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Physiological and Biochemical Characters of *Ralstonia solanacearum*

Jayesh Pawaskar, M. S. Joshi, Sudhir Navathe* and R. C. Agale
Department of Plant Pathology,
Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth
(Agricultural University), Dapoli, Maharashtra-415712 India
*Corresponding Author's Email: E-mail:sudhir.agro123@gmail.com

***Abstract* – Ralstonia *solanacearum* causes bacterial wilt of *solanaceous* crop plants a most devastating disease in humid tropic. Bacterium from roots of wilted plants was isolated on nutrient agar medium. It was identified as *Ralstonia solanacearum* on the basis of morphology as well as molecular marker basis. *R. solanacearum* was small straight rod shaped, measuring 1.5–3.12µ X 0.25–2.5µ and Gram negative in reaction with KOH positive test. The colonies of *R. solanacearum* on nutrient agar medium were smooth circular, raised and dirty white. The bacterium was visible in the form of thin pellicle on the surface of nutrient broth in 24 hrs. Later on, the growth became a little thick and medium became turbid with putrefactive odour. The bacterium was positive in acid and gas production tests and negatively responded to starch hydrolysis, hydrogen sulphide production and cellulose decomposition tests.**

***Keywords* – Chilli, Ralstonia, Wilt, Biochemical Characters.**

I. INTRODUCTION

Ralstonia solanacearum, the causal agent of bacterial wilt disease, is a severe obstacle to the production of solanaceous plants in both tropical and temperate regions. As a diverse species complex, R. solanacearum has developed an extremely broad host range throughout the world, including >450 host species representing 54 plant families (Wicker et al. 2007). The bacterium normally invades plant roots from the soil through wounds or natural openings, colonizes the intercellular space of the root cortex and vascular parenchyma, and eventually enters the xylem vessel and spreads up into the stem and leaves. Affected plants suffer chlorosis, stunting, wilting, and usually die rapidly.Among all these diseases bacterial wilt caused by *Ralstonia solanacearum* (Smith), is one of the most serious diseases of chilli. The pathogen exhibits wide variability and diversity. *Ralstonia solanacearum* was formerly known as *Pseudomonas solanacearum*, causing wilt on wide range of solanaceous crops(chilli, tomato, brinjal, and potato), peppers (*Capsicum*) and also bitter gourd and beans. The bacterial wilt caused by *Ralstonia solanacearum*is the most common disease of solanacious crops including chilli occurring in all parts of chilli growing region. The disease is becoming the major hurdle in successful cultivation of solanacious crops particularly chilli and brinjaland hence it was felt to conduct the studies on different physiological and biochemical characteristics of the causal bacterium.

II. MATERIALS AND METHODS

Whole experiment was carried out under *in vitro* conditions at

A. Physiological studies of Ralstonia solanacearum

Simple staining: Fresh pure culture of the bacterium was stained by Ziehl'scarbolfuchsin as recommended in the 'Manual of microbiological methods, 1957'. The slides were examined under microscope for determination of size, shape and arrangement of cells.

Gram staining: The young culture was stained by Kopeloff and Beerman's modified method of Gram staining (Anonymous, 1957).

KOH Test: On a glass slide a loopful of bacterial culture from a week old colony was mixed with a drop of 3 per cent aqueous KOH solution for 5 seconds with the help of a toothpick. The toothpick was then raised for a few centimeters from the glass slide and observed for formation of strands of viscid material for confirmation of Gram reaction.

Cultural studies of the bacterium: Cultural characteristics of the bacterium such assize and shape of colonies, growth on nutrient agar(NA) and in nutrient broth and mortality on stab culture were studied by following the standard procedures.

B. Biochemical characteristics of R. solanaearum

Acid and gas production: Acid production was tested by inoculating the culture on nutrient broth containing 2 per cent glucose, adjusted to pH 7.0. The tubes were incubatedfor 7 days at room temperature (28 ± 1^0C). The production of acid was tested by adding few drops of methyl red indicator. A distinct pink or red colour indicated the presence of acid.

The ability of the culture to produce gas was tested by growing the organism in nutrient broth containing 2 per cent glucose. The medium was distributed in test tube containing inverted Durham's tube. These were sterilized by autoclaving at 15 lbs psi for 20 minutes. The tubes were inoculated with 0.5 ml of bacterial suspension and incubated at room temperature (28 ± 1 ^{0}C) for seven days. Gas production was indicated by air bubbles in the inverted Durham's tube.

Starch hydrolysis: The ability of bacterium to hydrolyse starch was studied by growing on nutrient agar containing one per cent soluble starch. The sterilized liquefied nutrient agar was poured to sterilize Petri plates and allowed to solidify. The culture was inoculated in the center of the plates and incubated for seven days at room temperature (28 ± 1 ^{0}C). The plates were then flooded with

Lugol's iodine (Iodine 1g, potassium iodide 2 g and distilled water 300 ml.). Clear zone around bacterial culture indicates positive test.

Production of hydrogen sulphide: The ability of the culture to produce hydrogen sulphide was tested by inoculating the tubes of nutrient broth containing 3 per cent additional peptone. The tubes were sterilized at 15 lbs psi for 20 minutes. Filter paper strips were soaked in super saturated solution of lead acetate and then dried was inserted in the tubes with cotton plug, at the time of inoculation taking care that the strips do not touch the medium. The tubes were incubated for seven days at room temperature (28 ± 1 ^{0}C). The blackening of strips indicates the production of hydrogen sulphide gas.

Cellulose decomposition: The ability of bacterium to decompose cellulose was tested by inoculating the nutrient broth. The sterilized filter paper strips were immersed in the medium. The tubes were incubated at room temperature (28 ± 1 ^{0}C) for 30 days. Un-inoculated tubes containing filter paper strips served as control. Decomposition of the cellulose is indicated by maceration of the filter paper strips at the site of growth of the bacterium.

III. Results and Discussion

A. Physiological studies of Ralstonia solanacearum:

Morphology and staining reaction of R. solanacearum: The bacterium *Ralstonia solanacearum* was isolated on nutrient agar medium from infected chilli roots and its pathogenicity was confirmed.The results of staining reactions revealed the cells *R. solanacearum*were short straight rods, measuring 1.5–3.12µ X 0.25–2.5µ and Gram negative in reaction. Several researchers like Rath and Addy (1977),Khetmalas (1984), Venkatesh (1988), Chaudhry and Rashid (2011) reported similar morphological and staining reaction of*R. solanacearum*

KOH test of *R. solanacearum* indicated that the bacterium was confirmed as Gram negative as it produced strands of viscid materials on treating bacterial culture with 3 % KOH on glass slide.Chaudhry and Rashid (2011) also reported similar results for *R. solanacearum* grown on nutrient agar medium.

Cultural studies: The results of cultural studies revealed that the colonies of *R. solanacearum* on nutrient agar medium were smooth circular, raised and dirty white. The optical feature of the colony was opaque and measured around the average of 3 mm in size.The findings are in close conformity with Stanford and Wolf (1917) who described the colonies of *R. solanacearum* as white, wet, shining, circular, raised and smooth. Khetmalas (1984) and Tahat and Sijam (2010) also recorded similar observations regarding colony characters of *R. solanacearum.*The nutrient agar slants were inoculated with the bacterial culture in a straight line. In the beginning (after 24 hrs.) the growth of *R. solanacearum*was more along the line, filiform, raised and also equally spreading sidewise from line of inoculation. After 2 days, the growth was plain, smooth and dirty white in colour. Stanford and Wolf (1917) described the growth of *P. solanacearum* as filiform and usually spreading at the bottom of the slant. Khetmalas (1984) reported growth of *P. solanacearum* in slant as filiform and rose. Similarly, Das and Chattopadhyay (1955) reported the growth of *P. solanacearum* on nutrient agar slant as umbilicate, rough, dull, filiform, margin entire and opaque.

The growth of the bacterium along the stab was spreading uniformly away from the line of inoculation. There was uniform growth spreading away from the line of inoculation. These results are in confirmation with Khetmalas (1984).

The results of growth of the bacterium in nutrient broth indicated that bacterium was visible in the form of thin pellicle on the surface of nutrient broth in 24 hrs. Later on, the growth became a little thick and medium became turbid. There was also putrefactive odour. The growth after 12-15 days was little yellowish and breaking of pellicle was also seen.The results are in conformation with findings of Patel *et. al.* (1952);Das and Chattopadhyay (1955). Similarly, Rangaswami and Sannegowda (1962) reported growth of *P. solanacearum* from various places in nutrient broth as turbid with ring formation, pellicle formation and flocculation.

B. Biochemical characteristics of R. solanacearum

The results of biochemical studies revealed that when bacterium was grown on nutrient broth containing 2 per cent glucose, it produced acid within eighteen hours of incubation.This result is in agreement with Okabe (1933), Das and Chattopadhyay (1955) and Rath and Addy(1977). They have reported acid production by *P. solanacearum* when medium contains either of dextrose, sucrose, lactose and glycerol. Khetmalas (1984) also reported *P. solanacearum* isolated from groundnut to produce acid when grown in glucose containing medium. These reports indicated that the bacterium has a wide range of enzymes responsible for fermentation of different sugars.

Gas production test indicated that the bacterium (*R. solanacearum*) produced gas from glucose within eighteen hours of incubation.Anonymous (2004) reported *R. solanacearum* positive in gas production with 2 per cent glucose. The result are is also in conformation with Rath and Addy (1977) who reported that *P. solanacearum* produce gas from dextrose, glucose and salicilin. Khetmalas (1984) also reported *P. solanacearum* to be positive in gas production in presence of glucose.

Starch hydrolysis test of the bacterium showed that the bacterium was unable to hydrolyse starch. Anonymous (2004) reported *R. solanacearum* as negative in starch hydrolysis. SimilarlyBhide (1948), He *et al.* (1983), Khetmalas (1984) and Hsu *et al.* (1993) also reported*P. solanacearum* to be negative in starch hydrolysis. However, Das and Chattopadhyay (1955) found the positive results from *P. solanacearum* isolated from brinjal.

Hydrogen sulphide gas production test of *Ralstonia solanacearum* the bacterium was negative in H_2S production. This result is in agreement with the report of Bhide (1948), Das and Chattopadhyay (1955) and Hsu *et al.* (1993); While Rath and Addy (1977) and He *et al.*

(1983) reported *P. solanacearum* to be positive in gas production.

The test for cellulose decomposition was negative as *R. solanacearum* failed to decompose cellulose even after 30 days of incubation.Khetmalas (1984) also reported negative test for cellulose decomposition.

IV. Conclusion

*R. solanacearum*was small straight rod shaped, measuring 1.5–3.12µ X 0.25–2.5µ and Gram negative in reaction with KOH positive test. The colonies of *R. solanacearum* on nutrient agar medium were smooth circular, raised and dirty white. The bacterium was positive in acid and gas production tests and negatively responded to starch hydrolysis, hydrogen sulphide production and cellulose decomposition tests.

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Author's Profile

Mr. Jayesh Pawaskar

B. Sc. in Agriculture and M.Sc. Plant Pathology from Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth (Agricultural University), Dapoli. His area of research at M.Sc. was studies on bacterial wilt disease of chili. He is presently working various research projects by pesticides companies related to product testing in various crops like tomato, onion and maize.

Dr. M. S. Joshi

Associate professor of Plant Pathology atDepartment of Plant Pathology, Dr. BalasahebSawant Konkan Krishi Vidyapeeth (Agricultural University), Dapoli, Maharashtra- 415712 India. He has completed his B. Sc. in agriculture and M.Sc. Plant Pathology from Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth (Agricultural University), Dapoli. He has completed his doctoral studies from Marathwadakrishi Vidyapeeth Parbhani.

He has published more than 50 research articles and popular articles and many radio talks. He has worked with various research projects on paddy, vegetables and fruit crops related to disease management. More than 10 students havecompleted M.Sc. and Ph.D. under his guidance in plant pathology.

Mr. Sudhir Navathe

B.Sc. in agriculture and M.Sc. Plant Pathology from Dr. Balasaheb Sawant Konkan KrishiVidyapeeth (Agricultural University), Dapoli.

He is awarded with gold medal in plant pathology by Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth and INSPIRE RESEACH FELLOWSHIP by DST India. He started his research career with research in cultivation technology of milky white mushroom and oyster mushrooms with innovative technique and further in phytopathology.He has published eight research papers in international and national journals of repute.He has published two papers in national conferences on mushroom biodiversity and production technology. He is actively involved in extension activities too.

Mr. R. C. Agale

has completed B. Sc. in agriculture from Mahatma Phule Krisi Vidyapeeth, Rahuri and M.Sc. Plant Pathology from Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth (Agricultural University), Dapoli. He started his research career by working on Purple blotch disease of onion.

Fig.1. (a) Gas Production by *R. solanacearum*

Fig.1. (b) Acid production by *R. solanacearum*

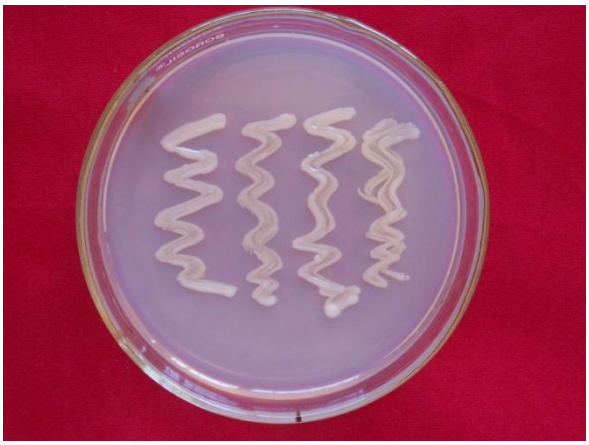
Fig.2. Colony of *R. solanacearum*

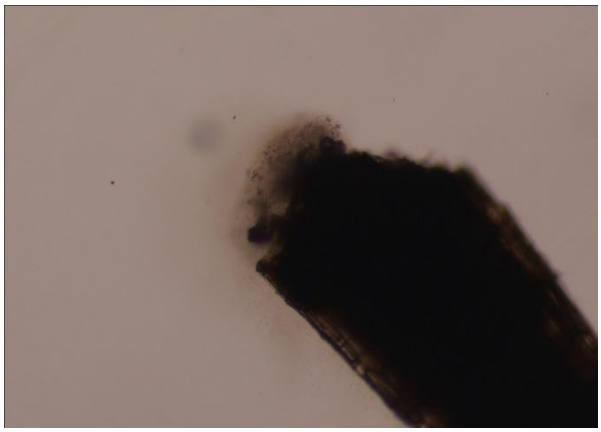
Fig.3.Bacterial oozing from infected cells.