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Identification and Biochemical Characterization of *Ralstonia solanacearum* causing Bacterial Wilt of Brinjal in Jharkhand

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Abstract: Bacterial wilt of brinjal is caused by *Ralstonia solanacearum* and is a highly destructive disease in humid tropical regions. A survey was conducted to determine the prevalence and severity of bacterial wilt in key brinjal-growing areas, and to identify and describe the *R. solanacearum* isolates responsible for brinjal bacterial wilt in Jharkhand. Bacterial wilt incidence was recorded highest in Bokaro district (28.33 %), followed by Ranchi (17.33 %) and Ramgarh (15.33 %), while the lowest wilt incidence (5.33%) was recorded in Garhwa district. Similarly, at the time of the survey, Bokaro recorded the highest wilt severity (4.33), while Garhwa had the lowest (1.33).

Based on the places from which they were collected, the *R. solanacearum* isolates were grouped into seven categories. The results of the Gram's staining and potassium hydroxide solubility test indicated that every group of *R. solanacearum* isolates is gram-negative. The four fundamental sugars (lactose, mannitol, sucrose, and dextrose) were fermented by *R. solanacearum* isolates. The pathogenicity test and the results of all biochemical assays verified that the isolates were *R. solanacearum*, which is responsible for the bacterial wilt of brinjal. After being incubated for 24 hours, all groups of *R. solanacearum* isolates were determined to be virulent, exhibiting a pink or light red color or the distinctive red core and whitish rim on TZC medium. The biovar test revealed that all groups of *R. solanacearum* isolates oxidized disaccharides (sucrose, lactose, and maltose) and sugar alcohols (mannitol, sorbitol, and dulcitol) within 3-5 days, confirming the biovar III. Thus, it could be verified that *R. solanacearum* from Biovar III and Race I is responsible for the bacterial wilt of brinjal in Jharkhand.

Keywords: Biochemical characterization, *Ralstonia solanacerum,* Bacterial wilt, Brinjal, *Solanum melongena* L.

INTRODUCTION

Brinjal (*Solanum melongena* L.), belonging to the family Solanaceae, is one of the popular vegetable crops grown worldwide, encompassing around 450 host species that belong to 54 plant families (Wicker *et al*., 2007). It has been grown in India for the past 4,000 years and is one of the most popular, most prevalent, well-liked, and important vegetable crops grown in India. Brinjal is locally known as "Begun," and its early European name is "Eggplant." Despite being an annual crop, it is perennial. India produces a variety of cultivars, with consumer preference based on fruit colour, size, and form. It contains many nutrients like protein, carbohydrates, calcium, magnesium, etc. Fruits consist of a high percentage of polyunsaturated fatty acids (65%), which are helpful for liver problems and controlling high blood cholesterol (Daunay and Hazra, 2012). The extract of eggplant

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root and leaves also has medicinal properties against skin diseases and throat and stomach problems (Mak, 2015). Brinjal is cultivated in almost all regions of India except in high-altitude places. The land of the Jharkhand state is suitable for the cultivation of brinjal. Brinjal is now considered among the major vegetables grown in all seasons. It can be cultivated in all types of soil, like sandy soil, loamy soil, and the acidic soil of Jharkhand. In Jharkhand the brinjal cultivation area was 16.582 ha with the productivity of 184.484 tonnes (Anonymous, 2023-24).

Recently, *Ralstonia solanacearum* (Smith) (Yabuuchi *et al.*, 1995), causing bacterial wilt in brinjal (Kelman, 1953), has been identified as an emerging threat to brinjal cultivation. *Ralstonia solanacearum* is a type of bacteria that is found in the soil. It is classified as gram-negative, rod-shaped, aerobic, and non-spore-producing. The occurrence of this phenomenon is widespread in tropical, subtropical, and warm temperate climates across the world. *R. solanacearum* was previously divided into five races (Buddenhagen *et al*., 1962; He *et al*., 1983 and Pegg and Moffet, 1971). It leads to significant reductions in agricultural yields for major crops such as tomato, eggplant, potato, tobacco, banana, ginger, and others. *R. solanacearum* affects the brinjal plant by interacting with the plant's xylem vessels through colonization and production of extracellular polysaccharides (EPS), which results in the blocking of xylem vessels, leading to wilting of the entire plant. The typical symptoms of infected plants include sudden green wilting, drooping of leaves, and a flaccid appearance of shoots, which ultimately leads to the plant's mortality. The bacteria persist in the soil and plant detritus even after the plant has died, which impedes the removal of the inoculum (Hayward, 1991; He *et al*., 1983).

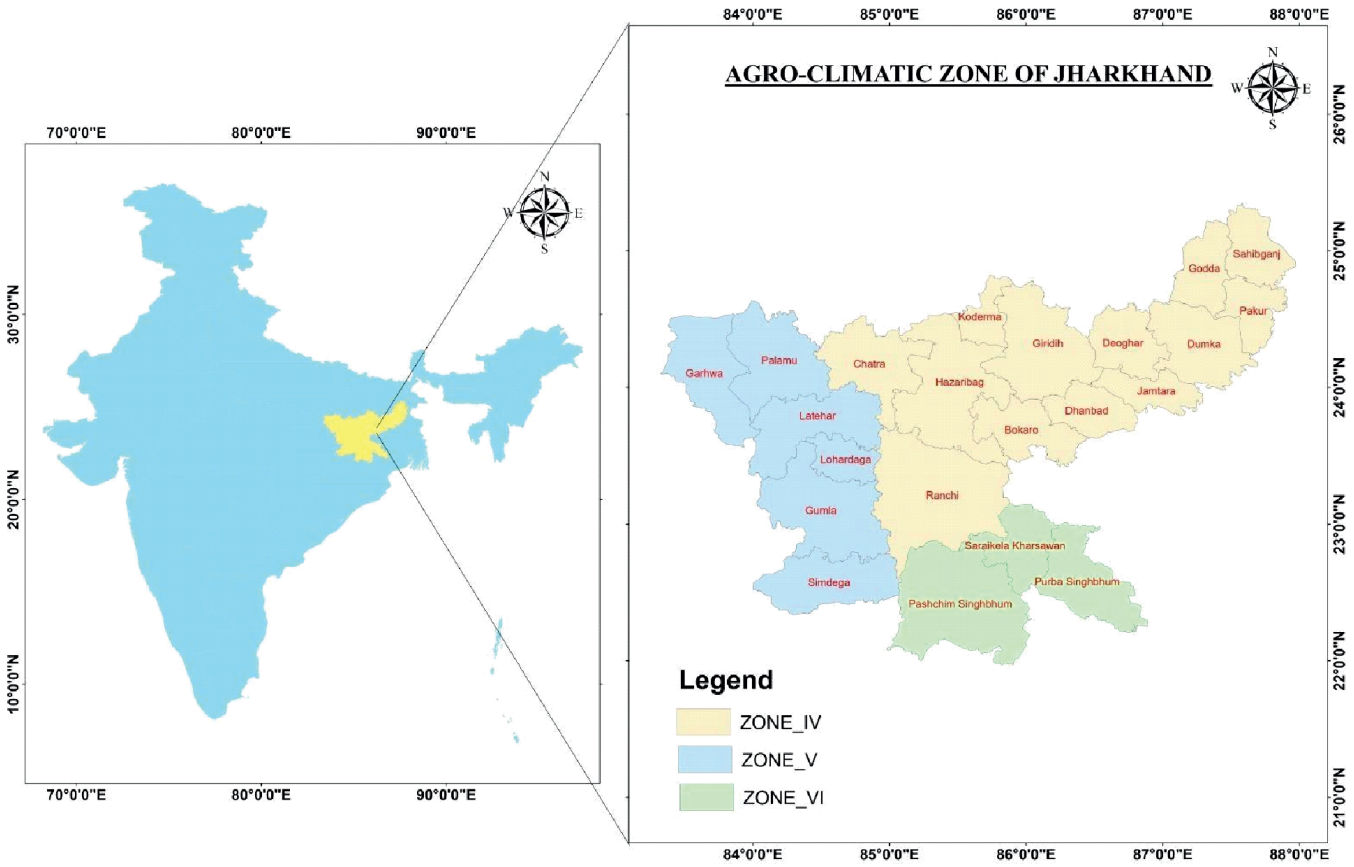

Fig. 1: Agro-climatic zone of Jharkhand

MATERIAL AND METHODS

Survey and surveillance of the disease

To determine the prevalence and severity of bacterial wilt of brinjal, a survey program was conducted in seven districts of Jharkhand, *viz.*, Ranchi, Ramgarh, Bokaro, Dhanbad, Palamu, Garhwa, and Lohardaga, from July to December 2023. At least four locations in each district and 100 plants from each location were randomly selected to record the bacterial wilt incidence and severity. For a quick field diagnosis of wilt caused by *R. solanacearum*, bacterial streaming from infected plant material was performed and confirmed by the streaming of milky white masses of bacterial cells (ooze) . At least five samples of the diseased plants were collected from each of the surveyed areas and brought into the laboratory for the isolation of different groups of isolates of *R. solanacearum*.

Based on the soil characterization, temperature, rainfall, and terrain, Jharkhand is divided into three agro-climatic zones. These are the Central and Northeastern Plateau sub-zone (Zone IV), Western Plateau sub-zone (Zone V), and Southeastern Plateau sub-zone (Zone VI). Zone IV has a total of 15 districts, Zone V has a total of 6 districts, and Zone VI has 3 districts. A survey was conducted in 2 agro-climatic zones of Jharkhand i.e. Zone IV and V; Ranchi, Bokaro, Ramgarh, and Dhanbad districts of Zone IV, and Lohardaga, Palamu, and Garhwa districts of Zone V was done.

Assessment of disease incidence and severity

The status of bacterial wilt of brinjal in different districts of Jharkhand was assayed based on wilt incidence and severity. Data on wilt incidence were recorded in at least four locations for each district and 100 plants from each location. Then the percentage of wilt incidence was calculated by the following formula:

$$\%\ Wilt\ incidence = \frac{Number\ of\ wilted\ plants\ in\ each\ field}{Total\ number\ of\ plants\ in\ each\ field} \times 100$$

$$Frequency(\%) = \frac{Number\ of\ isolations}{Total\ number\ of\ samples\ plated} \times 100$$

Ten plants were randomly selected from each location to calculate the wilt severity in each growing area. The severity of bacterial wilt was recorded based on the severity scale described as 0 = No symptom (0 %), 1 = One leaves wilting (1-25%), 2 = 2 or 3 leaves wilting (26-49%), 3 = Half plant wilting (50-74%), 4 = totally leaves wilting (75-100%), and 5 = Plant dies (Horita & Tsuchiya, 2001; Mwangi *et al*., 2011).

$$\%\ Disease\ severity = \frac{No.of\ Infected\ leaf\ of\ the\ wilted\ plant}{Total\ no\ of\ leaf\ of\ a\ plant\ sample} \times 100$$

Isolation, purification, identification, and preservation of *Ralstonia solanacearum*

The wilted brinjal plants were cut just from the upper side of the root, and they were kept in the glass beaker containing distilled water, taking care that the infected tissue was in contact with the water surface. After five minutes, the water in the glass beaker becomes turbid due to the oozing of bacterial cells from the cut ends of the diseased tissue, thus confirming the bacterial nature of the wilted plants. The vascular discolored tissues are cut into small pieces, then firstly surface sterilized with 0.5 percent sodium hypochlorite for 30 seconds. Then, the tissues were immersed in 70% ethanol for one minute. Finally, the tissues were rinsed with sterile distilled water for one minute to remove trace ethanol. The tissues were placed into a screw-capped vial that contained 2 mL of sterile distilled water. When you add tissues to the water, it gets cloudy. The bacterial suspension was streaked on Petri plates containing TTC medium (1 g of casein hydrolysate, 10 g of peptone, 5 g of glucose, and 5 ml of 1 percent triphenyl tetrazolium chloride for one liter of distilled water). The streaked plates were incubated at 28 ± 2°C for 36-48 h. The well-separated colonies were picked up and purified further

by single-colony isolation techniques. The virulent colonies with pink or light red colour or a characteristic red center and whitish margin, and avirulent, smaller, off-white, and non-fluidal colony strains of *R. solanacearum* were identified in Triphenyl Tetrazolium Chloride (TZC) medium containing 0.005% TZC (Kelman, 1954).

To isolate the pathogen from the soil, the soil samples were serially diluted, and the pathogen was isolated using a Nutrient Agar (NA) medium. At the end of the incubation period, the plates were observed for the development of both the virulent and avirulent colonies of *R. solanacearum*.

To confirm the isolates of *R. solanacearum*, the pathogenicity test was performed on a 15-day healthy brinjal plant by the needle inoculation method. Multiple colonies of *R. solanacearum* showing virulent, irregular, and creamy white with a pink colour at the center were collected in a conical flask, and a prepared bacterial cell suspension (10^8 CFU/ml). Bacterial suspensions were injected into the node of the plant with the help of a hypodermic syringe. Injected plants were observed daily, and up to 7-15 days of plants showed symptoms. The isolates of *R. solanacearum* were preserved at -20^0C for a long time (6 months to 1 year) and 4^0C for a short time (2-3 months) in the refrigerator for further biochemical studies.

Determination of biovars

The isolates of *R. solanacearum* were differentiated into biovars based on their ability to utilize disaccharides (sucrose, lactose, and maltose) and sugar alcohols (mannitol, sorbitol, and dulcitol) as described by He *et al*., 1983 and Ahmed, 2013. The biovars were determined in the mineral medium (Difco Bacto Peptone 1.0 g, KCl 0.2 g, $NH_4H_2PO_4$ 1.0 g, $MgSO_4.7H_2O$ 0.2 g, Agar 3.0 g, and Bromothymol blue 80.0 mg per liter). It contains 1% sugar. 200 µl of the melted medium is dispensed into the wells of the microtiter plate. Inoculum for each group of isolates was prepared by adding several loopfuls of bacteria from 24 to 48 hour-old cultures to distilled water to make a suspension containing about 10^8 CFU/ml. After that, 20 µl of bacterial suspension was added to the wells of the microtiter plate and incubated at 28-32^0C. The tubes were examined at 3 days after inoculation for a change in pH by a colour change (Schaad *et al*., 2001 and Rahman, 2010).

BIOCHEMICAL CHARACTERIZATION OF *RALSTONIA SOLANACEARUM*

Gram Staining

The Gram's staining reaction was performed using crystal violet. The purple dye, crystal violet, was picked up by the cell wall of both Gram-positive and Gram-negative bacteria. The cells are then dipped into an iodine solution. When ethanol (the decolorizing agent) is applied, the ethanol dissolves the lipids in the outer membrane of the cell wall of Gram-negative bacteria, causing the crystal violet-iodine (CV-I) complex to leave the cells. Gram-negative cells, therefore, appear colourless after the ethanol wash. As Gram-negative cells are colourless after the ethanol wash, they are counterstained with safranin, a pink or reddish- coloured dye. Therefore, Gram-negative cells appear pink after the Gram stain procedure.

KOH test

Placed approximately 50 µl of 3% (w/v) KOH on a clean glass slide. Aseptically transferred bacterial cells from an agar plate to a drop of KOH with a sterile toothpick. Agitate the cells with the toothpick. The viscosity of the drop increased, and a string of "goo" was easily picked up with the tip of the toothpick; the cells are gram-negative. If the viscosity does not greatly increase, the cells are gram-positive. The experiment was replicated thrice.

Kovacs oxidase test

Oxidase activity was detected by the method (Kovacs, 1956). Freshly grown (24 to 48 hrs) cultures from nutrient agar with 1% glucose were patched onto a filter paper moistened with a fresh oxidase reagent (1% w/v aqueous solution of tetramethyl-para-phenylene diamine dihydrochloride) using a wooden stick.

Catalase test

The catalase test was performed according to the methods described by He *et al*. (1983). Only 1 ml of a 3% solution of hydrogen peroxide was added to a Petri dish, and a loop of fresh culture grown on Casamino acid peptone glucose (CPG) agar medium was added to the solution. Release of bubbles from the culture was recorded as catalase positive (Sands, 1990).

Nitrate reduction test

This test was accomplished as suggested by Hayward (1964) using the medium of Fahy and Hayward (1991). It was a semi-soft agar medium containing (g/l): peptone 10g, NaCl 5g, KNO_3 2g, and agar 3g, which was boiled to dissolve the agar. The pH was adjusted to 7.0 with concentrated NaOH, and the medium was dispensed into test tubes and autoclaved. Tubes were stabbed, inoculated with a loop of a test strain, and then filled with sterile melted 3% water agar. Control test tubes were not inoculated with strains. Good growth in 5 days at 30ºC was taken as indicative of nitrate reduction into nitrite (Sands, 1990).

H2S production

This was detected, according to Sands (1990), by using a medium that constitutes (g/l) $NH_4H_2PO_4$ 0.5 g, K_2HPO_4 0.5 g, K_2HPO_4 0.5 g, $MgSO_4.7H_2O$ 0.2 g, NaCl 5 g, yeast extract 5 g, and cysteine hydrochloride (anhydrous) 0.1 g and dispensed in 5 ml aliquots into tubes and autoclaved. Lead acetate impregnated paper strips were prepared by dipping 5 mm strips into 5% lead acetate, drying, and autoclaving. These were hung above inoculated media using cotton plugs, and black discoloration of the lead sulphide was monitored as an indicator for H_2S production.

Starch Hydrolysis

Nutrient agar plates containing 0.2% soluble starch (w/v) were streaked by the tested isolates and incubated at 30ºC until heavy growth occurred. Then plates were flooded with IKI solution (iodine, 1 g; potassium iodide, 2 g; distilled water, 100 ml). A clear zone around a colony was recorded as a positive reaction (Sands, 1990).

Gelatin Hydrolysis

For this test, nutrient agar with 0.4% (w/v) gelatin was poured into petri dishes, cooled, and dried overnight. The following day, strains were inoculated onto each plate and incubated at 30ºC for 3 days. When good growth was observed, the plate surfaces were flooded with 5 ml of mercuric chloride solution ($HgCl_2$ 12 g; distilled water 80 ml, concentrated HCl 16 ml) (Sands, 1990). A clear zone surrounding bacterial growth indicates a positive reaction for the test (Dickey and Kelman, 1988).

Citrate Utilization

The citrate test was performed by inoculating 100 µl of a 10^4 CFU/mL of *R. solanacearum* into plates containing the Simmons' Citrate Agar medium, where sodium citrate is the only source of carbon and energy. Then all plates were incubated at 37 °C for 48 hrs. Bromothymol blue is used as an indicator for sodium carbonate, an alkaline product, which changes the color of the indicator from green to blue, and this constitutes a positive test.

Sugar fermentation test

Sugar fermentation tests were performed for confirmation of *R. solanacearum* isolates as described previously by Hossain *et al.* (2007).

The isolates of *R. solanacearum* can oxidize the sugars, which is indicated by color change (reddish to yellow).

RESULTS AND DISCUSSION

Survey and surveillance of the disease

A survey program was conducted in seven districts of Jharkhand, *viz*., Ranchi, Ramgarh, Bokaro, Dhanbad, Palamu, Garhwa, and Lohardaga. A significant variation was observed in terms of bacterial wilt incidence among the brinjal-growing districts surveyed (Table 1). The survey results showed that the highest 28.33% bacterial wilt incidence was recorded in Bokaro, followed by Ranchi and Ramgarh with 17.33% and 15.33% wilt incidence, while the lowest 5.33% bacterial wilt incidence was recorded in Garhwa. The incidence of bacterial wilt of brinjal was recorded in Lohardaga, Palamu, and Dhanbad is 15.00%, 11.33%, and 9.33%, respectively. In Jharkhand, the highest 4.33 bacterial wilt severity was recorded in Bokaro, while the lowest 1.33 bacterial wilt severity was recorded in Garhwa. The severity of bacterial wilt of brinjal was recorded as 3.68, 2.68, 2.67, 2.67 and 2.33 in Palamu, Dhanbad, Ranchi, Ramgarh and Lohardaga, respectively (Table 1). The result of the survey determines the regional variation in bacterial wilt incidence and severity. The differences in wilt incidence and severity were also reported due to the great diversity of host plants affected by the pathogen, the phenotype & genotype of *R. solanacearum,* its wide geographical distribution, and the wide range of environmental conditions conducive to bacterial wilt (Chatterjee *et al*., 1997).

Table 1: Incidence and severity of bacterial wilt of brinjal in different districts in Jharkhand

Districts	Wilt incidence (%)	Wilt severity (1-5 scale)
Ranchi	17.33 (24.56)	2.67 (9.36)
Ramgarh	15.33 (23.04)	2.67 (9.36)
Bokaro	28.33 (32.13)	4.33 (11.99)
Dhanbad	9.33 (17.76)	2.68 (9.36)
Lohardaga	15.00 (22.71)	2.33 (8.74)
Palamu	11.33 (19.65)	3.68 (10.86)
Garhwa	5.33 (10.95)	1.33 (5.42)
S.E.(m) 2.03 1.14 CD at 0.05 6.34 3.55		

The figure in parentheses is the angular transformed value

Isolation and identification of the *R. solanacearum* isolates

A total of 72 isolates of *R. solanacearum* were obtained from the wilted brinjal plant collected from different districts. There are

Table 2: Isolates of *Ralstonia solanacearum* collected from major brinjal growing areas in Jharkhand

Districts	*Sample No. of Isolates*	*Number of isolates collected from each area*	*Number of Virulent isolates*	*Virulent Culture of isolates in TZC medium*
Ranchi	Sample 1	12	3	Virulent colonies appeared white with pink centers
Ramgarh	Sample 2	10	3	Virulent colonies appeared white with pink centers
Bokaro	Sample 3	16	5	Virulent colonies appeared white with pink centers
Dhanbad	Sample 4	8	2	Virulent colonies appeared white with pink centers
Palamu	Sample 5	12	3	Virulent colonies appeared white with pink centers
Garwha	Sample 6	9	2	Virulent colonies appeared white with pink centers
Lohardaga	Sample 7	5	2	Virulent colonies appeared white with pink centers

12 isolates obtained from Ranchi, 10 from Ramgarh, 16 from Bokaro, 8 from Dhanbad, 12 from Palamu, 9 from Garhwa, and 5 from Lohardaga (Table 2). Although equal numbers of infected plants were collected from each of the surveyed areas, the number of isolates varied because of the failure of isolation of the bacterium from all the infected plants, or it might be due to other wilt symptoms. All of the *R. solanacearum* isolates collected from wilted brinjal plants produced 20 isolates show irregular-shaped, white-to-cream-colored, slimy colonies with pink color centers, and avirulent colony show dark red, round, and non-fluidal morphology with a narrow or absent white margin on TZC media after 24 to 48 hours of inoculation.

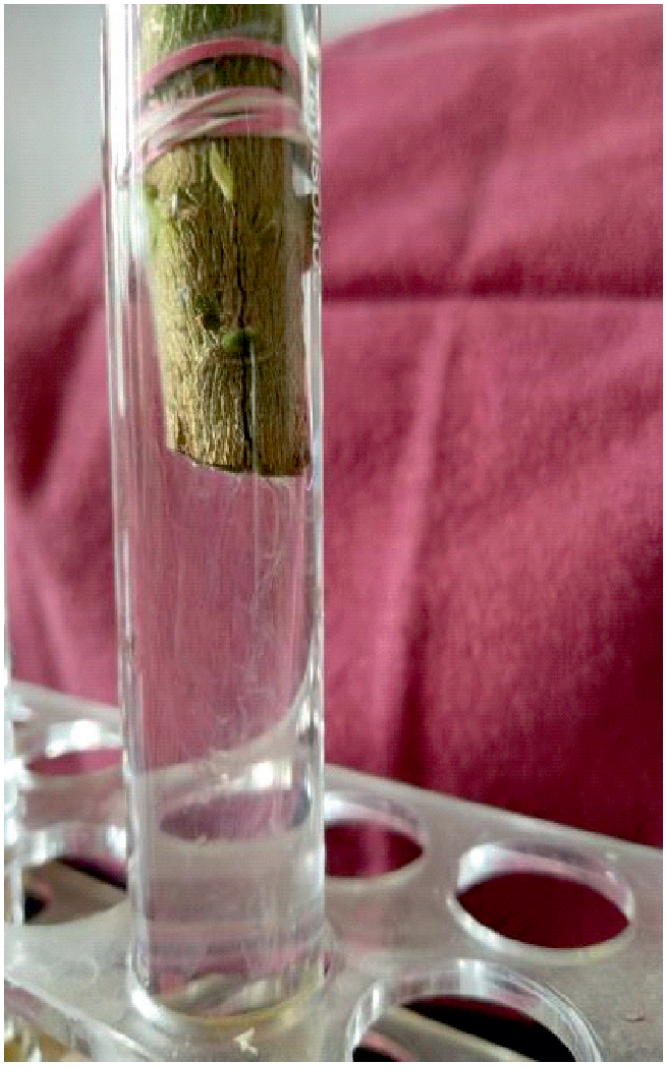

Fig. 2: Ooze test

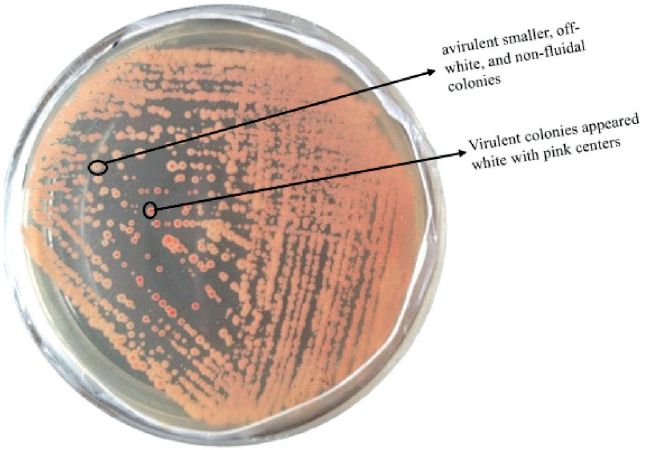

Fig. 3: Bacterial colony (Virulent and avirulent)

Determination of Biovars

In this study, the biovars of *Ralstonia solanacearum* isolates were determined by assessing their ability to metabolize a range of disaccharides (cellobiose, maltose, lactose) and sugar alcohols (dulcitol, mannitol, sorbitol). The change in medium color from green to yellow confirmed the oxidation of these carbon sources, which allowed the classification of the isolates as biovar 3 (Table 3). This method of biovar differentiation through carbohydrate utilization has been widely used, as first established by Hayward (1964) and later supported by He *et al*. (1983) and Kumar *et al*. (2013). Notably, Kumar *et al*. (2013) documented the predominance of biovar 3 isolates in India, especially those affecting solanaceous crops, which aligns with our findings in brinjal.

The prevalence of biovar 3 in India has also been reported by Singh *et al*. (2018b), who found that it accounted for 90.2% of the *R. solanacearum* population, while biovar 4 made up only 9.8%, largely restricted to Uttarakhand, Himachal Pradesh, and Jharkhand. Similarly, Gaitonde and Ramesh (2014) reported a strong dominance of biovar 3 in Western India. Interestingly, some studies have noted the occurrence of biovar 6 under the newer classification schemes, although its distribution remains limited.

The results indicate that biovar 3 is the dominant group of *R. solanacearum* across diverse agro-climatic regions of India, including the present study on brinjal isolates from Jharkhand. The persistence of this biovar under varying environments highlights the influence of both genetic and ecological factors in shaping the distribution of *R. solanacearum* populations.

Biochemical tests

Gram Staining

The Gram's staining reactions were performed using crystal violet. The purple dye, crystal violet, was obtained in the cell wall of both Gram-positive and Gram-negative bacteria. Then, after adding iodine solution. The microscopic results showed that all the isolates of *R. solanacearum* did not preserve violet colour, that is, the isolates retained counterstain (pink colour). Therefore, all isolates of *R. solanacearum* representing each group are gram-negative and straight or curved rod-shaped, which is the defining property of any plant-harmful bacteria (Tables 2 and 3). Several researchers (Pawaskar *et al*., 2014) reported similar morphological and staining reactions of *R. solanacearum*.

Potassium hydroxide solubility test

The gram-negative test of *R. solanacearum* was further validated by the potassium hydroxide solubility test. The existence of an elastic or viscous thread observed when the loop is lifted from the bacterial solution by a toothpick, a few centimeters from glass slides, shows a positive test for all groups of *R. solanacearum* isolates, showing that all of the isolates are gram-negative (Table 3). The KOH approach is significantly easier and faster to identify gram-negative and gram-positive bacteria than the classic Gram stain, in which dyes are applied (Suslow *et al*., 1982). Chaudhry and Rashid (2011) also reported similar results for *R. solanacearum* grown on nutrient agar medium.

Kovac's oxidase test

The Kovac's oxidase test was done to identify the oxidation ability of *R. solanacearum* isolates. This test result came from all groups of *R. solanacearum* isolates, which were able to create a deep blue colour with oxidase reagent within a few seconds, which suggested that the tested group of *R. solanacearum* isolates were gram negative (Table 3).

Catalase test

Release of bubbles from the culture was recorded as catalase positive. All the tested isolates were catalase-positive.

Nitrate reduction test

The nitrate reduction test *Ralstonia solanacearum* gives positive result the bacteria are able to reduce nitrate (NO_3^-) to nitrite (NO_2^-). The result is indicated by a clear color change such as blue to pink.

H2S production

The hydrogen sulphide gas production test of *Ralstonia solanacearum* showed that the bacterium was negative in H_2S production. This result is in agreement with the report of (Pawaskar *et al*., 2014).

Starch Hydrolysis

The starch hydrolysis test of the bacterium showed that the bacterium was unable to hydrolyze starch reported *R. solanacearum* as negative in starch hydrolysis. Similarly, He *et al*. (1983) and Pawaskar *et al*. (2014) also reported *P. solanacearum* to be negative in starch hydrolysis.

Gelatin Hydrolysis

Frequently, *Ralstonia solanacearum* does not hydrolyze gelatin. Studies on biochemical characterizations of this species show consistently negative results for the gelatin hydrolysis test.

Table 3: Biochemical test *of Ralstonia solanacerum* **of different sample groups**

Isolates Sample Nos.	*Pathogenicity test and colour test on TZC or TTC media*	*Gram staining reaction*	*KOH solubility test*	*Kovac's oxidase test*	*Catalase test*	*Nitrate reduction test*	*H2S production*	*Starch Hydrolysis*	*Gelatin Hydrolysis*	*Citrate Utilization*	*Sugar fermentation test*			
											Dextrose	*Sucrose*	*Mannitol*	*Lactose*
Sample 1	+	-	+	+	+	+	-	-	-	+	+	+	+	+
Sample 2	+	-	+	+	+	+	-	-	-	+	+	+	+	+
Sample 3	+	-	+	+	+	+	-	-	-	+	+	+	+	+
Sample 4	+	-	+	+	+	+	-	-	-	+	+	+	+	+
Sample 5	+	-	+	+	+	+	-	-	-	+	+	+	+	+
Sample 6	+	-	+	+	+	+	-	-	-	+	+	+	+	+
Sample 7	+	-	+	+	+	+	-	-	-	+	+	+	+	+

Sample 1= Ranchi, Sample 2= Ramgarh, Sample 3= Bokaro, Sample 4= Dhanbad, Sample5= Palamu, Sample 6= Garhwa, and Sample 7= Lohardaga

Citate Utilization

The isolates of *R. solanacearum* in the Simmons' Citrate Agar medium turned blue color after 48 hours; this indicates a positive citrate test for *R. solanacearum*.

Sugar fermentation test

The isolates of *R. solanacearum* can oxidize the sugars, which is indicated by a color change (reddish to yellow). The results of the sugar fermentation test clearly showed that all groups of *R. solanacearum* isolates obtained from the wilted brinjal plants were able to oxidize the four (4) basic sugars (dextrose, sucrose, mannitol, and lactose) by producing acid and gas (Table 3). The acid production in the sugar fermentation test by bacterial isolates was indicated by the colour change from reddish to yellow, gas production was noted by the appearance of gas bubbles in the inverted Dhuram's tubes, and the oxidation of sugar mannitol by the bacterial isolates was indicated by the production of yellow to red colour.

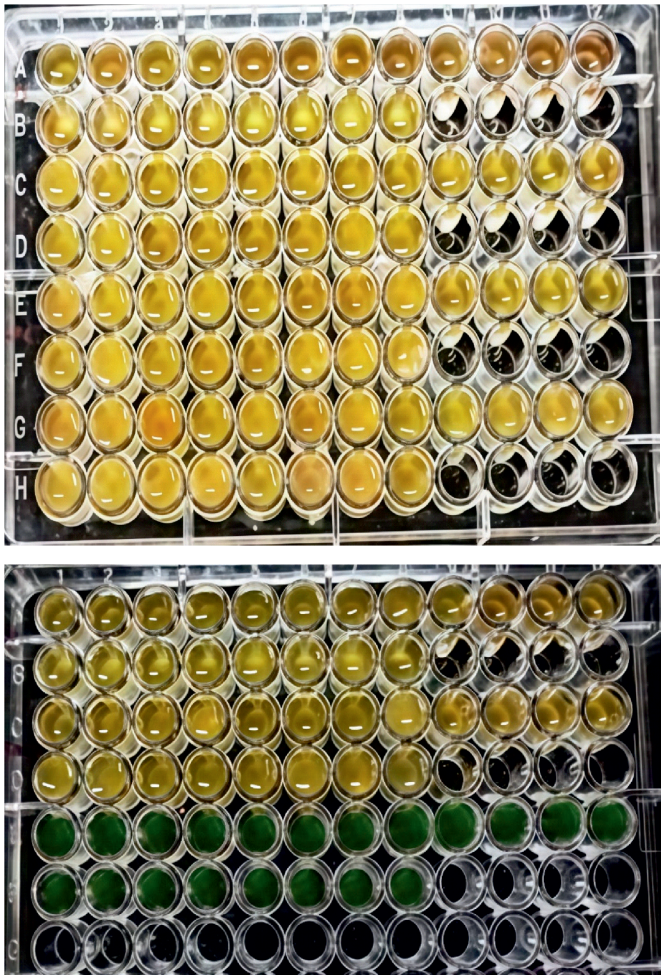

Fig. 4: Biovar test showing positive yellow color reaction, indicating utilization of sugars and alcohols by *R. solanacearum* isolates.

Fig. 5: Sugar fermentation test

SUMMARY AND CONCLUSION

A survey was carried out to determine the prevalence and severity of bacterial wilt in important brinjal growing in Jharkhand. Bokaro showed the highest wilt incidence (28.33), followed by Ranchi (17.33) and Ramgarh (15.33), while Garhwa recorded the lowest wilt incidence (5.33). All 72 collected isolates from different sources showed a positive reaction on the TZC test. All the tested isolates were oxidase positive, catalase positive, reduced nitrate to nitrite, hydrolyzed starch, and oxidized glucose. Bacterial wilt incidence varied in the major brinjal growing areas, which may be due to the species complex of the pathogen at the molecular level and various soil factors. Pathotypes and biotypes of bacterial wilt pathogens of brinjal remained the same in major brinjal-growing areas of Jharkhand. Biovar III and Race I of *R. solanacearum* were only prevalent in all growing areas of the country. The findings of the present study will step forward in the determination of the population structures of *R. solanacearum* to design an effective molecular-based analysis of *R. solanacearum*

causing bacterial wilt disease, with special emphasis on its integrated management.

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