

Full Length Research Paper

Characterization of *Erwinia chrysanthemi* isolates inciting stalk rot disease of sorghum

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Stalk rot of sorghum plants caused by *Erwinia chrysanthemi* is one of the most destructive diseases of sorghum crop. Twenty one of bacterial isolates were isolated from stalk of sorghum plants from different location of U.S. Nagar district of Uttarakhand, India. Biochemical, physiological and morphological characterization of *E. chrysanthemi* was done for confirmation of the bacterium specie. Bacterial suspension [0.7% Tween-40 (v/v) + 2×10^8 cell/ml] of each isolate was inject-inoculated with a 21G hypodermic needle into the stalk of plants for pathogenicity testing of the test bacterium. Reactions of biochemical and physiological testing are clearly evident enough to support the confirmation of test bacterium to taxonomic assignation of *E. chrysanthemi* causing soft roton sorghum plants. Out of 31 diseased samples of different locations examined, in 21 samples, the pathogen was detected as *E. chrysanthemi* by using set of biochemical and physiological testing. As all the bacterium produced typical stalk rot disease symptoms on sorghum plant and water-insoluble blue pigment (indigoidine) on nutrient glycerol $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (2 mM) agar medium it was confirmed as *chrysanthemi* species.

Key words: Blue pigment, characterization, *Erwinia chrysanthemi*, Stalk rot, sorghum.

INTRODUCTION

Soft rot erwinias are very important primary pathogens of both growing plants and the harvested crop (Pérombelon and Kelman, 1980). However, strains from different host plants differ in their specific host range as well as in the pathogenic and phenotypic properties (Dickey, 1979; Janse and Ruissen, 1988). The genus *Erwinia* is named after one of the founder of phytobacteriology, Erwin Frink Smith, and was established by Winslow et al. (1917) to include the plant pathogenic enterobacteria. Like other enterobacteria, the *Erwinia* are motile by means of

several to many peritrichous flagella usually 8-11 (Burkholder et al., 1953; Dickey, 1981), gram-negative, non-spore forming, straight rod with rounded ends, and occurs singly or in pairs. Its size varies from $0.8\text{-}3.2 \times 0.5\text{-}0.8 \mu\text{m}$ (average $1.8 \times 0.6 \mu\text{m}$) depending on carbon source present in the medium and growth conditions (Gruha, 1970). Stalk rot of sorghum caused by *Erwinia chrysanthemi* Burkholder, McFadden, and Dimock is one of the most destructive diseases of sorghum crop. Saxena et al. (1991) reported this bacterium causing

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stalk and top rot of sorghum under natural conditions in India during 1987-1988 crop season in sorghum field at Pantnagar, Uttarakhand. The disease was wide spread and affected 60 to 80% of plants in different sorghum genotypes. Recently, the occurrence of disease incidence ranging from 7.50 to 46.85 in Tarai region of Uttarakhand has been also shown by Kharayat and Singh (2013).

Early and accurate diagnoses of plant disease are necessary to predict outbreaks and allow time for development and application of mitigation strategies. A number of different biochemical methods are presently being employed for microbial identification and taxonomic classification. Moreover, each method has its advantages and disadvantages; with regard to ease of application, reproducibility, requirement for equipment and level of resolution. Present investigation was aimed to devise the set of biochemical testing to characterize the isolates of destructive soft rot bacterium, *E. chrysanthemi*.

MATERIALS AND METHODS

On the basis of visual observation of suspected stalk with typical soft rot symptoms, sampling was done from 31 different locations in the growing season 2011-2012. Samples were brought to the laboratory and kept in refrigerator at 4°C. Isolations were made from samples and the pathogen was identified by using set of biochemical and physiological testing.

Isolations and purifications of *E. chrysanthemi*

Isolation of bacterium was done as per the method described by Janse (2005). Pieces of tissue taken from the margin of healthy and diseased tissues were disinfected with 70% alcohol and placed in a sealed tube with sterile water and tissues were left for 30 min in suspension so that the bacteria could diffuse out of the tissues. Subsequently 100 µl of the suspension was plated onto crystal violet sodium polypectate (CVP) medium. Characteristically deep-pit forming colony on CVP medium purified on yeast dextrose calcium carbonate medium by streaking using freshly growing single colony and these plates were incubated at 28°C for five days.

Pathogenicity tests

To confirm the pathogenicity of isolates from various locations [Figure 1, 1-Pantnagar-1, 2-Pantnagar-2, 3-Pantnagar UTMC-535, 4-Majra Farm, 5-Banjari farm, 6-Chhinki Farm, 7-Sailanigot, 8-Tanakpur, 9-Khetalsanda, 10-Kashipur, 11-Haldhwani-1 (HLD1), 12-Kisanpur (Haldhwani), 13-Bajpur, 14-Bajpur (Doraha), 15-Rudrapur, 16-Kashipur (Sultanpur), 17-Tanda Kajal (Kashipur), 18-Gadarpur, 19-Barhani (Bajpur), 20-Sitarganj and 21-Nagina (Sitarganj)], stem inoculation was done on of 21 days old susceptible sweet sorghum plants variety SPSSV 6 under controlled glasshouse conditions. Isolates were grown on Luria Broth for 24 h at 28°C. The bacterial cells were suspended in sterile distilled water and the cell density adjusted to 2×10^8 cfu/ml. Bacterial suspension [0.7 % Tween-40 (v/v) + 2×10^8 cell/ml] of each isolate was inject-inoculated with a 21G hypodermic needle into the stalk of plants. Control plants were inject-inoculated with

sterilized water. Experiment was conducted twice to confirm the result.

Biochemical, physiological and morphological characterization of *E. chrysanthemi*

Test pathogen was screened for characterization upto species level by using a set of biochemical and physiological testing (Table 1) to detect the presumptive *E. chrysanthemi* which were selected according to keys of Schaad et al. (2001).

Scanning electron microscopy

SEM preparation for *E. chrysanthemi* Pantnagar isolate was done using procedure described by Kaláb et al. (2008). Twenty-four hours old actively growing bacterial cells on Luria broth medium were harvested by centrifuge at 6000 rpm. Then, the bacterial cells were fixed with 2.5% gluteraldehyde in 0.05 M sodium phosphate, pH 6.8, for 24 h at 4°C, washed with sodium cacodylate buffer three times (10 min each wash). They were finally fixed in 1% osmium tetroxide for 1 h and washed with 0.1 M sodium cacodylate buffer as before. Further, the cells were dehydrated through a series of graded acetone (10, 20, 30, 40, 50, 70, 80, 90 and 100%). These cells were soaked for 15 min at each concentration. The drying was completed by placing the sample in a flow of CO₂ in critical point dryer. The cells were mounted on aluminum stubs and coated with gold using Hummer V sputter coater, and viewed and photographed under a scanning electron microscope.

RESULTS AND DISCUSSION

Reactions of biochemical and physiological testing presented in Table 1 are clearly evident enough to support the confirmation of test bacterium to taxonomic assignation of *E. chrysanthemi* causing soft roton sorghum plants. Out of 31 diseased samples of different locations (Figure 1) examined, in 21 samples, the pathogen was detected as *E. chrysanthemi* by using set of biochemical and physiological testing. These set of test also has been used by several other investigators to detect the presumptive *E. chrysanthemi*, viz. oxidative/fermentative test (Hugh and Leifson, 1953), oxidase test, deep pit formation on crystal violet sodium polypectate, CVP medium + 0.4% tetrazolium chloride solution (Cuppels and Kelman, 1974; Tomlinson and Cox, 1987; Perombelon and Burnett, 1991; Bdliya et al., 2004; Kaneshiro et al., 2008; Zhu et al., 2010), indole production, sensitivity to erythromycin at 15 µg/ml (Jensen et al., 1986; Olabiyi, 2010), colony morphology on yeast dextrose calcium carbonate agar medium (YDC) (Dye, 1968; Goto, 1979; Kaneshiro et al., 2008), and Growth at 37°C (Pérombelon and Kelman, 1980; Lelliott and Dickey, 1984; Pérombelon and Hyman, 1986; Hyman et al., 1998). As the bacterium produced water-insoluble blue pigment (indigoidine) on NGM (Nutrient Glycerol MnCl₂.4H₂O (2 mM) agar medium it was confirmed as *chrysanthemi* species. As it has been already reported that *chrysanthemi* is only species under the genus which produced water-insoluble blue pigment

Table 1. Biochemical, physiological and morphological characterization of isolates of *Erwinia chrysanthemi* isolated from sorghum plants.

<i>E. chrysanthemi</i> isolates	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Indole production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methyl red	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrate utilization	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Glucose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adonitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Arabinose	+	+	+	+	+	-	+	+	+	+	-	+	+	-	+	+	-	+	+	+	+
Lactose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sorbitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sucrose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gram reaction	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3% KOH test	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Deep pit on CPV medium ^ψ	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Blue pigment on NGM [*]	+	+	+	+	+	+	+	+	+	+	-	-	+	+	-	+	+	-	+	+	+
Sensitivity to erythromycin (15 µg/ml)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 37°C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Undulate margin on NYDA ^φ	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pathogenicity test	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

^ψSize of deep pit was found variable it may be due to variability; ^φAllformed colony with undulate or lobate margin after 4 days, later becomes feathery on NYDA; [□]Mostly all isolates produced blue pigment but some were lack.

(Starr et al., 1966; Lee and Yu, 2006; Olabiya, 2010). SEM analysis showed that the shape and size of bacterium, straight rod with rounded ends (Figure 2) and 1.50 × 0.50 µm respectively. In pathogenicity test, the bacterium found potential pathogen as it produced typical symptom on stalk of sorghum plant as naturally occurred in field conditions after 4 days of inoculation (Figure 3). The symptom mainly affect sorghum stem showing water-soaked symptoms that later turned reddish dark brown color. The infected stem pith disintegrated and showed slimy soft-rot symptoms

after 7 days of inoculation. Several other workers also reported same symptoms (Zummo, 1969; Hepperly and Davila, 1987; Saxena et al., 1991; Hseuet et al., 2008).

Conclusion

Biochemical and physiological methods are easy to use, reproducible and less costly than molecular and serological methods and can be readily used for identification of *E. chrysanthemi*.

Conflict of Interest

The authors have not declared any conflict of interest.

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Figure 1. The number in map showing various locations of *Erwinia chrysanthemi* isolates.

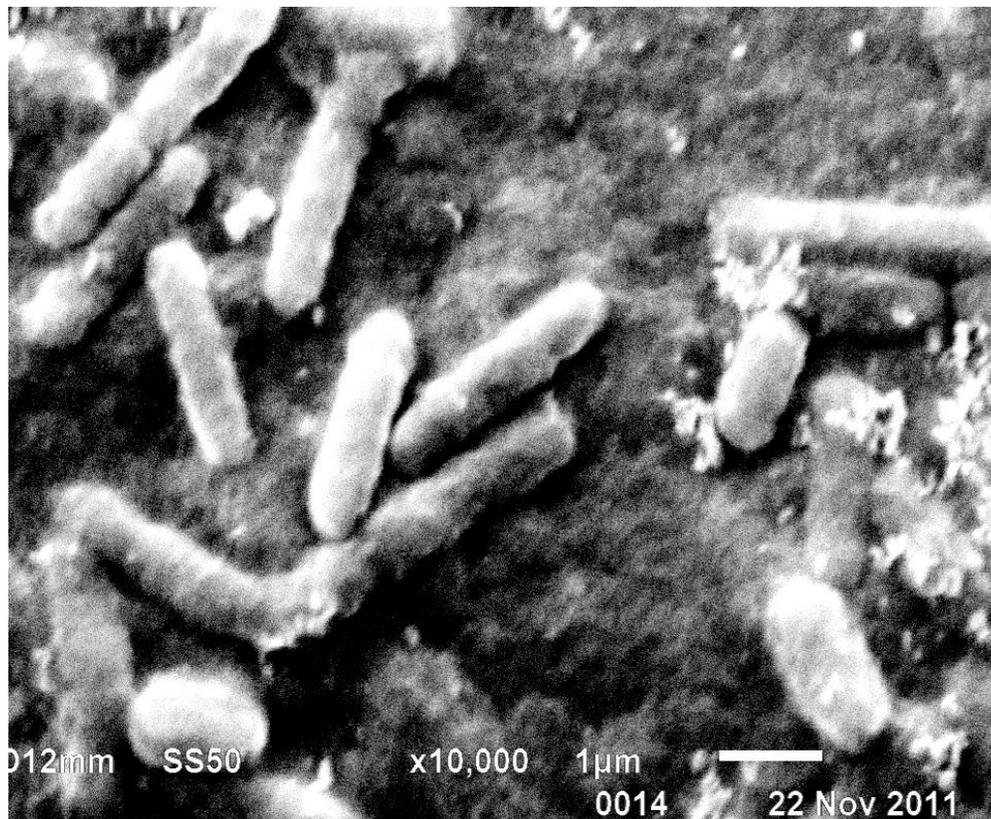


Figure 2. Scanning electron micrograph showing *E. chrysanthemi* cells with straight rod.



Figure 3. Typical stalk rot symptom produced by *E. chrysanthemi* on 21 days old susceptible sorghum plant.

glasshouse facilities during the course of investigation.

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