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Cultural and Morphological Characterization of *Ralstonia solanacearum* causing Chilli Bacterial Wilt

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ABSTRACT: In the coastal vegetable belt of Odisha, *Ralstonia solanacearum*-caused chilli bacterial wilt is a fairly well-known disease. It causes around 100 percent yield loss in the farmer's field. The condition is extremely complex, and numerous physical, cultural, chemical, and biological approaches have been tried and failed to control it. The purpose of this study is to determine the diversity of the various isolates and their chances of survival. Five isolates were obtained for this study from the Odisha coastal vegetable region. RS 1, RS 2, RS 3, RS 4, and RS 5 were the names given to the various isolates. They were also examined for diversity studies, including cultural and physical differences. They were all evaluated for colony growth in various growth medium, and it was discovered that TZC media and CPG media were the best. *Ralstonia solanacearum*'s cultural traits, including colony color, colony count, and colony form, were examined in vitro using the four-culture media. The TZC agar plate had the highest average colony count (72.00), which featured white fluidal colonies with pink spherical centres, and the lowest average colony count (56.00), which featured irregular yellow colonies. From this study, we got an idea about the diversity among various isolates of *Ralstonia* which in turn will help to manage this particular problem efficiently.

Keywords: *Ralstonia solanacearum*, culture media, Chilli, Triphenyl tetrazolium chloride, Nutrient Agar, Mac conkey agar, CPG agar.

INTRODUCTION

Several diseases like damping off, Fusarium wilt, Cercospora leaf spot, Alternaria leaf spot and bacterial wilt infect chilli crop worldwide (Dhaliwal, 2015). Moreover, bacterial wilt, one of the most infectious disease which is very widely distributed among chilli growing tracts of the world (Hayward, 1991; Denny, 2006). The said disease is largely seen in the coastal regions of India. The pathogen *Ralstonia solanacearum* (Yabuuchi *et al*., 1995) causing same disease of chilli. This particular disease is ranked one as the most notorious plant diseases worldwide. It is responsible for100 per cent yield loss in almost all solanaceous vegetables (Jyothi *et al*., 2012). First signs of the disease are wilting of new leaves mostly during the hottest part in the day and come back as normal during cooler hours (Ahmed *et al*., 2022). With high temperature as well as rainfall condition, whole plant exhibit wilting and suddenly the plant death occurs (Cerkauskas, 2004). Most of the time, in the starting stage of the disease, plant leaves remain green in most of the cases (Manda *et al*., 2020).

Chilli bacterial wilt caused by *Ralstonia solanacearum* is a very notorious disease in the coastal vegetable belt of Odisha. The disease is highly intricate and many physical, cultural, chemical and biological control methods have been tested to manage it with all in vain. Moreover, the resistant varieties of chilli are failing to manage the disease due to unknown reason specific to the abovesaid part of Odisha. Considering this problem, the present investigation was undertaken. This study is targeted to find out the diversity among the various isolates and its survival on the basis of morphological as well as cultural characteristics.

MATERIAL AND METHODS

Isolation of the pathogen. Chilli wilted plants from the field were gathered and subjected to isolation on a specific media. Vascular discolored tissues were cut into small, small pieces and finally put in the beaker containing distilled water which is sterilized earlier.

After five minutes, its been observed that a white small thread of fluid was coming down from the cut end of the injured-stem. It acted as a confirmatory test for bacteria presence. 1 ml of the above-said suspension was then discharged into previously sterilized Triphenyl tetrazolium chloride agar (TZC) agar plates. Further, they are perfectly allowed to solidify at room temperature i.e. around 28°C for the next forty-eight hours. After incubation period completed, the media containing petriplates were observed for the advancement of the colonies of *Ralstonia* and thus been said virulent as well as avirulent bacterial colonies. After this advancement, bacterial virulent colonies were streaked into the above said media containing petriplates for pure culture development. Slight Creamy white colonies with pink center observed on TZC medium. These colonies were further transferred to five ml of sterile water containing 16 cm^3 Eppendorf tubes and been used as stock suspensions for future use.

Cultural characteristics. To investigate the effects of colony color, colony count, and colony form on various culture medium, a total of four culture media were used. The four-test media were autoclaved for 15 minutes at 15 $lbs/inch^2$ pressure before being chilled. All media were additionally poured (at a rate of 20 ml per plate) into sterile Petri plates (90 mm in diameter), where they were then left to harden at room temperature. 0.1ml of a bacterial culture that has been in existence for 48 hours, taken with the aid of a micropipette, placed in the middle of a solidified media, and spread evenly to reach bacterial colonies that are well-developed. After that, infected plates were incubated at 28°C for 24, 48, 72, and 96 hours to see if the bacteria continued to thrive. Characteristics of the colonies, such as their number, color, and shape, were noted.

RESULTS AND DISCUSSION

A. Isolation and purification of R. solanacearum isolates

Chilli plants exhibiting typical symptoms of bacterial wilt such as loss of turgidity of the leaves, further advanced as drooping of the leaves with sudden wilting were collected from vegetable belts of Odisha. The infected plants exhibited brownish discoloration of roots. Presence of bacterial pathogen was detected through ooze test (Fig. 1.). Milky white bacterial ooze was released from the cut ends placed in sterile water. Bacterial colonies of all five isolates were cultured on TZC agar medium where the colonies were well separated and irregular with smooth and white margin, and characteristic pink center on TZC media. All five isolates used in the present study showed the typical characteristics of virulent strains which is round to irregular, highly fluidal with white margin with pinkish center. The five isolates were collected from different blocks during the survey on the basis of wilt incidence for further studies on the basis of distinctiveness were further given name RS1, RS2, RS3, RS4 and RS5 respectively.

Fig. 1. Ooze test for collected bacterial wilt chilli plants**.**

Fig. 2. Bacterial ooze streaming from the chilli stem.

Characterization of bacterial isolates. The bacteria isolated from different hosts were identified as per the standard microbiological procedures on the basis of morphological and cultural characteristics.

Cultural and morphological Characteristics. Cultural and morphological characteristics of *R. solanacearum* were evaluated on different culture media. Significant variation has been observed in all culture media for bacterial colonies**.** The influence of media on the growth of *Ralstonia* was tested by using four culture media under *in-vitro* conditions. The media used were nutrient agar medium, tetrazolium chloride agar medium, casamino acid-peptone-glucose agar (cpg) medium and mac-conkey agar media.

Table 1: Cultural characteristics of *Ralstonia* in NA media.

Sr. No.	Isolates	Morphology on NA media
1.	RS1	Small, smooth, circular colony, creamy white, round colonies
2.	RS2	Spherical, dull white colonies with translucent, slightly raised surface
3.	RS3	Round, convex, slightly raised surface
4.	RS4	Small, smooth, circular colony, creamy white, round colonies
5.	RS5	oval with smooth margin, convex, translucent, slightly raised surface

On Nutrient agar (NA) medium. The bacterial colonies of all the isolates of brinjal (RS1, RS2, RS3, RS4 and RS5) showed very small, smooth, circular colonies with creamy white, slightly mucoid, translucent, and raised surface on nutrient agar medium.

On Tetrazolium chloride agar medium. The bacterial colonies of all the isolates of brinjal (RS1, RS2, RS3, RS4 and RS5) showed fluidal, irregularly round, creamy, convex, dull white colonies with red coloured center on TZC media.

On Casamino acid peptone glucose medium. Bacterial Colonies of *R. solanacearum* isolates formed on CPG medium were white, round, slightly yellow and opaque and dry.

Table 2: Cultural characteristics of *Ralstonia* on TZC media.

Sr. No.	isolates	Morphology on TZC media
1.	RS1	Convex, Spherical, pink centered with cream border colonies
2.	RS2	dull white colonies, Spherical, pink at centre
3.	RS3	convex, Spherical, pink centered with cream border colonies
4.	RS4	dull white colonies, Spherical, pink at centre
5.	RS5	convex, creamish colony with light pink centre, oval with smooth margin

Table 3: Cultural characteristics of *Ralstonia* on CPG media.

Sr. No.	Isolates	Morphology on CPG media
1.	RS1	Irregularly round, dry, slightly yellow colour
2.	RS2	Cream coloured irregularly round, dry, slightly yellowcolour
3.	RS3	Irregularly round, dry, slightly yellow colour
4.	RS4	white coloured, round, dry, slightly yellow colour
5.	RS5	Irregularly round, dry, slightly yellow colour

Table 4: Cultural characteristics of *Ralstonia* on Macconkey agar media

Sr. No.	Isolates	Morphology on Macconkey agar media
1.	RS1	white coloured, irregularly round, dry and small colonies
2.	RS2	Cream coloured irregularly round, dry and small colonies
3.	RS3	White, irregularly round, dry and small colonies
4.	RS4	Small colonies, round, dry, slightly yellow
5.	RS5	white coloured, irregularly round, dry and small colonies

Table 5: Growth of *R. solanacearum* on different culture media.

Treatment no.	Treatment	Mean bacterial colonies per plate (cfu)			
		24hr	48hr	72hr	96hr
T_1	Triphenyl Tetrazolium Chloride Agar (TZC)	58.00	64.00	68.00	72.00
T_2	Casamino Peptone Glucose Agar (CPG)	46.00	54.00	66.00	69.00
T_3	Nutrient agar(NA)	37.00	43.00	52.00	57.00
T_4	Macconkey agar (MCA)	33.00	45.00	53.00	56.00
	S.E. (m) ±	2.96	2.29	2.53	1.97
	CD (α= 0.01)	9.65	7.47	8.24	6.43

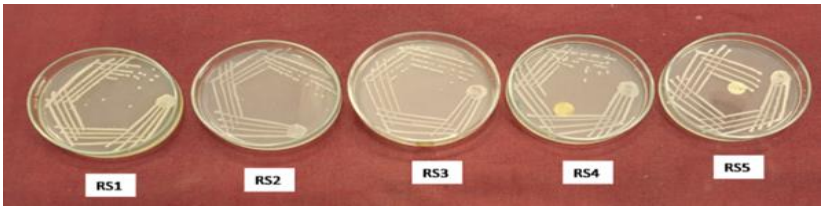

Fig. 3. Bacterial colony on Nutrient Agar media.

Fig. 4. Bacterial colony on TZC media.

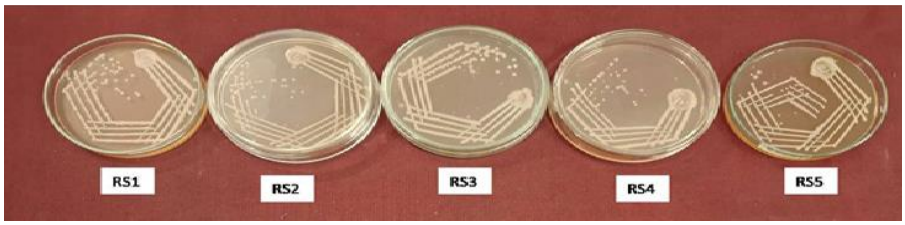

Fig. 5. Bacterial colony on CPG media.

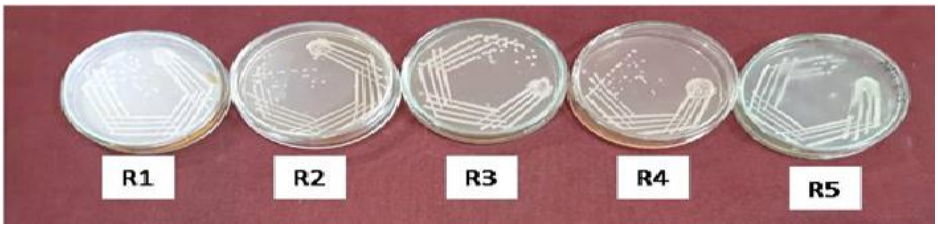

Fig. 6. Bacterial colony on Macconkey agar media.

On Mac conkey agar medium. Bacterial colonies of all isolates of chilli cultured on Mac conkey agar media were Round colonies, rough, opaque, slightly raised at the centers and dry.

Colony count. The findings were shown in Table 5, which revealed that the average colony count across all test media ranged from 56.00 (Mac conkey agar) to 72.00 (Triphenyl tetrazolium chloride). Triphenyl tetrazolium chloride agar media had the highest average colony count (72.00), followed by Casamino peptone glucose agar (69.00), Nutrient agar (57.00), and Mac conkey agar (56.00). Therefore, it was discovered that TZC agar media at 96 hours was best for the bacterial colony growth.

Colour of the colonies. Color of bacterial colonies were greatly influenced by culture media. The results of colony color study depicted in Table 1-4 reveals that on Triphenyl tetrazolium chloride agar media, the colonies were white, fluidal with a pink center. On Casamino peptone glucose agar (Table 3), the bacterial colonies were creamy white to off white. With little variation, the colonies were creamy and dull white on nutrient agar and white on Macconkeyagar media.

Colony Shape. Results from Tables 1-4 showed that circular, small-shaped colonies were created on the remaining test media, such as Nutrient Agar and MacConkey Agar Medium, whereas smooth, extremely fluidal, irregular-shaped colonies were developed on Triphenyl Tetrazolium Chloride (TZC) and Casamino Peptone Glucose (CPG).

In an experiment, Bannihatti *et al*. (2018) examined the cultural traits of isolates of *R. solanacearum* in various media. They used test media such as Triphenyl Tetrazolium Chloride Agar (TZC), Yeast Extract Milk Agar (YEMA), Casamino Peptone glucose Agar (CPG), Potato Dextrose Agar (PDA), Yeast Extract Peptone Agar (YPA), Yeast Extract Agar (YEA), Nutrient Agar (NA), and Yeast Extract Chalk Agar (YEA), and they discovered that the TZC agar and SMSA media; cream or off-white colored colonies on CPG agar, Potato dextrose agar and Yeast extract agar; creamy white and dull white colonies on NA media, Yeast extract milk agar Yeast extract peptone agar and yellow colored colonies on Yeast extract chalk agar were created.

After 24 hours of incubation, *Ralstonia* generated fluidal colonies with pink color colonies on TZC medium, according to Rahman *et al*. (2010). When diseased tissues from the patient were cut and submerged in sterile water, milky-white ooze was seen coming from the cut ends (Vanitha *et al*., 2009).

The results closely matched those of Tahat and Sijam (2010), who described *Ralstonia* colonies as white, round, shiny, smooth, and grown on NA media.

Sharma *et al*. (2019) concluded after the same experiment conducted where she found out Colonies of *Ralstonia solanacearum* formed on CPG medium were white or cream-colored, irregularly round, fluidal and opaque which found similar to this experimental result.

CONCLUSIONS

According to the above test results, TZC media is the best media for the growth of *R. solanacearum* among the other media. To justify it TZC media showed highest amount of growth of the bacteria i.e. 72.00.

FUTURE SCOPE

Now-a-days several new synthetic media are coming to facilitate the growth of the said bacterium. These new media can be taken up in the research so that a new media can be justified as the best media for the growth of *R. solanacearum.*

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Conflict of Interest. None.

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