *Xcc* cells in seeds, improving seed lot sanitation and disease control strategies (Laala et al., 2015). This advanced diagnostic approach enables early detection of infected seeds before sowing, significantly reducing the risk of disease transmission and ensuring healthier crop establishment. These advancements underscore the need for rapid and reliable diagnostic tools to mitigate the impact of *Xcc* on castor bean and other crops. Integrating molecular diagnostics with precision agriculture techniques, such as remote sensing and real-time pathogen monitoring, could further enhance disease forecasting and management, ultimately improving castor bean productivity and sustainability.

This study aims to isolate and biochemically characterize *X. campestris* from infected castor bean plants to establish a foundation for improved detection and management practices. By identifying its key biochemical properties, this research seeks to contribute to the broader understanding of bacterial leaf spot disease in castor bean cultivation and support the development of effective disease control measures.

2. Materials and Methods

2.1. Sample Collection

The infected leaves of Castor bean plant were collected from nearby fields of Yogi Vemana University, Kadapa. From the infected leaves pure culture of *Xanthomonas campestris pv. ricini* was isolated. Isolated organisms were maintained on Nutrient agar (PDA) (HiMedia) for further experiments.

2.2. Isolation of plant pathogens

Infected leaves were taken and surface layer was removed aseptically by wearing glove. Small piece of infected portion of leaf was taken and kept on surface of Nutrient Agra Media plate. The plate with the infected leaf piece was incubated at room temperature for 24 hours and observed for bacterial growth. After 24 hours of incubation bacteria growing on the edges of the infected leaf pieces was taken and sub cultured in to fresh Nutrient Agra Media. Pure culture was prepared using this method.

2.3. Gram staining for Morphological Charecterisation

Gram staining was performed to determine the Gram reaction of *Xanthomonas campestris* isolates following the standard protocol described by Cappuccino and Sherman (2008). A bacterial smear was prepared by transferring a loopful of the culture onto a clean glass slide, air-dried, and heat-fixed. The smear was stained with crystal violet for 60 seconds, followed by Gram's iodine for 60 seconds to enhance stain retention. Afterward, the smear was decolorized using 95% ethanol for 10–15 seconds and counterstained with safranin for 30 seconds. The slide was air-dried and examined under a light microscope using an oil immersion objective (100×) (Cappuccino and Sherman, 2008).

2.4. Biochemical Charecterisation

- **Catalase Test:** A loopful of 48-hour-old bacterial culture was transferred onto a clean glass slide. A drop of 3% hydrogen peroxide (H_2O_2) was added to the culture, and the reaction was observed. The presence of immediate bubble formation indicated a positive catalase reaction, confirming the bacterium's ability to break down H_2O_2 into water and oxygen (Sunil et al., 2023).
- **Potassium Hydroxide (KOH) Test**: The KOH solubility test was performed to confirm the Gram-negative nature of the bacterial isolate. A drop of 3% KOH solution was placed on a clean glass slide, and a loopful of 48-hour-old bacterial culture was mixed into the solution using a sterile inoculation loop. The suspension was stirred for 10 seconds and observed for the formation of a viscous, slimy thread. The formation of a string-like consistency confirmed a positive result, indicating the Gram-negative nature of X. campestris (Naik et al., 2018).
- **Methyl Red Test:** The methyl red test was used to assess the bacterial strain's ability to produce stable acid end-products from glucose fermentation. Five drops of methyl red indicator were added to the broth culture. A red colour change indicated a positive reaction, confirming the bacterium's ability to perform mixed acid fermentation, while a yellow colour indicated a negative result. These biochemical tests were crucial in confirming the physiological and metabolic characteristics of Xanthomonas campestris isolated from the castor bean plant (Schaad NW, 1992).

3. Results and Discussion

3.1. Sample Collection

The infected leaves of castor bean plant have been successfully collected from the nearby fields of Yogi Vemana University, Kadapa.

Figure 1 Infected Castor bean leaves.

3.2. Isolation of plant pathogens

From the infected leaves placed on a petri plate containing nutrient agar media the bacteria was isolated successfully. By taking the small part from the isolated bacteria the streaking was done and the pure cultures are observed successfully.

Figure 2 A) Infected leaf material on Nutrient Agar Media, B) 24 hours Culture and 3) Pure Culture

3.3. Gram staining for Morphological Charecterisation

The Gram staining test confirmed that the isolated *Xanthomonas campestris* was Gram-negative. When observed under an oil immersion microscope (100× magnification), the bacterial cells appeared pink, indicating their inability to retain the crystal violet stain after decolorization with ethanol. Morphologically, the cells were rod-shaped, consistent with the characteristics of X. campestris. This result aligns with previous findings, confirming the Gram-negative nature of the pathogen (Naik et al., 2018).

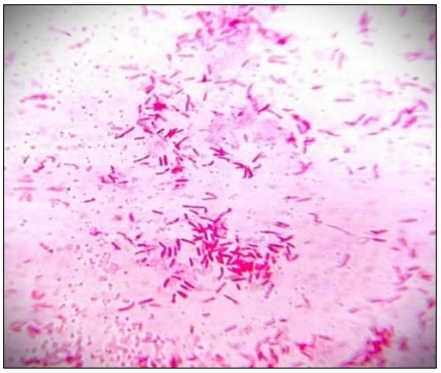

Figure 3 Gram staining of Isolated Bacteria (100X)

3.4. Biochemical Charecterisation

3.4.1. Catalase Test

The presence of immediate bubble formation indicated a positive catalase reaction, confirming the bacterium's ability to break down H_2O_2 into water and oxygen (Sunil et al., 2023).

Figure 4 Catalase Test

3.4.2. Potassium Hydroxide (KOH) Test

The KOH solubility test was performed to confirm and observed for the formation of a viscous, slimy thread. The formation of a string-like consistency confirmed a positive result, indicating the Gram-negative nature of X. campestris (Naik et al., 2018).

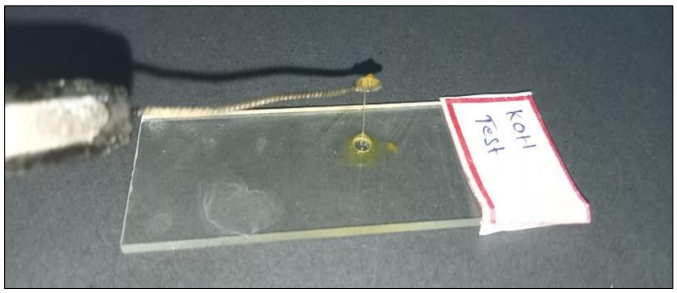

Figure 5 KOH Test

3.4.3. Methyl Red Test

A red colour change indicated a positive reaction, confirming the bacterium's ability to perform mixed acid fermentation These biochemical tests were crucial in confirming the physiological and metabolic characteristics of Xanthomonas campestris isolated from the castor bean plant (Schaad NW, 1992).

Figure 6 Methyl Red Test

4. Conclusion

The biochemical characterization of Xanthomonas campestris pv. ricini isolated from castor bean plants confirmed its Gram-negative, rod-shaped morphology. The results of the catalase, KOH, and methyl red tests were consistent with the expected characteristics of X. campestris. These findings contribute to the basic understanding of the pathogen and highlight the importance of biochemical assays in bacterial identification. Further research, including molecular characterization and pathogenicity testing, is recommended to gain deeper insights into the genetic diversity and virulence of this pathogen, aiding in the development of effective disease management strategies.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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