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RESEARCH ARTICLE

Population diversity and pathogenicity of *Ralstonia solanacearum* causing bacterial wilt of tomato in Odisha

Samikshya Sankalini Pradhan[1*], Subrata Sarkar[1], Biswajit Jena[2], Anita Mohanty[3], Deepali Mohapatra[1], Saswati Sibani Panda[4], Lipikant Sahoo[1] & Debashis Dash[2]

[1]Department of Plant Pathology, Odisha University of Agriculture & Technology, Bhubaneswar 751 003, India
[2]Department of Agriculture and Allied Sciences, C.V. Raman Global University, Bhubaneswar 752 054, India
[3]All India Coordinated Research Project on Vegetable Crops, Odisha University of Agriculture & Technology, Bhubaneswar 751 003, India
[4]Centre for Training, Diagnosis and Services for Management of Plant Viral Diseases in Odisha, Odisha University of Agriculture & Technology, Bhubaneswar 751 003, India

*Correspondence email - samikshyapradhan8@gmail.com

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Abstract

Bacterial wilt, caused by *Ralstonia solanacearum*, is one of the most destructive diseases limiting tomato production in tropical and subtropical regions. To understand the pathogen's biology in Odisha, twenty isolates were collected from wilt-affected tomato fields and characterized for incidence, morphology, biochemical properties, metabolic traits, pathogenic variability and race/biovar grouping. Wilt incidence ranged from 13.33 % to 73.33 %. The most virulent isolates-ORS1, ORS3 and ORS20 (where the ORS series denotes isolates of *Ralstonia solanacearum* collected from wilt-affected tomato plants across Odisha) caused severe wilt and showed high bacterial populations, whereas isolates such as ORS10 and ORS19 were weakly pathogenic. On tetrazolium chloride (TZC) medium, most isolates formed fluidal, white-margined, red-centred colonies, though variations such as irregular or slimy types were observed. Growth was vigorous on TZC agar, moderate on nutrient agar and poor on King's B agar. Biochemical and physiological characterization confirmed the identity of all isolates as *Ralstonia solanacearum*. The isolates exhibited generally conserved metabolic behaviour with limited variability in certain carbohydrate and amino acid utilization patterns, indicating a genetically uniform yet adaptable population across Odisha. All isolates were classified as Biovar III and Race I, infecting tomato, chilli and brinjal but not banana or ginger. These results confirm that tomato wilt in Odisha is dominated by a virulent Biovar III, Race I population with intraspecific variability, underscoring the need for integrated management and resistance breeding against aggressive local isolates.

Keywords: biovar; Odisha; pathogenicity assays; race; *Ralstonia solanacearum;* tomato; variability

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated vegetable crops in India and serves as both a staple food and an important cash crop. However, its production is severely constrained by bacterial wilt, a devastating vascular disease caused by *Ralstonia solanacearum*. This soil-borne, Gram-negative, rod-shaped bacterium (0.5-0.7 × 1.5-2.0 µm) thrives under warm, humid and aerobic conditions, with optimal growth between 28-32 °C (1-5). The disease is particularly severe in tropical and subtropical regions, where conducive environments accelerate pathogen multiplication and host colonization. Yield losses of up to 80–90 % in tomato and eggplant due to bacterial wilt have been reported from different parts of India, highlighting the economic significance of the pathogen.

The host range of *R. solanacearum* is exceptionally broad, encompassing several solanaceous crops (tomato, brinjal, chilli, potato) as well as non-solanaceous hosts such as banana, ginger, groundnut, tobacco and mulberry (6, 7). For epidemiological and taxonomic clarity, the pathogen has been categorized into races based on host range and into biovars based on carbohydrate utilization patterns. Race I isolates infect a wide spectrum of solanaceous crops and certain weeds, Race II is restricted mainly to banana and *Heliconia*, Race III is recognized as the potato race, Race IV is specific to ginger and Race V is associated with mulberry (8). Five biovars (I-V) are defined according to the ability to utilize or oxidize specific disaccharides and hexose alcohols (9). However, a consistent relationship between race and biovar classifications has often been lacking, as isolates within a single race may belong to different biovars and vice versa.

Despite advances in molecular diagnostics-including Polymerase chain reaction (PCR) based assays, Deoxyribonucleic Acid (DNA) hybridization and gene sequencing-that have enhanced the resolution of strain differentiation, information on the diversity and prevalence of *R. solanacearum* lineages in India remains incomplete. Recent reports confirm that Indian populations are dominated by Biovar III and Race I, particularly in tomato and eggplant, though notable genetic and pathogenic variability exists within this grouping (10). Such diversity has direct implications for breeding durable resistance and tailoring location-specific disease management strategies.

Although *R. solanacearum* has been widely reported in India, Odisha lacks a harmonized, multi-district assessment that links field wilt incidence with soil inoculum, in-plant bacterial loads, pathogenicity spectrum and race/biovar status under a single methodological framework. This gap constrains evidence-based resistance screening and rotation planning. We therefore (i) surveyed major tomato belts across Odisha and recovered representative isolates, (ii) quantified field incidence and soil inoculum, (iii) assessed cultural/biochemical traits and pathogenicity under controlled conditions with disease-reaction scaling and (iv) assigned race/biovar groups. We interpret the findings for epidemiology and integrated management in the state.

Materials and Methods

Study area, sampling and handling of samples

Surveys for bacterial wilt of tomato were conducted between December 2023 and November 2024 in major tomato-growing belts across the coastal, central, northern, western and southern agro-climatic zones of Odisha. Wilted plants showing vascular browning and bacterial ooze were sampled from farmers' fields. Symptomatic tissues and their rhizospheric soil were placed in sterile polyethene bags, labelled with location, date and transported to the laboratory within 24 hr to minimize desiccation and contamination.

Isolation and purification of the pathogen

Infected soil samples were collected from wilt-affected tomato fields and used for pathogen isolation. Approximately 10 g of soil from the rhizosphere region of diseased plants was suspended in sterile distilled water, serially diluted and plated onto modified TZC agar containing (g L^{-1}): peptone 10, casamino acids 1, agar 17, glycerol 5 mL and 1 % TZC (5 mL) added after autoclaving. Plates were incubated at 28 °C for 48 hr. Colonies showing the characteristic fluidal, irregular, cream-white morphology with pink to red centres were purified by sub-culturing on fresh TZC medium. Twenty isolates were successfully recovered and coded ORS1 to ORS20 according to the collection site. Pure cultures were maintained on TZC slants at 4 °C.

Pathogenicity Test

Pathogenicity of *R. solanacearum* isolates was assessed on the susceptible tomato variety, 'Pusa Ruby'. Four- to five-leaf seedlings were transplanted into sterilized soil-sand mix and inoculated by stem wounding with bacterial suspensions of approximately 10^8 colony-forming unit (cfu/mL) prepared from 48 hr TZC cultures. Ten plants per isolate were maintained at 28 ± 2 °C and observed for wilting up to 20 days. Disease incidence was recorded as per standardized scale (11) and bacterial populations in stem tissues were estimated by dilution plating on TZC agar. The pathogen was re-isolated from symptomatic plants to confirm Koch's postulates.

Field disease incidence and soil inoculum density

The disease incidence was estimated by using the following equation:

Incidence (%) = (number of wilted plants ÷ total assessed plants) × 100 per field (Eqn.1)

Soil inoculum density (cfu g^{-1}) was estimated by dilution plating on TZC and counting colonies with characteristic morphology. Ten 'Pusa Ruby' plants per isolate were inoculated (≈10^8 cfu mL^{-1}) and scored at 5, 10, 15, 20 days after inoculation (DAI) using standardized disease-reaction scale (mortality %) (11) to assign pathogenicity status: highly susceptible (HS) (> 80 %), susceptible (S) (61-80 %), moderately susceptible (MS) (41-60 %), moderately resistant (MR) (21-40 %), resistant (R) (1-20 %), highly resistant (HR) (0). We report the mean status at 20 DAI; re-isolation confirmed Koch's postulates.

Biochemical tests

Fresh cultures (18-24 hr) were examined for Gram reaction using crystal violet/iodine staining with ethanol decolorization and safranin counterstain. Potassium hydroxide (KOH) (3 %) string test, catalase with 3 % hydrogen peroxide (H_2O_2) and oxidase with 1 % tetramethylphenylenediamine (TMPD) assays were performed to confirm gram-negative, catalase and oxidase-positive characters.

Race identification

Seven representative isolates, along with the full set of twenty, were assessed for host specificity under glasshouse conditions. Bacterial suspensions (7×10^7 cfu mL^{-1}; OD_{480} = 0.8-1.0, where OD_{480} refers to Optical Density at 480 nm) were prepared from 48-hour cultures. A differential panel comprising tomato, chilli, banana, ginger and mulberry (*Morus* spp.) was used. Seedlings of solanaceous hosts (20 days old) were root-dipped for 10 min in the inoculum, transplanted into sterilized soil and watered regularly. Banana suckers, ginger rhizomes and mulberry cuttings were inoculated in situ by applying 20 mL of bacterial suspension around the root zone. Sterile distilled water served as the control. Plants were maintained in a glasshouse and inspected weekly for the development of wilt; race designation was based on the host reaction spectrum.

Biovar typing

Biovar grouping using carbohydrate oxidation was performed as per standardized procedure (12). A basal medium consisted of ammonium dihydrogen phosphate ($NH_4H_2PO_4$) 1.2 g, magnesium sulfate ($MgSO_4 \cdot 7H_2O$) 0.24 g, potassium chloride (KCl) 0.24 g, peptone 1.2 g and bromothymol blue (1 %) adjusted to pH 7.2 per liter. The medium was dispensed (150 mL $flask^{-1}$), autoclaved, cooled to 45 °C and supplemented with 10 % filter-sterilized solutions of cellobiose, lactose, maltose, dulcitol, mannitol and sorbitol (heat-stable polyols autoclaved at 110 °C for 20 min). Aliquots (5 mL) were dispensed into sterile tubes, inoculated with 20 µL bacterial suspension and incubated at 30 °C. Colour change from green to yellow on days 3, 7 and 14 indicated acid production; patterns of positive reactions were used for biovar assignment.

Results

Field incidence and isolation

A total of twenty isolates of *R. solanacearum* were collected from wilt-affected tomato fields across different regions of Odisha. The incidence of bacterial wilt exhibited marked variability among the tested isolates, indicating considerable differences in their pathogenic potential. Wilt incidence ranged from as low as 13.33 % in isolates ORS10 and ORS19 to as high as 73.33 % in ORS1, reflecting the wide diversity in virulence among the *Ralstonia solanacearum* populations evaluated (Table 1). Isolates such as ORS1, ORS3, ORS4, ORS5 and ORS20 exhibited high wilt incidence, each exceeding 60 %. Moderate incidence (40-53.33 %) was recorded for ORS6, ORS14, ORS16 and ORS17, while the remaining isolates showed comparatively lower values between 20-40 %.

Table 1. Details of isolates collected from different tomato-growing regions of Odisha

S. No.	Location	Districts	Coordinate	Isolates name
1	Bhubaneswar	Khordha	20°16'36.38"N, 85°47'6.22"E	ORS1
2	Begunia	Khordha	20°11'56.24"N, 85°27'6.17"E	ORS2
3	Tangi	Khordha	19°55'11.89"N, 85°22'59.44"E	ORS3
4	Banki	Cuttack	20°22'29.31"N, 85°31'53.75"E	ORS4
5	Salepur	Cuttack	20°28'57.78"N, 86°6'9.15"E	ORS5
6	Atopur	Keonjhar	21°37'57.70"N, 85°35'59.33"E	ORS6
7	Bargarh	Bargarh	21°20'48.43"N, 83°38'51.77"E	ORS7
8	Dhirapatna, Athagarh	Cuttack	20°30'59.36"N, 85°34'56.49"E	ORS8
9	Raisuan	Keonjhar	21°9'15.60"N, 85°28'38.49"E	ORS9
10	Kodavatta	Nabrangpur	19°38'12.51"N, 82°16'47.59"E	ORS10
11	Dasamantpur	Koraput	19°2'44.74"N, 82°55'19.33"E	ORS11
12	Raygada	Raygada	19°10'36.64"N, 83°23'24.51"E	ORS12
13	Hernamunda	Nuapada	20°49'9.31"N, 82°31'57.01"E	ORS13
14	Ishwarpal	Angul	20°35'29.47"N, 85°25'6.44"E	ORS14
15	Ganjiatikra	Bargarh	21°20'50.35"N, 83°37'55.26"E	ORS15
16	Sekhsari, Jaleswar	Balasore	21°46'45.43"N, 87°10'3.40"E	ORS16
17	Nayagarh	Nayagarh	20°12'26.76"N, 85°6'37.80"E	ORS17
18	Khairitikra	Sonepur	20°52'2.55"N, 83°47'42.90"E	ORS18
19	Chaurasipur	Sonepur	20°54'50.53"N, 83°40'54.78"E	ORS19
20	Bhagilakatta	Angul	21°1'56.19"N, 84°56'7.98"E	ORS20

The bacterial population in soil samples also showed significant variation. ORS1 recorded the highest bacterial load (33.4×10^5 cfu/g soil), followed closely by ORS3 (30.2×10^5 cfu/g) and ORS20 (22.4×10^5 cfu/g) (Fig. 1). In contrast, isolates such as ORS10 (3.4×10^5 cfu/g), ORS11 (4.6×10^5 cfu/g) and ORS19 (7.1×10^5 cfu/g) were associated with much lower pathogen densities (Table 2). Overall, both wilt incidence and pathogen population varied markedly among isolates, suggesting differences in virulence potential.

Cultural and morphological characteristics

When cultured on TZC medium, the isolates displayed clear colony variations. Several isolates, including ORS1, ORS4, ORS5, ORS12, ORS14, ORS16 and ORS18, produced raised, fluidal colonies with white margins and pink to red centers. Isolates such as ORS2, ORS8, ORS9 and ORS20 exhibited moderately fluidal, irregular colonies, while ORS3, ORS6, ORS10, ORS11, ORS13, ORS15, ORS17 and ORS19 showed round red-centered colonies with raised or flat margins (Fig. 2). ORS15 was distinctive, producing irregular, slimy colonies. Despite this variation in colony type, all isolates were rod-shaped, small in size and lacked pigmentation. Importantly, virulence was confirmed in all isolates.

Biochemical and physiological characterization

Biochemical profiling revealed uniformity across all 20 isolates (Table 3). They were consistently Gram-negative and tested positive for catalase and oxidase, confirming their aerobic and oxidative enzymatic nature. Indole production and starch hydrolysis tests were negative in all isolates, whereas all isolates were positive for gas production, KOH solubility and gelatin liquefaction. They also utilized citrate and succinate and produced urease. Representative assays, including Gram staining, catalase, oxidase, KOH solubility

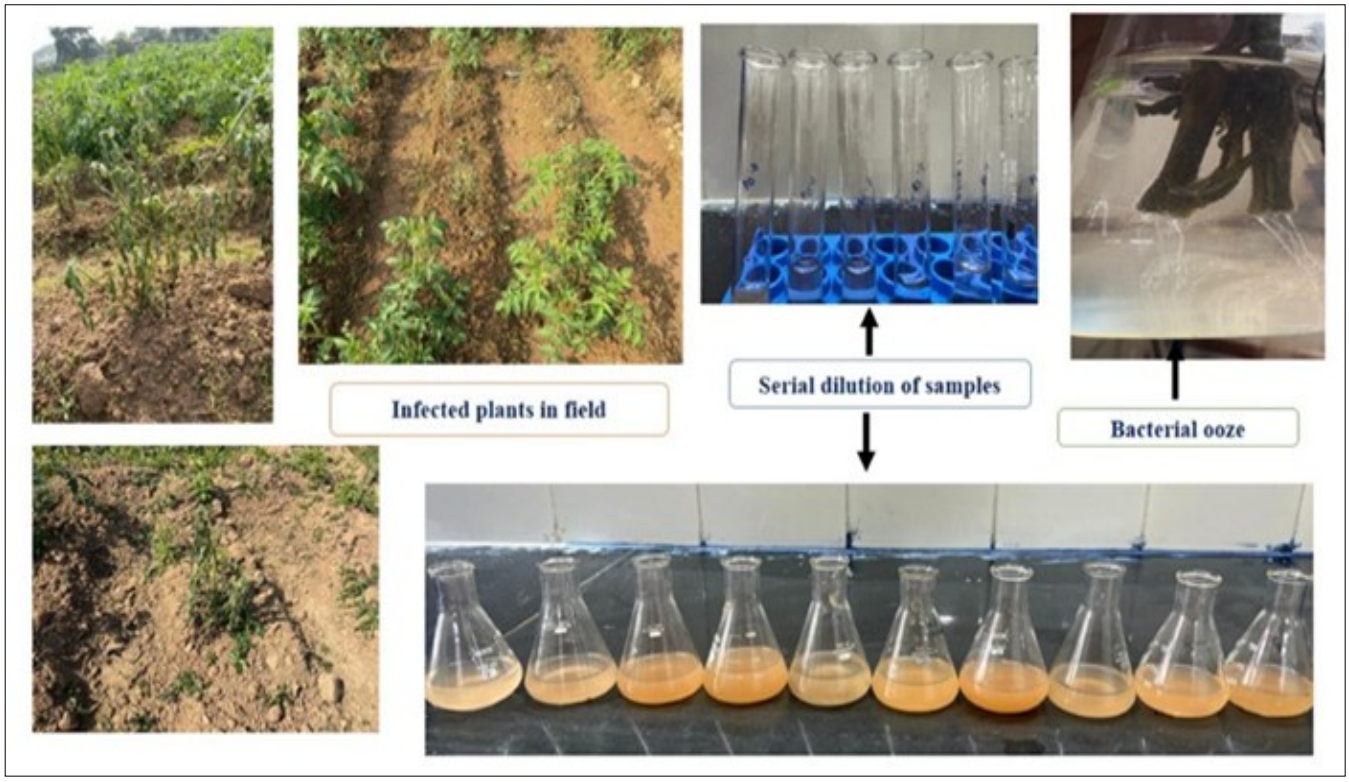

Fig. 1. Isolation of *Ralstonia solanacearum* from wilted tomato plants.

Table 2. Isolation details of different isolates from the wilt-affected tomato fields of Odisha

S. No.	Isolates	Stage of the crop	% wilt incidence	Bacterial population density ($\times 10^3$ cfu/g of soil)
1	ORS1	Flowering	73.33	33.4
2	ORS2	Flowering	60.00	28.6
3	ORS3	Flowering	66.66	30.2
4	ORS4	Fruiting	46.66	18.4
5	ORS5	Flowering	53.33	22.8
6	ORS6	Fruiting	46.66	15.9
7	ORS7	Flowering	26.66	8.9
8	ORS8	Flowering	33.30	12.6
9	ORS9	Fruiting	40.00	17.7
10	ORS10	Flowering	13.33	3.1
11	ORS11	Fruiting	20.00	4.6
12	ORS12	Flowering	26.66	7.8
13	ORS13	Flowering	40.00	12.4
14	ORS14	Fruiting	46.66	13.6
15	ORS15	Flowering	20.00	6.5
16	ORS16	Flowering	46.66	14.8
17	ORS17	Fruiting	40.00	5.3
18	ORS18	Flowering	26.66	14.7
19	ORS19	Flowering	13.33	7.1
20	ORS20	Fruiting	60.00	22.4

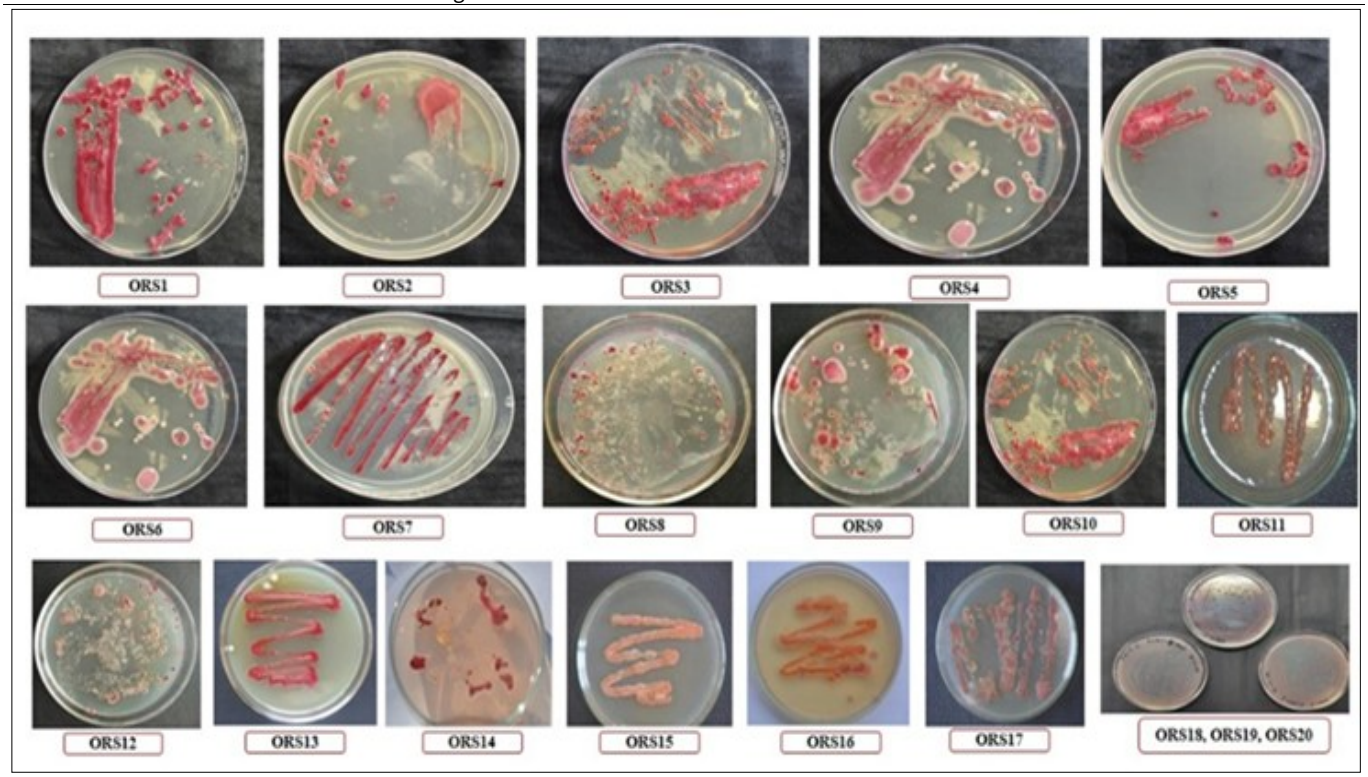

Fig. 2. Colony morphology of *Ralstonia solanacearum* isolates grown on TZC medium.

Table 3. Biochemical and physiological characterization of different isolates collected from different tomato growing regions of Odisha

S. No.	Isolates	Gram staining	Catalase test	Oxidase test	Indole production	Starch hydrolysis	Gas production	KOH test	Gelatin liquifaction
1	ORS1	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
2	ORS2	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
3	ORS3	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
4	ORS4	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
5	ORS5	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
6	ORS6	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
7	ORS7	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
8	ORS8	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
9	ORS9	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
10	ORS10	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
11	ORS11	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve
12	ORS12	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve

and citrate utilization tests, visually supported these results.

Utilization of sugars and amino acids

The isolates displayed broad yet variable utilization of different carbon and nitrogen sources. All isolates fermented glucose and consistently utilized sugars such as D-glucose, maltose, mannitol, sorbitol, sucrose and malonate. Enzyme activities including β-glucosidase, β-galactosidase and β-xylosidase were uniformly positive across the population. On the other hand, substrates like Ala-Phe-Pro-arylamidase, phosphatase and indole production remained consistently negative. Variability was observed in the utilization of L-arabitol, D-trehalose, L-proline arylamidase, lysine decarboxylase and ornithine decarboxylase, with only some isolates responding positively. This reflected metabolic heterogeneity among the isolates, despite their conserved core traits.

Pathogenicity and aggressiveness

Pathogenicity testing on the susceptible variety 'Pusa Ruby' revealed that the majority of isolates were highly virulent. Fourteen isolates (ORS1, ORS2, ORS3, ORS4, ORS5, ORS6, ORS7, ORS8, ORS9, ORS12, ORS14, ORS16, ORS18 and ORS20) induced severe wilting (+++ to ++++) within 15–20 days of inoculation and maintained high in-plant bacterial populations (3.08×10^7 to 8.23×10^7 cfu/100 mg) (Table 4). Three isolates (ORS11, ORS15 and ORS17) exhibited moderate virulence, causing limited wilting with bacterial populations ranging from 3.65×10^5 to 5.25×10^6 cfu/100 mg. Notably, ORS10 was identified as very weakly pathogenic, causing minimal wilting and harboring the lowest bacterial load (4.84×10^6 cfu/100mg). Koch's postulates were successfully confirmed in all isolates through re-isolation of the pathogen. The disease reaction scale used for determining the pathogenicity status of the isolates is provided in Table 5 (11).

Biovar differentiation

All twenty isolates were classified under Biovar III based on their carbohydrate utilization pattern. They uniformly utilized monosaccharides (dextrose, lactose, maltose), disaccharides and sugar alcohols (dulcitol, mannitol, sorbitol), as well as polysaccharides such as cellobiose (Fig. 3). This uniformity confirmed that Biovar III was the predominant strain associated with tomato wilt in Odisha (Table 6).

Table 5. Disease reaction scale (mortality %) for tomato bacterial wilt (*Ralstonia solanacearum*)

Reaction	% Mortality
Highly resistant	0
Resistant	1-20
Moderately resistant	21-40
Moderately susceptible	41-60
Susceptible	61-80
Highly susceptible	> 80

Race differentiation

Race identification using differential hosts showed that the most virulent isolates (ORS1, ORS2, ORS3, ORS5, ORS9, ORS14, ORS18 and ORS20) infected tomato, chilli and brinjal but did not induce symptoms on ginger or banana (Fig. 4). Based on this reaction pattern, all isolates were categorized as Race I, which is typically associated with solanaceous crops (Table 7).

Discussion

The present investigation provided a comprehensive account of the incidence, cultural traits, biochemical characteristics, pathogenic variability, biovar and race differentiation of *R. solanacearum* isolates associated with tomato wilt in Odisha. Although the isolates were collected from diverse locations and crop stages, several commonalities were observed, while considerable variation existed in aggressiveness, colony morphology and metabolic responses. Wilt incidence in farmers' fields ranged widely from 13.33 % to 73.33 %, accompanied by a corresponding variation in bacterial population densities. The highest incidence was recorded in isolates such as ORS1, ORS3 and ORS20, which also harbored the highest pathogen load, indicating that soil inoculum pressure played a central role in disease expression. However, cases of moderate incidence despite intermediate inoculum levels suggested that host phenology, soil environment and cultural practices also influenced wilt severity (13-15). On TZC medium, the isolates produced typical colonies of *R. solanacearum*, but subtle morphological differences were evident. Most isolates formed fluidal, white-margined, red-centered colonies, while others showed irregular or slimy growth patterns (e.g., ORS15). Such variation likely reflected differences in exopolysaccharide production, a known virulence

Table 4. Pathogenicity and aggressiveness of *Ralstonia solanacearum* isolates on the susceptible tomato variety Pusa Ruby

Sl. No.	Isolates	Wilting Index (in the experimental pot condition) Days after inoculation				Pathogenicity status	Re-isolation of bacteria and confirmation of Koch's postulates	In-plant Population (cfu/100mg of diseased plant sample)
		5	10	15	20			
1	ORS1	++	+++	++++	+++++	Highly pathogenic	YES	8.23×10^7
2	ORS2	++	++	++	++++	Highly pathogenic	YES	6.12×10^7
3	ORS3	++	++	++++	+++++	Highly pathogenic	YES	7.04×10^7
4	ORS4	++	++	+++	++++	Highly pathogenic	YES	4.89×10^7
5	ORS5	++	++	+++	++++	Highly pathogenic	YES	5.26×10^7
6	ORS6	+	++	+++	++++	Highly pathogenic	YES	4.32×10^7
7	ORS7	-	+	+	++	Pathogenic	YES	1.36×10^7
8	ORS8	+	++	+++	+++	Highly pathogenic	YES	3.16×10^7
9	ORS9	+	++	+++	++++	Highly pathogenic	YES	4.65×10^7
10	ORS10	-	-	-	++	Very weakly pathogenic	YES	4.84×10^6
11	ORS11	-	-	-	+	Pathogenic	YES	5.25×10^6
12	ORS12	+	++	++	++	Highly pathogenic	YES	2.86×10^7
13	ORS13	+	++	++	++	Highly pathogenic	YES	3.08×10^7

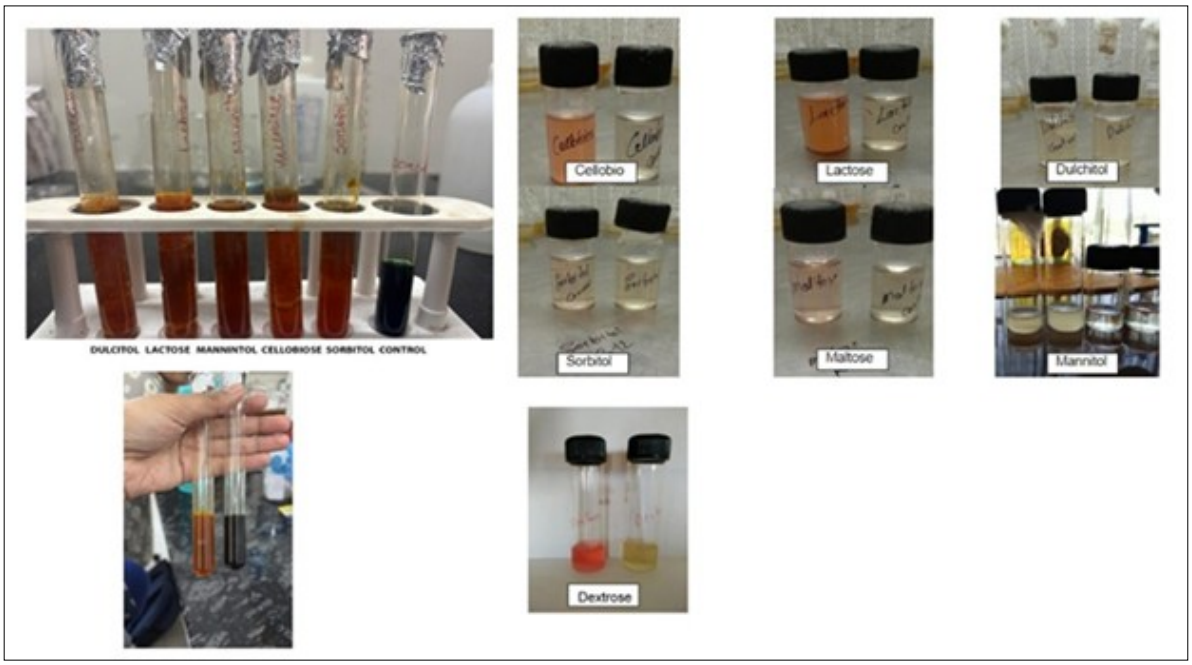

Fig. 3. Biovar characterization of *Ralstonia solanacearum* based on carbohydrate utilization.

Table 6. Biovars differentiation of *Ralstonia solanacearum* by utilization of different carbon sources

Isolates	Utilization of carbohydrates							
	Mono			Di			Poly	Biovar
	Dextrose	Lactose	Maltose	Dulcitolol	Mannitol	Sorbitol	Cellobiose	
ORS1	+ve	+ve	+ve	+ve	+ve	+ve	+ve	III
ORS2	+ve	+ve	+ve	+ve	+ve	+ve	+ve	III
ORS3	+ve	+ve	+ve	+ve	+ve	+ve	+ve	III
ORS4	-ve	+ve	+ve	+ve	+ve	+ve	+ve	III
ORS5	+ve	+ve	+ve	+ve	+ve	+ve	+ve	III
ORS6	+ve	+ve	+ve	+ve	+ve	+ve	+ve	III
ORS7	+ve	-ve	+ve	+ve	+ve	-ve	+ve	III
ORS8	-ve	+ve	+ve	+ve	+ve	+ve	+ve	III
ORS9	+ve	+ve	+ve	+ve	+ve	+ve	+ve	III
ORS10	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-
ORS11	+ve	+ve	+ve	+ve	+ve	-ve	+ve	III
ORS12	-ve	+ve	+ve	+ve	+ve	+ve	+ve	III
ORS13	+ve	+ve	+ve	+ve	+ve	+ve	+ve	III
ORS14	+ve	+ve	+ve	+ve	+ve	+ve	+ve	III
ORS15	+ve	-ve	+ve	+ve	+ve	-ve	+ve	III
ORS16	+ve	+ve	+ve	+ve	+ve	+ve	+ve	III
ORS17	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-
ORS18	-ve	+ve	+ve	+ve	+ve	-ve	+ve	III
ORS19	+ve	+ve	+ve	+ve	+ve	+ve	+ve	III
ORS20	-ve	+ve	+ve	+ve	+ve	+ve	+ve	III

Fig. 4. Screening of different hosts for wilt pathogen races.

Table 7. Detection of races of the most virulent isolates of *R. solanacearum* using different hosts

Isolates	Symptoms observed 15 days after inoculation					Races
	Chilli	Tomato	Brinjal	Ginger	Banana	
ORS1	++	+++	++	-	-	I
ORS2	+++	+++	++	-	-	I
ORS3	++	+++	++	-	-	I
ORS5	+	++	+	-	-	I
ORS9	+++	++	+	-	-	I
ORS14	++	++	++	-	-	I
ORS18	+++	++	+	-	-	I
ORS20	+	+++	++	-	-	I

factor, although pathogenicity results confirmed that colony morphology alone could not reliably predict aggressiveness (16). All isolates were small, rod-shaped and non-pigmented, which was consistent with previous descriptions of the pathogen.

Biochemical and physiological characterization revealed a consistent profile across all isolates. All were Gram-negative, catalase-positive, oxidase-positive and KOH-positive, while negative for indole production and starch hydrolysis (17). Universal positivity for gelatin liquefaction, citrate utilization and gas production suggested a high level of enzymatic activity contributing to vascular colonization and host wilt (18).

Carbohydrate and amino acid utilization patterns highlighted both conserved and variable traits. All isolates fermented glucose and consistently utilized sugars such as maltose, mannitol, sorbitol and sucrose. Enzymatic activity for β-glucosidase, β-galactosidase and β-xylosidase was also consistently positive, confirming the pathogen's strong carbohydrate-degrading capacity. In contrast, differential utilization of L-arabitol, trehalose and certain amino acids indicated intraspecific metabolic diversity (19). This variability may explain differences in aggressiveness among isolates despite their shared biovar identity, reflecting ecological adaptations to diverse soils and cropping systems (20).

All isolates belonged to Biovar III based on carbohydrate utilization and pathogenicity assays on differential hosts classified them under Race I, characterized by pathogenicity on solanaceous crops (tomato, chilli, brinjal) but not on banana or ginger. The uniformity of biovar and race across isolates suggested the predominance of a single lineage in the region, consistent with reports of Race I/Biovar III as the most widespread and destructive group infecting tomato in tropical Asia (21, 22). From a management perspective, this finding has practical implications for breeding and crop rotation, as resistance screening can focus on Race I/Biovar III challenges and solanaceous rotations should be avoided (23).

Despite the uniform biovar and race grouping, pathogenicity assays revealed a clear gradient of aggressiveness. Fourteen isolates were highly pathogenic, producing severe wilting within 15-20 days and maintaining high in-plant bacterial loads (3.08×10^7- 8.23×10^7 cfu/100 mg). Three isolates were moderately pathogenic, while one (ORS10) was very weakly pathogenic, causing minimal wilting. The parallel increase in wilt index and bacterial populations supported the established link between xylem colonization and symptom expression. Nevertheless, differences in symptom onset and intensity among isolates with comparable bacterial populations suggested that additional factors, such as effector proteins, quorum sensing, or exopolysaccharide dynamics, contributed to pathogenic variation (24, 25).

Despite uniform Race I/Biovar III status, the isolates displayed a broad virulence spectrum. This decoupling between taxonomic grouping and aggressiveness is consistent with reports that effector repertoires and Type III Secretion System (T3SS) regulation, rather than biovar, drive host colonization kinetics and wilt severity (26, 27). Divergence in type III effector alleles (28), horizontal gene transfer (27) and quorum-sensing control of exopolysaccharides (EPS I/II) and cell-wall-degrading enzymes (29) can yield markedly different disease trajectories within a single biovar/race framework. These mechanisms plausibly underlie the rapid wilt and high in-plant titers observed for ORS1/3/20 versus the delayed or weak reactions for ORS10/11. Colony fluidity on TZC, often linked to EPS overproduction (29), broadly aligned with higher wilt scores, supporting EPS as a virulence determinant; however, exceptions indicate additional layers (xylem fitness, detoxification, biofilm dynamics) modulate symptom expression. Likewise, modest variability in carbon/amino-acid utilization likely reflects ecological adaptation to local soil condition (carbon availability, pH, temperature), which can influence saprophytic survival and infection thresholds without altering race/biovar assignment.

Conclusion

Tomato wilt in Odisha is driven by a predominantly Race I/Biovar III *R. solanacearum* population that nonetheless exhibits substantial pathogenic diversity. Field incidence and soil inoculum density covaried with aggressiveness and most isolates caused rapid wilt with high in-plant titers. These results prioritize Race I-focused resistance screening, inoculum-suppressive practices (nursery hygiene, sanitation, non-host rotations) and district-level monitoring. Incorporating molecular typing (e.g., 16S/MLST/effector profiling) will further refine management and breeding targets against the most aggressive local lineages.

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Authors' contributions

SSP[1] conceived and designed the study, conducted field sampling, pathogen isolation, characterization and pathogenicity assays, analyzed the data, and prepared the original manuscript. SS supervised the research work, contributed to experimental design, data interpretation, and critically revised the manuscript. BJ participated in sample collection, laboratory experiments, and data analysis. AM provided technical guidance in plant pathological techniques and assisted in pathogenicity studies and interpretation of results. DM contributed to laboratory experimentation, statistical analysis, and preparation of tables and figures. SSP[2] assisted in field surveys, sample collection, and preliminary data analysis. LS

supported experimental execution, data validation, and manuscript editing. DD contributed to supervision of the study, interpretation of results, and critical review of the manuscript. All authors read and approved the final manuscript. [SSP[1] - Samikshya Sankalini Pradhan; SSP[2] - Saswati Sibani Panda]

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interest to declare.

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AI Declaration

The authors declare that no generative Artificial Intelligence (AI) tools were used in the conception of the research problem, experimental design, data collection, laboratory work, statistical analysis, or interpretation of results presented in this manuscript. Only standard writing-assistance tools like ChatGPT (OpenAI) were used for language improvement and all scientific content, data interpretation and conclusions are entirely the authors' original work. The authors take full responsibility for the integrity, accuracy and authenticity of the manuscript.

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