

Morphological and Biochemical Properties Useful for Identification of *Erwinia chrysanthemi*

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ABSTRACT

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Rhizome rot or tip over disease of banana has been occurring in serious form and affecting the yield wherever bananas are being cultivated. The morphological characteristics of the eighteen isolates of the bacterium revealed that cells were rod shaped, occurred singly, Gram negative and peritrichously flagellated. The pathogen was identified on the basis of cultural, morphological and biochemical characteristics and its pathogenicity in banana was proved using Koch's postulates. The identity of the pathogen was also confirmed by the manifestation of characteristic water soaked, necrotic lesion on tobacco leaves.

Introduction

Rhizome rot or tip over disease of banana incited by *Erwinia chrysanthemi* is a serious disease causing rotting of newly planted rhizomes with failure to sprout, stunting and yellowing of young plants. It is a major constraint for commercial cultivation of banana. Several workers have reported in the past from India. Edward *et al.*, (1973) noticed 'Tip over' or 'bacterial rot' in a small piece of land with alluvial soil located close to Yamuna River for the first time on variety Basarai banana in 1968 from India, and isolated a strain of *Erwinia carotovora* from rotting parts of rhizome. Chattopadhyay and Mukherjee (1986) reported the pseudostem of banana caused by *Erwinia chrysanthemi* pv. *paradisica* on Gaint Governor in West

Bengal. Lakshmanan and Mohan (1986) observed tip over disease or head rot caused by *Erwinia carotovora* in Tamil Nadu in North Arcot and Trichy districts. Dickey and Victoria (1980) reported that, the strain from *M. paradisiaca* in Colombia was very similar to *E. chrysanthemi* on the basis of biochemical and physiological properties. Snehalatharani and Khan, (2010) reported the bacterium showing positive for pectate degradation on CVP medium, growth at 36⁰C and 39⁰C, catalase production and negative for indole production. Further they gave positive result for the production of acid from various agars such as lactose, trehalose, maltose and cellobiose and soft rot of inoculated potato slices.

Biochemical and pathogenicity test indicated that the isolates are *Erwinia chrysanthemi*. This work deals with pathogenicity test, hypersensitive, morphological and biochemical characterisation of *Erwinia chrysanthemi* causing tip-over disease of banana.

Materials and Methods

Morphological characteristics

The morphological characteristics of the eighteen isolates with respect to shape, Gram reaction and flagella staining, were studied as described by Society of American Bacteriology (Anon., 1957), Stover (1959), Bradbury (1970) and Dickey (1981).

Pathogenicity test

The pathogenicity test was carried out on 20 banana plants. The bacterial isolates were multiplied on nutrient agar plates. The bacterial cultures were harvested and suspended in sterile water and adjusted turbidometrically to give a cell concentration of 5×10^7 cfu/ml and also viable cell counts were made by dilution plate method.

Proving the Koch's postulates

The healthy susceptible tissue cultured Grand Naine plantlets were used in this study. The plantlets were washed to remove the traces of soil adhering to the root surface and roots were injured by using sterile blade for entry of the pathogen. The rhizomes were dipped in the bacterial suspension for 45 minutes, control plants were dipped in sterile distilled water and planted in the earthen pots containing sterilized soil and pots were maintained in the polyhouse. Observations were recorded for the appearance and development of symptoms. After symptoms development, reisolation was done from the artificially infected plants. The isolate

obtained was compared with the original culture for confirmation.

Hypersensitive reaction on tobacco (hr)

The bacterial isolates obtained from the infected rhizome tissues and multiplied in the nutrient broth. The bacterial cells were suspended in sterile distilled water and the concentration of the cell suspension was adjusted to 2×10^8 cfu/ml. The bacterial cell suspension was adjusted in to the intercellular spaces of intact tobacco leaves (*Nicotiana tobaccum*) with a hypodermic needle. The infected plants were kept in the glass house at 28 °C to 30 °C. The plants were observed for the development of necrotic lesions. Leaves injected with sterilized distilled water served as control.

Biochemical characteristics

The bacterial isolates were tested for the physiological and biochemical characteristics with regard to potato soft rot, gelatine liquefaction, sensitivity to erythromycin, indole production, phosphatase test, growth at 36 to 37°C. The tests were conducted as per the methods described by Dickey and Victoria (1980) and Dickey (1981).

Results and Discussion

Morphological characteristics

The morphological characteristics of the eighteen isolates of the bacterium revealed that cells were rod shaped, occurred singly, Gram negative and peritrichously flagellated (Plate 1). Similar results were reported by Stover (1959) who reported that the bacterium is motile gram-negative, rod shape. Schaad and Brenner (1977) reported that cells of the bacterium were Gram-negative, peritrichous, rod shape. Dickey and Victoria (1980) reported that bacterium isolated from *M. paradisiaca* was a gram negative, non-spore

forming rod shape. Snehalatharani and Khan (2010) reported that the bacterium was rod shaped, gram negative, peritrichously flagellated, non-spore forming and non-acid fast.

Pathogenicity test

The bacterial cultures were artificially inoculated to 20 banana seedlings by rhizome dip technique. The leaves in the inoculated plants started turning yellow and which later on became brown and dried after 25-30 days after inoculation. The rhizomes were found discoloured dark brown to black, rotted and

emitted foul smell, re-isolation was done for the artificially infected plants. The isolates were compared with original isolates thus confirming the identity of the pathogen (Plate 2). Similar findings were made by Stover (1959) who reported that the disease symptoms in above ground parts appeared in 8 -10 weeks after inoculated rhizomes were planted in pots. Lakshmanan and Mohan (1992) reported that the pathogenicity was proved by soaking the suckers in a bacterial suspension of virulent strains (7×10^7 cfu/ml) for 15 minutes. Pathogenicity test of bacteria was successfully done by artificial inoculation method at Arabhavi centre (Anon., 2009).

Plate.1 Growth of *Erwinia chrysanthemi*



Plate.2 Pathogenicity test



Uninoculated

Inoculated

Plate.3 Hypersensitive reaction on Tobacco



Plate.4 Biochemical characteristics of *Erwinia chrysanthemi*

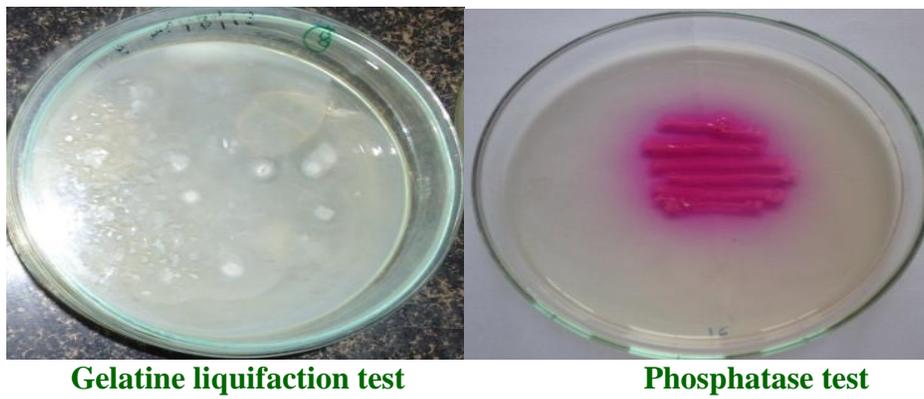
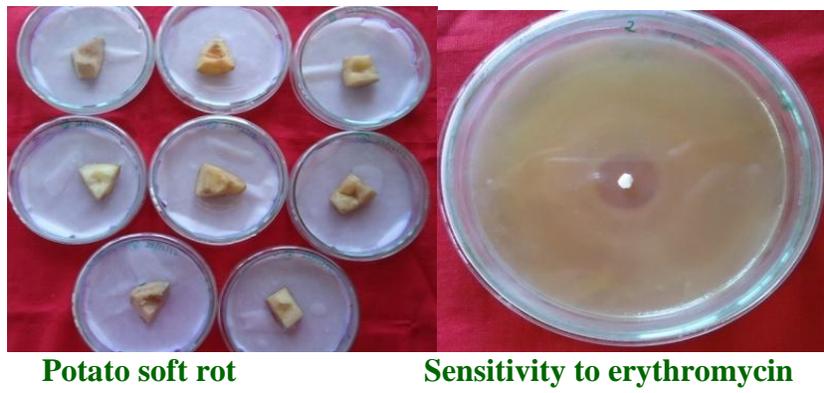


Table.1 Biochemical properties useful for identification of *Erwinia chrysanthemi*

Test	I ₁	I ₂	I ₃	I ₄	I ₅	I ₆	I ₇	I ₈	I ₉	I ₁₀	I ₁₁	I ₁₂	I ₁₃	I ₁₄	I ₁₅	I ₁₆	I ₁₇	I ₁₈
Potato soft rot	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sensitivity to erythromycin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 36-37 ⁰ C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gelatine liquefaction	-	-	-	-	-	-	-	-	-	-	V	-	-	-	-	-	-	-
Phosphatase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Indole test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

I: Isolate V: Variable

I₁- Dastikoppa

I₂- Yamakanamaradi

I₃- Hattaragi

I₄- Shivapur

I₅- Hidakal

I₆- Munavalli

I₇- Kittur

I₈- Nalawathad

I₉- Mudnal

I₁₀- Nagerbetta

I₁₁- Muddebihal

I₁₂- Siddapur

I₁₃- Yalahatti

I₁₄- Jamakhandi

I₁₅- Lokapur

I₁₆- Salahalli

I₁₇- Rabakavi

I₁₈- Rajapur

Hypersensitive reaction on tobacco (hr)

The results of the studies conducted on the hypersensitive reaction are presented in the (plate 3). The bacteria injected in the intercellular spaces of tobacco leaves. The injected leaves produced characteristic water soaked lesion within few seconds, yellowing at 48 hours and necrosis during the next 96 hours. The result was in confirmation with the studies of Ouf *et al.*, (1991) who reported four tested isolates of *E. carotovora* subsp. *carotovora* induced hypersensitive reaction in cactus (*Aloe* sp.) and *Nicotiana glutinosa* plants.

Biochemical characteristics

The results of various biochemical tests are given in Table 1 and Plate 4. All the eighteen isolates caused characteristic rotting of the potato slices. All the isolates showed sensitive to erythromycin, positive reaction for growth at 36-37⁰C, phosphatase test, indole test. All the isolates of the bacterium were negative to gelatine liquefaction but I₁₁ variable with gelatine test. On comparing the various

physiological and biochemical characters of the isolates with those type of typical *Erwinia* test mentioned by Snehatharani and Khan (2010) who reported that reported the bacterium showing positive for pectate degradation on CVP medium, growth at 36⁰C and 39⁰C, catalose production and negative for indole production. Dickey and Victoria (1980) reported that *Erwinia* sp. isolated from *Musa paradisiaca* was sensitive to erythromycin. The result of the findings confirmed the fact that all the isolates were sensitive to erythromycin.

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