

Original Research Article

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Isolation and Characterization of *Ralstonia solanacearum* causing Bacterial Wilt of Potato in Konkan Region of Maharashtra

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ABSTRACT

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The isolation of *R. solanacearum* was carried out by streak plate method on TZC agar medium from bacterial wilted potato plant. The *R. solanacearum* was identified on the basis of different Morpho-biochemical characteristics. The colonies of test bacterium were appeared as, dull white or creamy colour, irregularly round fluidal colonies and opaque with light pink or red center on TZC agar medium. The *R. solanacearum* was Gram negative in reaction and positive for KOH solubility, acid production, starch hydrolysis, catalase test, production of hydrogen sulphide, Motility test and Casein hydrolysis test.

Introduction

The Potato (*Solanum tuberosum* L.) belonging to family Solanaceae is a vitally important starchy food crop of the world, commonly known as the “Poor Man’s Friend”. Potato is the staple food in many parts of the world and is an important part of the world's food supply. The potato crop is infested by many bacterial, fungal, viral, viroidal, nematodal diseases, etc. Bacterial wilt disease is responsible for yield loss in potato upto 70%, in India (Staff, 2018). Bacterial wilt or brown rot is caused by the pathogenic bacterium *Ralstonia solanacearum* (Smith) Yabuuchi. The disease

pathogen has extensively been distributed in tropical, subtropical and some warm temperate regions of the world and is one of the major constraints in the production of potato crop.

R. solanacearum, is a rod shaped, gram negative, non-spore forming β proteobacterium and its size ranges from 0.5 - 0.7 x 1.5 - 2.0 μm , which causes bacterial wilt in more than 200 plant species of many economically important crops. It survives for longer period in soil, water, latently infected plants and can be spread by soil, water, farm equipments and infected plant materials (Hayward, 1991). The bacterial wilt of potato

was noted and reported in India from Pune district of Maharashtra (Cappel, 1892) (Fig. 1). Keeping these facts in mind, the present study focusing on bacterial wilt disease of potato was carried out at the Department of Plant Pathology, College of Agriculture, Dapoli.

Materials and Methods

Collection of disease samples

The potato plants showing typical symptoms of bacterial wilt were identified and such diseased samples were collected from wilt infested field of Central Experiment Station, Wakawali. Diseased plants were uprooted and kept in polythene bags and brought to the laboratory for isolation and further studies.

Stem streaming test

The test was conducted by placing a cut section of stem in test tube containing sterilized water. Within few minutes, viscous white spontaneous slime stream of bacterial cells exuded from cut end of the stem into sterilized water, which was the indication of presence of bacterium in infected stem tissue.

Isolation of the pathogen (*R. solanacearum*)

Isolation of the test bacterium was done by following streak plate method on Triphenyltetrazolium Chloride (TZC) agar medium. The infected plant tissue which was found positive in oozing was subjected for isolation. The surface sterilized stem pieces placed in test tube containing 10 ml sterile distilled water for 10-15 min.

The inoculation loop was dipped in test tube containing bacterial ooze and then streaked on petriplates containing TZC agar medium. Inoculated plates were incubated at $28 \pm 2^{\circ}\text{C}$ for 24 to 48 hours to observe bacterial colonies.

Pathogenicity test

Pathogenicity test were carried out by soil drenching and seed dipping method. The potato seed tubers of Cv. Kufri pukhraj was obtained from Central Experiment Station, Wakavali were used for conducting pathogenicity test.

Soil drenching method

Potato seed tubers were sown in pots containing sterilized potting mixture, soil + sand + FYM (2:1:1). The bacterial suspension was prepared from 72 hours old bacterial culture on TZC medium and poured around the root zone of 30 days old potato plants. The uninoculated pot served as a control.

Seed dipping method

The potato seed tubers were surface sterilized with 95% ethanol and then washed with sterile distilled water to remove traces of chemical. Then seed tubers of potato were soaked in 72 hrs old bacterial suspension for one hour. Then soaked tubers were sown in plastic pots containing sterilized potting mixture, soil + sand + FYM (2:1:1).

Characterization of the pathogen (*R. solanacearum*)

Different Morpho-biochemical tests were carried out to characterize the *R. solanacearum* are described below.

Gram staining

Gram staining test was performed as per standard method.

KOH solubility test

A clean glass slide was inoculated with a loop of 48 hrs old bacterial culture. Few drops of 3% KOH (Potassium hydroxide) were added

on the clean glass slide containing bacterial culture and mixed thoroughly for 5-10 seconds with the help of bacterial inoculation needle for formation of slime threads.

Acid production

Nutrient broth containing 2 per cent glucose (adjusted to pH 7.0) was prepared and 48 hrs old bacterial culture was inoculated in the test tubes containing nutrient broth. The inoculated test tubes were kept for incubation for 7 days at $28 \pm 2^{\circ}\text{C}$. After incubation, few drops methyl red indicator was added in the test tubes. A discrete red or pink colour indicates the positive reaction.

Starch hydrolysis

48 hrs old bacterial culture inoculated on petriplates containing sterilized starch agar medium and incubated for 96 hrs at $28 \pm 2^{\circ}\text{C}$. Then incubated plates were flooded with lugol's iodine solution. The clear zone around the bacterial growth indicates a positive reaction.

Production of Hydrogen sulphide (H_2S)

A nutrient broth with additional 3% peptone was prepared and poured into test tube. The test tubes were sterilized in autoclave and inoculated with 48 hrs old bacterial culture. Then, filter paper strips were soaked in super saturated solution of lead acetate and were kept for drying. After drying, they were inserted into test tubes with cotton plug. The inoculated tubes were incubated for seven days at $28 \pm 2^{\circ}\text{C}$. Filter paper strips turns into black colour indicates positive reaction.

Catalase test

On a clean glass slide add a loopful bacterial culture from a test tube containing 48 hrs old of test pathogen. Few drops of 3% hydrogen

peroxide (H_2O_2) was added on clean glass slide, and mixed with the help of bacterial inoculation needle.

Motility test

The test bacterium culture from 48 hrs old was streaked on petriplates containing motility agar medium with the help of sterile bacterial inoculation needle and inoculated plates were incubated for two days at $28 \pm 2^{\circ}\text{C}$.

Casein hydrolysis

A loopful of 48 hours old bacterial culture was streaked on petriplates containing sterilized skim milk agar medium and plates were incubated in an inverted position for two days at $28 \pm 2^{\circ}\text{C}$.

Results and Discussion

Isolation

The virulent colonies of test bacterium developed on TZC agar medium appeared as, dull white or creamy colour, irregularly round fluidal colonies and opaque with light pink or red center and non virulent colonies appeared dark red in colour (Fig. 2). This result is similar with the report of Dhital *et al.*, (2001), Ahmed *et al.*, (2013) and Chamedjeu *et al.*, (2018) isolated *R. solanacearum* on TZC medium from infected potato plants.

Pathogenicity test

The symptoms observed on inoculated potato plants by both methods as, tender leaves lost their turgidity, lower leaves pale yellowing, drooping of infected leaves, the leaves became flaccid and sudden wilting of the whole plant (Fig. 3). This result is conformity with Shweta *et al.*, 2018 who proved pathogenicity of *R. solanacearum* by soil

drenching method for potato.

Characterization

The results of gram staining revealed that test bacterium was gram negative; rod shaped and retained reddish pink coloured cells (Table 1 and Fig. 4). Several workers like Pawaskar *et al.*, (2014), Singh *et al.*, (2015) and Bannihatti, R.K. (2015) reported similar results of gram

staining. The bacterium formed slime threads (Fig. 4) when 48 hrs old bacterial culture was mixed with 3% KOH solution and showed positive reaction for test (Table 1). This result is similar with the report of Rahman *et al.*, (2010), Chakravarty and Kalita (2011) and Singh *et al.*, (2015) reported *R. solanacearum* to be positive in KOH solubility test.

Table.1 Morpho-biochemical characterization of *R. solanacearum*

Sr. No.	Tests	Reaction
1.	Gram staining	Gram negative
2.	KOH solubility	Positive
3.	Acid production	Positive
4.	Starch hydrolysis	Positive
5.	Catalase test	Positive
6.	Production of H ₂ S	Positive
7.	Motility test	Positive
8.	Casein hydrolysis	Positive

Fig.1 Typical symptoms of *R. solanacearum* on potato tubers



Bacterial wilted potato plant



Bacterial oozing from cut end of potato stem



Bacterial oozing from infected tuber eye

Fig.2 Colonies of *R. solanacearum* on TZC/ CPG medium

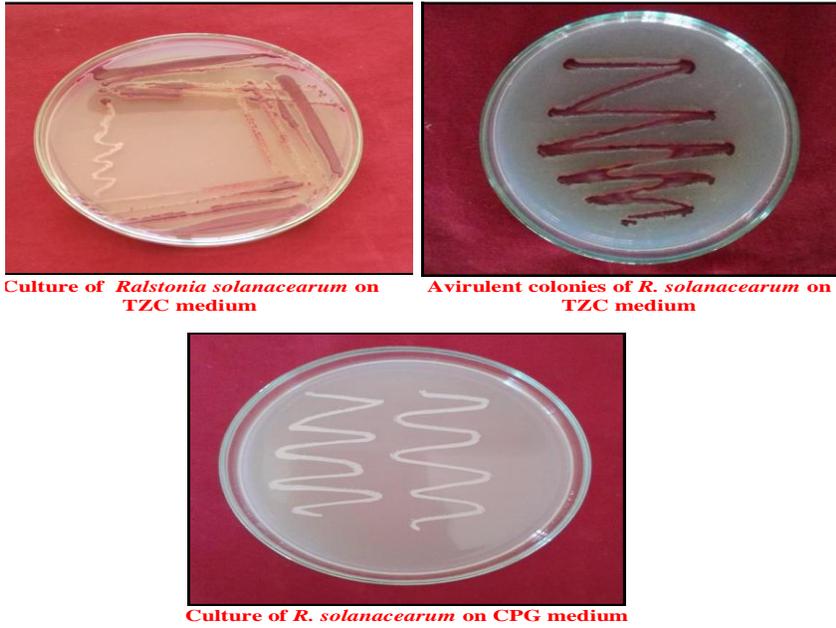
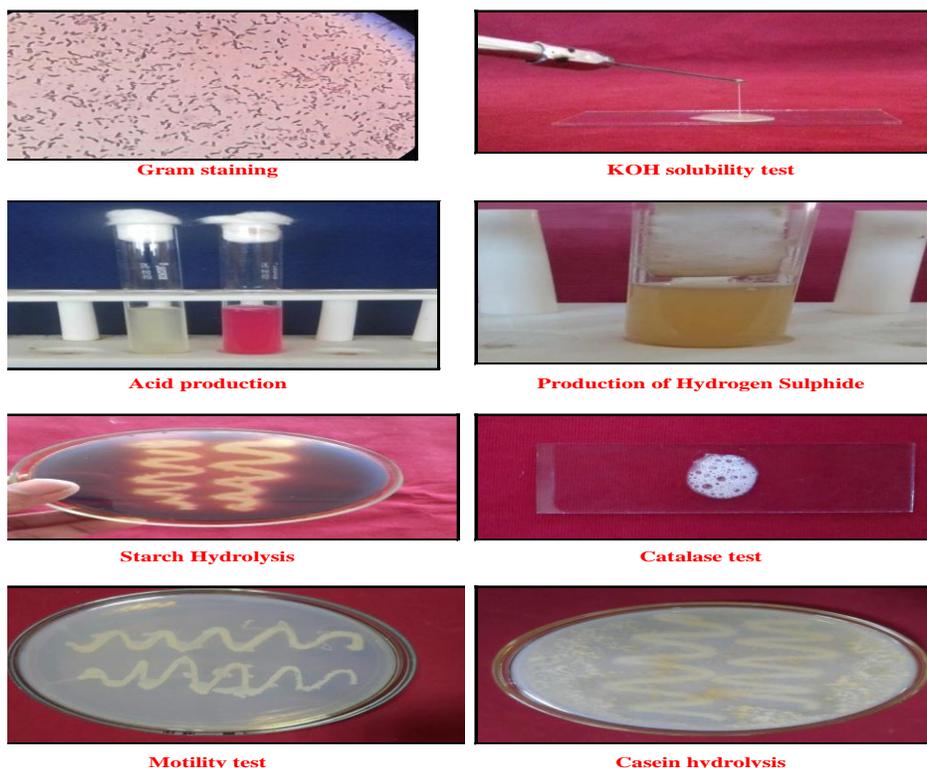


Fig.3 Pathogenicity test of *R. solanacearum* on potato



Fig.4 Morpho-Biochemical characterization of *R. solanacearum*



The bacterium produced acid (Fig. 4) when grown on nutrient broth containing 2 per cent glucose after addition of methyl red indicator and hence, showed positive reaction (Table.1) for test. Pawaskar *et al.*, (2014) reported similar results of Acid production test.

The bacterium was able to hydrolyse starch and formed clear zone (Fig. 4) around the bacterial colony growth on starch agar medium when flooded with lugol's iodine which indicated positive reaction (Table.1). This result is similar with the report of Bannihatti, R. K. (2015), Katakya *et al.*, (2017) and Salvi *et al.*, (2020) reported *R. solanacearum* to be positive in starch hydrolysis test.

Formation of gas bubbles (Fig. 4) indicated positive reaction for catalase test (Table.1). Gas bubbles formation revealed the presence of aerobic and facultative anaerobic bacteria. Bannihatti, R. K. (2015) reported similar results of catalase test.

The bacterium was able to produce Hydrogen sulphide and blackening of filter paper strips (Fig. 4) indicated positive reaction for test (Table.1). Salvi *et al.*, (2020) reported similar results of Production of Hydrogen sulphide test.

The bacterium formed spreading colony (Fig. 4) when grown on motility agar medium, it after 48 hrs of incubation, hence bacterium showed positive reaction for test (Table.1). Bannihatti, R. K. (2015) reported similar results of motility test.

The bacterium formed clear zone around the bacterial growth (Fig. 4) when grown on skim milk agar medium, and bacterium showed positive reaction for test (Table.1). Bannihatti, R. K. (2015) reported similar results of casein hydrolysis test.

From the results (Table. 1) it was concluded that the bacterium *R. solanacearum* was Gram negative in reaction. The bacterium showed positive reaction to all biochemical tests *viz.*,

KOH solubility test, acid production test, starch hydrolysis, catalase test, production of hydrogen sulphide, motility test and casein hydrolysis test.

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