International Journal of Advanced Research in Biological Sciences
ISSN : 2348-8069
www.ijarbs.com

Research Article

Biochemical characterization of the *Ralstonia solanacearum* associated with *Capsicum annum* L. plants in Marathwada Region, Maharashtra

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Abstract

Biochemical characterization of 20 isolates of *Ralstonia solanacearum* isolated from *Capsicum annum* (chilli) from the various locations of in Marathwada region of Maharashtra state. The bacteria were isolated on the TZC (2, 3, 5 tetrazolium chloride) media (Kelman's 1964) and they appeared as small creamy white colonies with pink center. Various type of biochemical tests were performed for the conformation of the bacterial such as solubility in KOH, Arginine Hydrolysis, Nitrate Production, Utilization of Carbohydrate and gelatin liquification, and oxidization of Lactose, Maltose, Ducitol, Sorbitol, Manitol and cellobiose. On the basis of utilization of the sugars the pathogen is classified in different races and biovars.

Keywords: *Ralstonia solanacearum, Capsicum annum (*chilli), triphenyltetrazolium chloride (TTC), Kelman's medium.

Introduction

Ralstonia solanacearum is one of the pathogens that cause disease on at least 200 different plant species in the world. *R. solanacearum* represents a major concern, given that it can seriously affect the production of ornamental plants and economically valuable crops such as tomato, banana, peanut, brinjal, chilli, potato and others. It affects a wide range of plants, including herbaceous plants, shrubs, and trees. Monocots and dicots plants belong to *solanaceae* family including Chilli (*Capsicum annum*), potato (*solanum tuberosum*), brinjal (*solanum melongena*) and tomato (*solanum lycopersicon*) production in India is always threatened by bacterial wilt there are various factors causing wilt disease **(**Elphinstone 2005). It was first reported in Asia and South America in the late 1880's it was firstly described by E.F. Smith (1920).

The present investigation deals with isolation and characterization of the bacterial pathogen and biochemical characterization of the *R. solanacearum*, in chilli (*Capsicum annum*) plant. Sub-spesific categories amongst the isolates were established based on the difference in cultural and biochemical properties. *R. solanacearum* has extremely wide host range (Meng et. al.2013). Different pathogenic varieties (races) within the species, though, may show more restricted host ranges (He et al 1983). Characterization of the *R. solanacearum* are generally based on utilization of disaccharides and hexose alcohols, into biovars and based on host range and hypersensitivity reaction on specific hosts, into races and biovars. (Kelman and Haywards 1964).

Materials and Methods

Isolation of *Ralstonia solanacearum*

From different locations of Nanded district such as Loha, Kandhar, Ardhapur, Bhokar, Biloli, Naigaon,

Narsi, and Hadgaon plants are identified and collected. For the isolation of the bacteria surface sterilization of the infected tissue is carried out by 1% $HgCl_2$ for 1-2 min. The isolation of the pathogenic bacteria carried out on the Kelmen's medium i.e. 2, 3, 5, triphinyltetrazolium chloride and then various biochemical tests were performed for the identification of the bacteria. For the morphological characterization of the bacteria Gram staining and motility test were performed. Series of biochemical tests such as Kovac's Oxidase test, levan production, carbohydrate utilization, Arginine production, Aesculin hydrolysis, Gelatin liquification and Tween 80 lypolysis, Tyrosinase activity and Hydrogen per oxide test were also performed as described by Hayward 1964. All the isolates of *R. solanacearum* show the production of the circular isolated colonies with pink center with entire margin on TZC medium (Kelmen's 1964).

Determination of biovars:

On the basis of the utilization of the disaccharides (Dextrose, lactose, and maltose) and sugar alcohols (mannitol, sorbitol and dulcitol) the bacteria can be differentiated into biovars as described by Hayward (1964). It can be determine by the medium (NH4H2PO4 1.0g, KCl 0.2g, MgSO4.7H2O 0.2g, Difco bacto peptone 1.0g, Agar 3.0g and Bromothymol blue 80.0 mg per litre) with 1% respective sugar. Inoculation of the 48-72 hrs old bacterial suspension into the microtitre plate containing the media and incubate at 28 to 32^0C for 2 to 3 days. Then the tubes showed positive result when colour and pH changes.

Races identification:

The races are differentiated by the pathogenesis test on wild host range as described by the (Schaad *et al.*, 2001). Seedlings of chilli were raised in tray and one month old seedlings were inoculated by soil inoculation method. The incubated plants were then kept in the net house until symptoms development. The results are recorded at regular intervals of time.

Results and Discussion

On the specific Kelman's medium the bacterial colonies were appeared as mucoid oval and fluidal in nature with pink center. The morphological test reveals that the bacteria are short rod and Gram negative in nature after isolation the isolates are further tested for the characterization of biochemical reactions as described by Kelman and Hayward (1964).

Pathogenicity and hypersensitivite reaction (HR) test:

The results of pathogenicity tests of the isolates showed that all the isolates can cause the wilting symptoms on young seedlings of chilli plant. The isolation of the isolates were carried out for the infected chilli leaves and tested for the hypersensitivity reaction on tobacco, It showed all the isolates can cause wilt and in sever condition it can results into the death of the tissue of the tobacco leaves. This results in the accordance with the findings reported by Dhital *et al.* (2001) who observed that *R. solanacearum* was able to produce HR in tobacco leaves.

The 10 isolates were selected for the characterization of biochemical properties collected from different location of Marathwada district. The isolates are coded as RSC1 (Loh), RSC2 (Kan), RSC3 (Nan), RSC4 (Bho), RSC5 (Ard) were collected from Jintur, Kandhar, Nanded, Bhokar, and Ardhapur. The rest of the isolates RSC6 (Bil), RSC7 (Nae), RSC8 (Nar), RSC9 (Nan2) and RSC10 (Had) collected from Biloli, Naigaon, Narsi, Nanded and Hadgaon. Out of which the isolates shows positive results in utilization of carbohydrate and starch hydrolysis. In Levan production isolates RSC5 (Ard) and RSC6 (Bil) shows weakly positivity and rest of which are positive in results where as in Aesculin hydrolysis activity all the isolates shows negative results, as well as in the Kovac's oxidase test all the isolates are negative. The Arginine hydrolysis of the isolates represents positive results as well as in Tween 80 all the isolates are positive. In Gelatin liquification there are RSC2 (Kan), RSC4 (Bho) and RSC9 (Nan2) shows highly positive as compared toRSC1 (Loh), RSC3 (Nan), RSC5 (Ard), RSC6 (Bil), RSC7 (Nae), RSC8 (Nar), and RSC10 (Had). Tyrosinase activity results in positive reaction in all the isolates.

Table 1: Biochemical characterization of different isolates of *Ralstonia solanacearum* isolated from chilli (*Capsicum annum*).

Name of the tests	Name of the isolates									
	RSC1 (Loh)	RSC2 (Kan)	RSC3 (Nan)	RSC4 (Bho)	RSC5 (Ard)	RSC6 (Bil)	RSC7 (Nae)	RSC8 (Nar)	RSC9 (Nan2)	RSC10 (Had)
Oxidase	-	-	-	-	-	-	-	-	-	-
Utilization of carbohydrate	+	+	+	+	+	+	+	+	+	+
Levan production	+	+	+	+	±	±	+	+	+	+
Arginine hydrolysis	+	+	+	+	+	+	+	+	+	+
Aesculin hydrolysis	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+
Tween 80	+	+	+	+	+	+	+	+	+	+
Tyrosinase activity	+	+	+	+	+	+	+	+	+	+
Gelatin liquification	+	++	+	++	+	+	+	+	++	+
Hydrogen peroxide test	+	+	+	+	+	+	+	+	+	+

"-" = negative, "+" = positive, "++" = strongly positive, "±" = weakly positive

Table 2: Utilization of disaccharides and sugar alcohol by chilli:

Sr.no.\ sugars	RSC1 (Loh)	RSC2 (Kan)	RSC3 (Nan)	RSC4 (Bho)	RSC5 (Ard)	RSC6 (Bil)	RSC7 (Nae)	RSC8 (Nar)	RSC9 (Nan2)	RSC10 (Had)
Dextrose	+	+	+	+	+	+	+	+	+	+
lactose	+	+	+	+	+	+	+	+	+	-
Maltose	-	-	-	-	-	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	-	-
Sorbitol	+	+	+	+	+	+	+	+	+	+
Dulcitol	+	-	+	-	-	-	-	-	+	-

"-ve" = Negative, "+"= Positive

On the basis of the utilization of the sugar by the isolates they are differentiated in the Races and Biovars. All the isolates of the Chilli shows positive results to utilize the Lactose, Dextrose and Maltose. Some variations are recorded in the utilization of the Maltose RSC1 (Loh) RSC2 (Kan) RSC3 (Nan) RSC4 (Bho) and RSC5 (Ard) shows negative rest of the isolates shows positive results. All the isolates shows positive results in the utilization of sugar alcohols such as sorbitol and mannitol whereas in the utilization of the dulcitol RSC1 (Jin), RSC3 (Nan) and RSC9 (Nan2) shows positive whereas rest of the isolates are negative and on the basis of the results all are designated as Biovar III (He. et al 1983). On the other hand, all the control plates of different disaccharides and sugar alcohol remain unchanged. On the hypersensitivity reaction all the isolates produces the symptoms on chilli leaves but dark necrosis appears after 24 hrs incubation at 30^0 C results in the yellowing of the leaves, This was followed by extensive wilting and yellowing of leaves this will concluded as Race 1 (Kumar et. al. 2013).

Conclusion

The isolation on TTC media and morphological tests revealed that the isolates belong to Gram negative short rods. The series of biochemical tests were conducted for the determination of the bacteria isolated from host wilted chilli plant and according to the results of the panel of tests, it is concluded as the isolated *Ralstonia solanacearum* is associated with the Race 1 Biovar 3. The findings of the present study will be useful for designing the study of the population structures of *R. solanacearum* using the molecular approaches with special emphasis on its integrated management.

Acknowledgments

Asrar siraj is grateful to the University Grant Commition, New Delhi for providing grants Moulana Azad National fellowship.

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