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Characterization of *Ralstonia solanacearum* (Smith) Yabuuchi inciting bacterial wilt in brinjal (*Solanum melongena* L.)

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Abstract
The present investigation were carried out at the Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.) during 2019-20 and 2020-21. A survey was conducted for collection of bacterial wilt samples on different solanaceous crops from different vegetable growing regions of Chhattisgarh plains. Fourteen isolates of *R. solanacearum* were collected from wilt affected plants of brinjal (11 isolates), tomato (2 isolates) and potato (1 isolate) from different vegetable growing regions of Chhattisgarh. Isolation of the *Ralstonia solanacearum* was done on TZC medium. Virulent and a virulent colonies were differentiated on TZC medium. Bacteria were negative in gram reaction and KOH test. Bacterial isolates were identified on the basis of morpho-cultural and biochemical characterization.

Keywords: Brinjal, bacterial wilt, *R. solanacearum*, pathogenicity, virulence, morpho-cultural, biochemical characterization

Introduction
Bacterial wilt is one of the most destructive diseases of cultivated plants and most widespread in tropical, subtropical and warm temperate regions of the world. The wilt caused by *Ralstonia solanacearum* is wide spread, affecting many solanaceous vegetable crops including brinjal with 10 to 100% incidence of bacterial wilt. This plant pathogenic organisms is regarded as most important due to its lethality, persistence, wide host range, wide variation, broad geographical distribution and long survival in soil. Incidence of bacterial wilt disease is prevalent in areas with acidic soil (pH <7) and humid climates. The disease development is favored by high moisture and temperature. *Ralstonia solanacearum* causes important losses on a large range of economic crops worldwide. Although yield losses vary according to host, cultivars, climate, soil type, cropping practices and pathogens strains (Elphinstone, 2005) [11]. Bacterial wilt disease can destroy entire crop when conditions are favorable. *Ralstonia solanacearum* enters plant roots through wounds and multiplies rapidly in the vascular system. Rapidly multiplying bacterial cells clog the xylem elements and further block the xylematic flow, leading to fast drooping of foliage followed by wilting and eventually plant death. The yield loss in India due to this disease has been estimated up to 10-90 per cent. *Ralstonia solanacearum* is aerobic non spore forming Gram negative plant pathogenic bacterium. It is soil borne and motile with polar tuft of flagella. *R. solanacearum* doesn't behave as single bacterium with uniform biology and host range but as complex of variants such as groups, races, biovars, biotypes, sub races and strains. Isolates of *Ralstonia solanacearum* are also grouped into 6 biovars based on utilization of carbohydrates and organic acid.

Materials and Methods
1. Survey and collection
Survey was carried on the occurrence of *Ralstonia solanacearum* causing bacterial wilt on different solanaceous crops from different vegetable growing regions of Chhattisgarh plains and wilt affected plants *viz.*, brinjal, chilli, tomato and potato plants showing typical symptoms of bacterial wilt were collected. The collected plants were labeled, packed in polythene bags and kept at 4 °C for the purpose of isolation of the causal organism.

2. Isolation of *Ralstonia solanacearum*

Brinjal, chilli, tomato and potato plants showing typical symptoms of bacterial wilt caused by *R. solanacearum* collected from different vegetable growing regions. In field, Bacterial nature of the wilted plants was confirmed by performing bacterial ooze test.

Triphenyl tetrazolium chloride (TZC) medium was used as basal medium for isolation and purification of *R. solanacearum*. Multiplication of *R. solanacearum* was done on Casamino acid Peptone Glucose Agar (CPG) medium. The bacterial suspension obtained from oozing was serially diluted in nine ml sterile distilled water. 100 µl of the diluted bacterial suspension was poured on the surface of solidified triphenyl tetrazolium chloride agar (TZC) medium (Kelman, 1954) [13] in sterilized Petri plates. The bacterial suspension was spread on the surface of TZC medium with a sterilized spreader and also streaked with the help of inoculation needle in other Petri plate containing TZC medium. The inoculated plates were incubated at 28-30°C for 48 hours in BOD incubator. At the end of the incubation period, the plates were observed for the development of both avirulent and virulent colonies of *R. solanacearum*.

3. Purification and preservation of the bacteria isolated from wilted plants

Well isolated typical virulent colonies of *Ralstonia solanacearum* on TZC medium were picked and streaked separately on TZC medium in sterilized Petri plates. The plates were incubated at 28- 30°C for 48 hours in BOD incubator. The well-separated virulent colonies of *Ralstonia solanacearum* were picked up with sterile inoculation loop and suspended in sterile distilled water in sterile propylene culture collection tubes and stored at 4°C in refrigerator for further use· as stock culture.

4. Glycerol stock culture of *Ralstonia solanacearum*

Well separated typical virulent colonies of *Ralstonia solanacearum* were picked with a sterile toothpick and suspended in two milli litre of CPG broth (casamino acid peptone glucose broth without Triphenyl tetrazolium chloride salt) and incubated at 28°C for 36 hours in rotary shaker at 160 rpm. About 850 µl of this bacterial culture of *Ralstonia solanacearum* was pipetted into sterile microfuge tubes and 150 µl of 70 per cent autoclaved glycerol was added and stored at 4°C in refrigerator for further use.

5. Identification and characterization of bacterial isolates

Identification of the bacterial isolates were done based on morph-cultural, biochemical and molecular characterization.

5.1 Morpho -cultural characterization

For identification of bacteria Gram staining and KOH test was performed**.** Various colony characters were recorded for all isolates *in vitro*. The colony characters on TZC media plate were size, pigmentation, form, margin and elevation (Cappuccino and Sherman, 1996). Virulent and avirulent colonies of bacteria were differentiated based on their colony morphology on TZC medium (Kelman, 1954) [13], Nutrient agar media and Mac conkey agar media.

5.2 Biochemical characterization

Biochemical tests *viz*., oxidase test, indole test, starch hydrolysis, gelatin liquefaction, levan formation, arginine dihydrolase production, catalase oxidation, decarboxylase test, triple sugar iron test, motility test, nitrate reductase test and NaCL tolerance @ 1%, 2% and 3% growth at 41ºC temperature were carried out as per the methods described in the Manual of Microbial methods (Anon, 1957) and Laboratory guide for identification of Plant pathogenic bacteria (Schaad, 1992). Antibiotic sensitivity test for bacterium was also performed. Biovars of *R. solanacearum* were determined by their ability to utilize different carbohydrates and organic acids (Hi-media carbo kit).

Result and Discussion

1. Survey and collection

14 isolates of *R. solanacearum* were collected from wilt affected plants of brinjal (11 isolates), tomato (2 isolates) and potato (1 isolate) from different vegetable growing regions of Chhattisgarh plains (Table 1). Bacterial wilt samples from brinjal plants were collected from single location of Bilaspur, Raipur, Durg, Kawardha, Bemetara, Rajnandgaon, Balod districts as well as from two different locations of Balodabajar and Mahasamund districts. Bacterial wilt samples of tomato were collected from Bemetara and Mungeli districts, whereas samples from potato plants were collected from Raipur district. All isolates were assigned with a specific code. Codes assigned to bacterial isolates collected from brinjal *viz.* RS-1, RS-2, RS-3, RS-4, RS-5, RS-6, RS-7, RS-8, RS-9, RS-13 and RS-14; tomato *viz*. RS-10 and RS-12 and potato *viz*. RS-11.

2. Isolation of *Ralstonia solanacearum*

Isolation of the bacterium was made directly from cut stem piece of brinjal, tomato and potato plants showing typical symptoms of bacterial wilt as well as from infected soil samples by serial dilution technique. The samples formed typical virulent colonies of *R. solanacearum* which were observed within 48 hours after inoculation via streaking on 2, 3, 5-tetrazolium chloride medium (TZC medium). Virulent colonies with typical characters like fluidal, irregular slimy, dull white coloured with pink to red coloured center were observed. Whereas, avirulent colonies were non fluidal, round to convex shaped butyrous with red/pink coloured center and regular narrow dull white coloured margins. A typical single colony of *R. solanacearum* was picked and sub culturing of bacterial isolates were done by multiplying in TZC media plates.

Similar to our findings, Champoiseau (2009) [9] also isolated *R. solanacearum* from wilted tomato plants and cultured on TZC media. He distinguished virulent and avirulents isolates of bacteria on TZC media plate. Artal *et al*. (2012) [4] also isolated *R. solanacearum* from freshly wilted tomato, brinjal and chilli plants separately and streaked loopful of flowing bacterial ooze on sterile TTC (Triphenyl Tetrozolium Chloride) agar plate and incubated at 30 °C for 48 to 72 hours. Isolation of *R. solanacearum* were also done by Ahmed *et al*. (2013) [2] from the wilted potato plants on nutrient agar media and further purified by streaking a single colony of each isolate on Triphenyl Tetrazolium Chloride (TTC) plate.

3. Identification and characterization of bacterial isolates

3.1 Morpho -cultural characterization

Result presented in Table 2(a) revealed that, all the tested 14 isolates of *R. solanacearum* i.e. RS-1, RS-2, RS-3, RS-4, RS-5, RS-6, RS-7, RS-8, RS-9, RS-10, RS-11, RS-12, RS-13, RS-14 did not retain violet color and showed pink to light red coloured cells under microscopic observation. Bacteria were straight or curved rod shaped under microscope which is the characteristic feature of plant pathogenic bacteria. Hence, that all isolates were negative

for Gram's reaction. Gram negative reaction of *R. solanacearum* isolates were further subjected to KOH test for confirmation. Strands or thread like viscous material were observed when bacterial solution were raised upto few centimeter through the wire loop from glass slides. Hence, the bacterium was Gram negative. Similar, results in gram's staining reaction and KOH test were obtained by Rahman *et al.* (2010) [20], Chakravarty and Kalita (2011) [8], Chaudhry and Rashid (2011) [10], Ahmed *et al*. (2013) [2], Seleim *et al*. (2014) [22], Pawaskar *et al*. (2014) [18], Mulani and Siraj (2015) [16], Shahbaz *et al*. (2015) [23], Popoola *et al*. (2015) [19], Khasabulli *et al*. (2017) [14], Bannihatti *et al*. (2019) [5] and Hosssain *et al*. (2021) [12].

Result presented in table 1(a) revealed that, all isolates obtained from brinjal were similar with respect to morpho-cultural characteristics. Virulent colonies appeared on TZC media by some isolates obtained from brinjal *viz.* RS-1, RS-3, RS-4, RS-6, RS-8 and RS-14, whereas other isolates remained avirulent *viz.* RS-2. RS-5, RS-7, RS-9 and RS-13. Virulent isolates formed fluidal, viscous, slimy colonies with dull white coloured irrregular margin along with dark red center (RS-1), pink coloured center (RS-4 and RS-6), pink to red center (RS-4 and RS-8), pink to dark red coloured center (RS-14) on TZC media. Avirulent isolates of brinjal appeared as convex shaped slimy colonies having regular elevated dull white coloured margins with pink to red center (RS-2), dark red center (RS-5), pink coloured center (RS-7, RS-9 and RS-13) on TZC media.

Virulent isolates of tomato and potato i.e. RS-10 and RS-11, respectively were appeared as fluidal, viscous, slimy colonies with irregular dull white coloured margin as well as dark red to reddish brown coloured center on TZC media. Avirulent isolate of tomato produced convex shaped slimy colonies with regular dull white coloured margin and pink to red center coloured center on TZC media.

In case of growth pattern (Table 2. b), isolate RS-1, RS-3, RS-4, RS-5, RS-8, RS-10 and RS-14 showed comparatively fast growth, whereas isolate RS-2, RS-6 and RS-11 showed moderate growth and slow colonial growth was observed in isolate RS-7, RS-9, RS-12 and RS-13. Colony size produced by each isolates were also observed, five isolates namely RS-2, RS-4, RS-7, RS-9 and RS-13 formed large colonies, whereas moderate colonies were formed by isolate RS-3, RS-5, RS-6, RS-8 and RS-14. Isolates RS-10, RS-11 and RS-12 produced small colonies on TZC media. All isolates formed small, round, dull white coloured, mucoid, translucent and slightly raised surface colonies on Nutrient agar media.

In corroboration to the findings of present investigation, Kelman (1954) [13] also studied the colony morphology of *R. solanacearum* and observed irregular round, smooth, fluidal and opaque colonies by virulent isolates on TZC media. Rahman *et al.* (2010) [20], Ahmed *et al*. (2013) [2] and Mulani and Siraj (2015) [16] distinguished appearance of virulent and avirulent colonies of *R. solanacearum* on Triphenyl Tetrazolium Chloride (TTC) medium. Pawaskar *et al*. (2014) [18] observed smooth, circular, raised and dirty white bacterial colonies of *R. solanacearum* on nutrient agar(NA) medium.

3.2 Biochemical characterization

Result presented in Table 3(a) revealed that, all *R. solanacearum* isolates tested positive for oxidase test, levan production, catalase oxidation test and motility test. All isolates of test bacterium were negative for indole production, starch hydrolysis, Gelatin liquefecation and growth at 41ºC. All isolates were tolerant to sodium chloride concentration upto 1%. Whereas its growth was inhibited at 2% and 3% concentration of sodium chloride. Among all tested isolates of *R. solanacearum*, isolate RS-12, RS-13 and RS-14 considered as positive for Arginine dihydrolase reaction. Isolate RS-13 and RS-14 tested positive for both arginine as well as ornithine decarboxylation (Table 3.b). Whereas, isolate RS-9 as well as RS-10 tested positive for ornithine decarboxylation and isolate RS-12 gave positive reaction for arginine decarboxylation. In Triple sugar iron test (Table 3.c), none of the isolates were able to ferment glucose, sucrose and lactose except isolates RS-2, RS-13 and RS-14 which were fermented all three sugars. Gases like CO_2 and H_2 were produced by isolates RS-3, RS-4, RS-10, RS-12 and RS-13. Except isolate RS-12 all other isolates produced H_2S gas. Most of the isolates tested positive for nitrate reduction except isolates RS-2, RS-5 and RS-12, which remained negative. Antibiotic sensitivity test revealed that (Table 3.d), bacterial isolates RS-1, RS-2, RS-3, RS-4, RS-6, RS-8, RS-9, RS-12, RS-13 and RS-14 were resistant on both antibiotics i.e. Kanamycin and Carbenicillin, whereas isolate RS-7 and RS-11 were sensitive to both antibiotics. Isolate RS-5 and RS-10 showed resistance to Kanamycin but remained sensitive to Carbenicillin.

Results of biochemical tests were in agreement with the findings of earlier workers *viz*., Bhide (1948) [6], Rahman *et al.* (2010) [20], Chakravarty and Kalita (2011) [8], Chaudhary and Rashid (2011) [10], Murthy and Srinivas (2012) [17], Ahmed *et al*. (2013) [2], Makhlouf and Hamedo (2013) [15], Seleim *et al*. (2014) [22], Shahbaz *et al*. (2015) [23], Khasabulli *et al*. (2017) [14], Verma *et al.* (2017) [24], Anitha *et al.* (2018) [3], Bannihatti *et al*. (2019) [5], Hossain *et al.* (2021) [12] and Zaoti *et al*. (2018) [25].

Tables 1: Survey and collection of bacterial wilt samples from different vegetable growing regions of Chhatisgarh plains

S. No.	Location of survey (village)	lattitude and longitude	Districts	Host species (Common name)	Host species (Botanical name)	Isolate Code
1	Sendari	21.96208, 81.87075	Bilaspur	Brinjal	*Solanum melongena*	RS-1
2	IGKV Farm	21.23320, 81.71105	Raipur	Brinjal	*Solanum melongena*	RS-2
3	Sirsakala- Bhilai	21.19680, 81.48206	Durg	Brinjal	*Solanum melongena*	RS-3
4	Lohara	21.83351, 81.12553	Kawardha	Brinjal	*Solanum melongena*	RS-4
5	Bhinoda	21.61460, 82.86523	Balodabajar	Brinjal	*Solanum melongena*	RS-5
6	Dhansir	21.56202, 82.71067	Balodabajar	Brinjal	*Solanum nigrum*	RS-6
7	Kisdi	21.24847, 83.07189	Mahasamund	Brinjal	*Solanum melongena*	RS-7
8	Jhilmila (Saraipali)	21.31591, 83.02473	Mahasamund	Brinjal	*Solanum melongena*	RS-8
9	Umariya	21.21100, 81.89097	Bemetara	Brinjal	*Solanum melongena*	RS-9
10	Saja	21.66387, 81.31318	Bemetara	Tomato	*Lycopersicon esculentum*	RS-10
11	IGKV Farm	21.23537, 81.70844	Raipur	Potato	*Solanum tuberosum*	RS-11
12	Padampur	22.09319, 81.87036	Mungeli	Tomato	*Solanum melangena*	RS-12
13	Dundera	21.14667, 80.80435	Rajnandgaon	Brinjal	*Solanum nigrum*	RS-13
14	Dondi	20.49000, 81.08459	Balod	Brinjal	*Solanum melongena*	RS-14

Table 2(a): Morpho- cultural characterization of different *Ralstonia Solanacearum* isolates

Isolates	Morpho- cultural characterstics			Gram staining Test	KOH Test
	Colony characters on TZC medium	Shape	Occurrence		
RS-1	Fluidal, viscous, slimy, irrregular margin with, dull white coloured with dark red center and dense colonies	Small rods	Single	-Ve	+Ve
RS-2	Convex shaped, regular elevated margins, dull white coloured with pink to red center, slimy colonies	Small rods	Single	-Ve	+Ve
RS-3	Irregular margins, dull white coloured with pink center, slimy, fluidal colonies	Small rods	Single	-Ve	+Ve
RS-4	Irregular margins, dull white coloured with pink to red center, fluidal, slimy colonies	Small rods	Single	-Ve	+Ve
RS-5	Convex shaped, regular margins, dull white coloured with dark red center, fluidal, slimy, dense colonial growth	Small rods	Single	-Ve	+Ve
RS-6	Irregular margins, dull white coloured with pink center, fluidal, slimy colonies	Small rods	Single	-Ve	+Ve
RS-7	Convex shaped, regular margin, viscous, slimy, dull white coloured with light pink center, slimy colonies	Small rods	Single	-Ve	+Ve
RS-8	Irregular dull white coloured margin with red to pink coloured center, fluidal, viscous, slimy colonies	Small rods	Single	-Ve	+Ve
RS-9	Convex shaped, regular dull white coloured margin with pinkish center, smooth textured colonies	Small rods	Single	-Ve	+Ve
RS-10	Irregular margin, dull white coloured with dark red center, fluidal, viscous, slimy colonies	Small rods	Single	-Ve	+Ve
RS-11	Irregular margin, dull white coloured with reddish brown center, viscous, slimy colonies	Small rods	Single	-Ve	+Ve
RS-12	Convex shaped with regular margin, slimy, dull white coloured with pink to red colonies	Small rods	Single	-Ve	+Ve
RS-13	Convex shaped with regular margin, dull white coloured with pink center, slimy colonies	Small rods	Single	-Ve	+Ve
RS-14	Irregular margin, dull white coloured with pink to dark red center, fluidal, viscous, slimy colonies	Small rods	Single	-Ve	+Ve

Table 2(b): Morpho- cultural characterization of different *Ralstonia solanacearum* isolates

Isolates	Morpho-cultural Characterisation	
	Growth pattern	Colony size
RS-1	Fast growth	Medium
RS-2	Moderate growth	Large
RS-3	Fast growth	Medium
RS-4	Fast growth	Large
RS-5	Fast growth	Medium
RS-6	Moderate growth	Medium
RS-7	Slow growth	Large
RS-8	Fast growth	Medium
RS-9	Slow growth	Large
RS-10	Fast growth	Small
RS-11	Moderate growth	Small
RS-12	Slow growth	Small
RS-13	Slow growth	Large
RS-14	Fast growth	Medium

Table 3(a): Biochemical characterization of different *R. solanacearum* isolates

Isolates	Biochemical characteristics													
	Oxidase test	Indole test	Starch hydrolysis test	Gelatin liquefaction test	Levan formation test	Arginine dihydrolase production test	Catalase Oxidation test	Urease test	Motility test	Nitrate reductase test	Growth at 41ºC	NaCl tolerance @1%	NaCl tolerance @2%	NaCl tolerance @3%
RS-1	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve
RS-2	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve
RS-3	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve
RS-4	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve
RS-5	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve
RS-6	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve
RS-7	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve
RS-8	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve
RS-9	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve
RS-10	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve
RS-11	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve
RS-12	+ve	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	+ve	-ve	-ve
RS-13	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve
RS-14	+ve	-ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve

Table 3(b): Decarboxylase test

Test isolates	Result Interpretation		
	Lysine decarboxylation	**Arginine decarboxylation**	**Ornithine decarboxylation**
RS-1	-ve (yellow colour broth)	-ve (yellow colour broth)	-ve (yellow colour broth)
RS-2	-ve (yellow colour broth)	-ve (yellow colour broth)	-ve (yellow colour broth)
RS-3	-ve (yellow colour broth)	-ve (yellow colour broth)	-ve (yellow colour broth)
RS-4	-ve (yellow colour broth)	-ve (yellow colour broth)	-ve (yellow colour broth)
RS-5	-ve (yellow colour broth)	-ve (yellow colour broth)	-ve (yellow colour broth)
RS-6	-ve (yellow colour broth)	-ve (yellow colour broth)	-ve (yellow colour broth)
RS-7	-ve (yellow colour broth)	-ve (yellow colour broth)	-ve (yellow colour broth)
RS-8	-ve (yellow colour broth)	-ve (yellow colour broth)	+ve (Purple colour broth)
RS-9	-ve (yellow colour broth)	-ve (yellow colour broth)	+ve(Purple colour broth)
RS-10	-ve (yellow colour broth)	-ve (yellow colour broth)	+ve(Purple colour broth)
RS-11	-ve (yellow colour broth)	-ve (yellow colour broth)	-ve (yellow colour broth)
RS-12	-ve (yellow colour broth)	+ve(Purple colour broth)	-ve (yellow colour broth)
RS-13	-ve (yellow colour broth)	+ve(Purple colour broth)	+ve(Purple colour broth)
RS-14	-ve (yellow colour broth)	+ve(Purple colour broth)	+ve(Purple colour broth)

Table 3(c): Carbohydrate fermentation, CO_2, H_2 and H_2S gases Production by different isolates of *R. solanacearum* in Triple suagr iron test

Test result	***R. solanacearum* isolates**														
	RS-1	**RS-2**	**RS-3**	**RS-4**	**RS-5**	**RS-6**	**RS-7**	**RS-8**	**RS-9**	**RS-10**	**RS-11**	**RS-12**	**RS-13**	**RS-14**	**Control**
Alkaline slant(red) / Alkaline butt(red) K/K	✓	×	×	✓	×	×	✓	×	×	×	✓	✓	×	×	✓
Alkaline slant(red) / acid butt(yellow) K/A	×	×	✓	×	✓	✓	×	✓	✓	✓	×	×	×	×	×
acid slant(yellow)/ Alkaline butt(red) A/K	×	×	×	×	×	×	×	×	×	×	×	×	×	×	×
Acid slant (yellow) / acid butt (yellow) A/A	×	✓	×	×	×	×	×	×	×	×	×	×	✓	✓	×
Bubbles or cracks	×	×	✓	✓	×	×	×	×	×	✓	×	✓	✓	×	×
Black precipitate	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	×	✓	✓	✓

Table 3(d): Antibiotics sensitivity test

Isolates	Antibiotics	
	Kanamycin	**Carbenicillin**
RS-1	Resistance	Resistance
RS-2	Resistance	Resistance
RS-3	Resistance	Resistance
RS-4	Resistance	Resistance
RS-5	Resistance	Sensitive
RS-6	Resistance	Resistance
RS-7	Sensitive	Sensitive
RS-8	Resistance	Resistance
RS-9	Resistance	Resistance
RS-10	Resistance	Sensitive
RS-11	Sensitive	Sensitive
RS-12	Resistance	Resistance
RS-13	Resistance	Resistance
RS-14	Resistance	Resistance

Summary and Conclusion

Present survey indicated that the bacterial wilt depicted as major issue in production of brinjal and other solanaceous crops in vegetable growing regions of Chhatishgarh plain i.e. Raipur, Bilaspur, Rajnandgaon, Mahasamund, Balodabazar, Bemetara, Kawardha, Mungeli and Balod district. Bacteria were isolated from wilt affected brinjal, tomato and potato plants and cultured on 2, 3, 5-tetrazolium chloride medium (TZC) and Virulent as well as avirulent colonies were differentiated. Bacterial isolates were identified on the basis of morpho-cultural and biochemical characterization. All 14 isolates *R. solanacearum* were rod shaped, Gram negative, non-capsulated, non-spore forming and appeared as single colonies on TZC medium. Brinjal isolates slightly varied with respect to isolates of tomato and potato on morphological characters.

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