Pertanika 5(1), 84–89 (1982)

Studies of Bacterial Stalk Rot Disease of Corn[1]

HIRYATI ABDULLAH
Department of Plant Protection, Faculty of Agriculture, Universiti Pertanian Malaysia, Serdang, Selangor, Malaysia.

Key words: Corn *(Zea mays* L.*)*; **Bacterial stalk rot**; *Erwinia chrysanthemi.*

RINGKASAN

Reput bakteria batang jagong (Zea mays *L.*) *telah diperhatikan dan bakteria penyebabnya diasingkan daripada pokok-pokok jagung yang berpenyakit. Berdasarkan kajian gejala-gejala, kepatogenan, kultura, biokimi dan fisiologi, patogennya telah kenalpasti sebagai* Erwinia chrysanthemi *Burk., McFadden dan Dimock. Akan tetapi, strain jagong didapati hanya mempunyai virulen yang rendah kepada kultivar-kultivar nenas yang peka, mencadangkan yang ia mungkin suatu patova bakteria itu yang berlainan.*

Kajian kepatogenan telah menunjukkan yang pokok jagung yang berumur 3 minggu dan 5 minggu, varieti yang disukai, Metro, dan hibrid baharu jagung manis, Bakti, yang ditanam di Malaysia, adalah peka pada penyakit ini.

SUMMARY

Bacterial stalk rot of corn (Zea mays *L.*) *was observed and the causal bacterium isolated from infected corn plants. Based on the symptoms, pathogenicity, cultural, biochemical and physiological studies, the pathogen was identified as* Erwinia chrysanthemi *Burk., McFadded and Dimock. However, the corn strains were found to be only weakly virulent to the susceptible pineapple cultivars tested, suggesting that it may be a distinct pathovar of the bacterium.*

Pathogenicity test showed that the 3 wk. and 5 wk. old corn plants, of a popular corn variety, Metro, and a recent sweet corn variety, 'Bakti', grown in Malaysia, were susceptible to the disease.

INTRODUCTION

Bacterial stalk rot of corn *(Zea mays* L.*)* is a destructive disease and has been described in various parts of the world (Hingorani *et al,* 1959; Ludbrook, 1942; Pauer, 1964; Prasad, 1930; Sabet, 1954 and 1957; Volcani, 1961; and Zachos *et al,* 1963). In India, the disease was reported and attributed to *Phytomonas dissovens* Rosen *(Erwinia dissolvens* (Rosen) Burkholder*)* (Prasad, 1930). In 1959, Hingorani *et al.* reported the disease to be prevalent in the principal maize-growing areas of India and the organism was said to be similar to or identical with *Erwinia caratovora* f. sp. *zeae* as reported by Sabet (1954) from Egypt. Several investigators have questioned the proper taxonomic position of this pathogen (Dye, 1969; Graham, 1964; Hoppe and A. Kelman 1969, Sabet *et al.* 1964; and Starr and Mandel 1969). Hoppe and Kelman (1969) in their study concluded that the corn stalk pathogen showed closer affinity to *E. chrysanthemi* than to *E. caratovora* while Dye (1969) considered it to be synonimous with the 'chrysanthemi' group.

In Malaysia, the disease has been observed by the author since 1977. So far, the incidence remains low and sporadic. A random survey on the Universiti Pertanian Malaysia farm in May 1980 revealed that 5.2% of the 6.07 ha field of corn was infected by stalk rot. Even though the disease has existed for quite some time, up till now, there is a lack of information concerning the disease in Malaysia. The primary objectives of this investigation were to record the incidence of this disease, to study the etiology of the disease and to determine their pathogenicity on our local commercial varieties.

[1] Part of this paper was presented at the 3rd Malaysian Microbiology Symposium, 15-17 Oct. 1980, Universiti Pertanian Malaysia, Serdang, Selangor, Malaysia.

Key to author's name: A. Hiryati.

MATERIALS AND METHODS

Comparisons of the isolates of *Erwinia* from corn were made with those of *Erwinia caratovora* var. *caratovora* from cabbage *(Brassica oleracea* L.*)* and Irish potato *(Solanum tuberosum* L.*)* with respect to its pathogenicity, cultura, biochemical and physiological characteristics.

Cultures

Three isolates each of *E. caratovora* var. *caratovora* from cabbage and potato and five isolates from corn were used thoughout this study. All isolates were maintained on nutrient agar (Difco) and yeast extract-salt agar slopes (YS) (Dye, 1968) at approximately 4-6° C.

Cultural test

Cultural characteristics of all the isolates were observed on modified yeast extract dextrose calcium carbonate medium (YDC) (Dye, 1968), Endo agar (Difco) and sucrose peptone (SP) medium (Hayward, 1960). All cultures, unless otherwise indicated, were incubated at 30° C.

Biochemical and Physiological test

All tests were carried out using 24-48 hr. cultures from YDC and were repeated at least twice, unless otherwise indicated. The basal medium used for acid production from sucrose, glucose, lactose, maltose, cellobiose, dulcitol, mannitol, sorbitol, arabinose, trehalose and ramnose were that of Bacto OF medium (Difco). A 10% (w/v) aquous solution of the above sugars, which were filter sterilized, were aseptically added to the basal medium to give a final concentration of 1.0% (w/v). A change in the color of the medium from green to yellow was scored as a positive reaction. Durham tubes were used to test for the production of gas from glucose. Tubes were sealed with 2 cm of sterile mineral oil to provide anaerobic condition. Readings were done at regular intervals of 48 hrs. up to 2 wks. The tests for indole, methyl red, H_2S production, detection of catalase, hydrolysis of casein, gelatin and starch were done as described by Bradshaw (1963). Stabbed tubes to test for gelatin hydrolysis were incubated at 20° C and readings done after 1 and 2 weeks. Bacto Malonate broth (Difco) and Bacto Koser citrate medium (Difco) were used to test for the utilization of malonate and citrate respectively. Readings were taken regularly up to 1 week. Gram stain, nitrate reduction and the oxidase test were carried out according to Bradbury (1970), while phosphatase production and the test for reducing substance from sucrose were determined as described in Bergey's Manual (Lelliott, 1957). Isolates were tested for growth at 40° C by inoculating Bacto nutrient broth (Difco) and yeast extract salt broth (Dye, 1968) and incubated at 40° C in a waterbath shaker for up to 1 week.

Inoculation test

Tests for soft rot of potato, carrot and green tomato slices were made as described by Bradbury (1970). Twenty-day old corn pseudostem (Metro variety) and 5 week old padi sheath (*Bahagia* variety) were cut approximately 9-10 cm in length, surface sterilized and placed in boxes lined with three layers of moist blotter papers. These were innoculated at the centre, by injecting a heavy bacterial suspension of the respective isolates, using a microsyringe. Cabbage leaves were trimmed to smaller sizes, surface sterilized and placed in boxes as indicated above. These were inoculated by pricking the leaves with an inoculating needle through a drop of a heavy bacterial suspension of the isolates. Readings were taken after 24 to 72 hrs. Three-week and 5-week old corn plants of the variety Metro and a sweet variety 'Bakti' were inoculated in the glasshouse by the whorl inoculation method as described by Hartman and Kelman (1973). Bacterial suspension, consisting of approximately 8.5×10^9 bacterial cells/ml as determined by the dilution plate technique were used with 0.7% Tween 40. Control plants were similarly inoculated but using sterile distilled water and 0.7% Tween 40. Inoculation of corn isolates on susceptible pineapple leaves, (cultivar Red Spanish and Nangka) were carried out as described by Lim (1971).

RESULTS AND DISCUSSION

Symptoms

The disease was observed on 2-to 3-week old corn seedlings and on mature plants. The initial symptom consisted of wilting of the innermost leaves (Plate 1a) accompanied by water soaked decay of the inner leaves and stalk tissues which eventually turned brown. At this stage the inner whorl of leaves were easily pulled out (Plate 1b). Rotting of the stalk progressed rapidly after this, until the top broke and toppled over (Plate 1c). Rotting was characterised by an offensive odour. Similar symptoms were observed on inoculated plants (Plate 2). These symptoms were similar to those described by Hoppe and Kelman (1969).

Cultural characteristics

On modified YDC, the corn isolates sometimes produced a blue pigment which also stained the media after 24 to 72 hrs. while the cabbage and potato isolates were never observed to produce any blue pigment. All cultures grew very well after 24 hrs.; colonies were similar and could not

Plate 1a. Initial symptom of bacterial stalk rot of corn showing wilting of the innermost leaves.

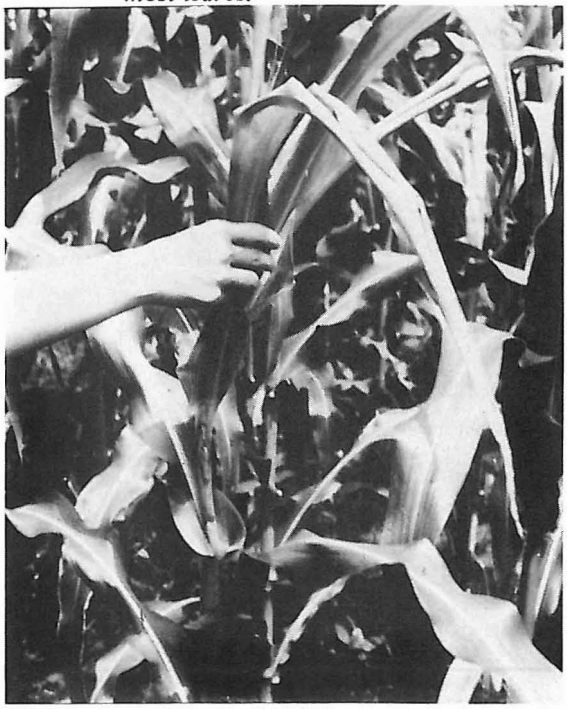

Plate 1b. Inner whorl of leaves can be easily pulled out due to decay of the inner leaves and stalk tissues.

Plate 1c. As the rotting of the stalk progresses, the top breaks and topples over.

Plate 2. Three weeks old corn plants inoculated in the greenhouse. Right – plants showing the same symptoms as in the field. Left – control plants showing no symptom.

be differentiated. On SP medium, all cabbage and potato isolates were whitish while the corn isolates were cream in color. On Endo agar, colonies of corn isolates were generally small, 1 to 3 mm diameter, pale pink, round, convex and did not change the colour of the medium. Colonies of cabbage and potato isolates were similar on Endo agar; were generally larger, 2 to 4 mm diameter,

TABLE 1

Comparison of the physiological and biochemical properties of *Erwinia* isolates from corn, cabbage and potatoes.

Property	*Erwinia* isolates		
	Corn	Cabbage	Potatoes
Acid production from:			
Glucose (aerobic & anaerobic)	+	+	+
Maltose	—	—	—
Lactose	+	+	+
Cellobiose	+	+	+
Trehalose	—	+	+
Ramnose	+	+	+
Arabinose	+	+	+
Sucrose	+	+	+
Sorbitol	—	—	—
Dulcitol	—	—	—
Mannitol	+	+	+
Gas from glucose	+	—	—(1/3)*
Hydrolysis of:			
Casein	+	+	+
Gelatin	+	+	+
Starch	—	—	—
Utilization:			
Sodium citrate	+	+	+
Sodium malonate	+	—	—
Growth 40°C	+	—	—
Blue pigment on YDC*	V*	—	—
Indole	+	—	—
Methyl Red	—	+	+
H_2S production	—	—	—
Phosphates test	+	—	—
Kovac's oxidase	—	—	—
Catalase	+	+	+
Nitrite from nitrate	+	+	+
Reducing sub. from sucrose	+	+	+
Gram stain	—	—	—

* YDC = Yeast extract dextrose calcium carbonate agar
V = Variable
( ) = No. of samples showing positive reaction
\+ = Positive reaction
— = Negative reaction

TABLE 2
Pathogenicity of *Erwinia* isolates on corn (plants and pseudostem), rice (sheaths), cabbage (leaves, carrot, green tomatoes and potatoes (slices).

Host	*Erwinia* isolates from		
	Corn	Cabbage	Potatoes
Corn (plant)	++	—	—
Corn (pseudostem)	+	—	—
Rice (sheath)	—	—	—
Cabbage (leaves)	+	++	++
Carrot (slices)	++	++	++
Green tomatoes (slices)	++	++	++
Potatoes (slices)	++	++	++

++ Severe infection
\+ Slight infection
— No infection

round, convex, dark red with greenish metallic sheen and changed the colour of the medium to dark red.

Biochemical and physiological characteristics

The isolates tested showed several differences (Table 1) which, as pointed out by Dickey (1979), may be useful as a presumptive test for *E. chrysanthemi.* Even though all isolates tested were able to liquefy gelatin, their pattern of liquefaction was distinctly different. Corn isolates produced the infundible pattern while the potato and cabbage isolates produced the crateriform pattern. Properties of the corn isolates tested showed that they were distinctly different from *Erwinia caratovora* and should be classified as *E. chrysanthemi* (Lelliott, 1975; Dye, 1969; and Dickey, 1979). This work supports the work of other workers (Hoppe and Kelman 1969; and Dye, 1969).

Inoculation tests

Results of inoculation tests are given in Table 2. The corn isolates were less virulent on cabbage as shown by the limited rotting on cabbage leaves after 48-72 hrs. Pathogenicity test on corn showed that the corn isolates were pathogenic to both varieties and age group tested, while none of the isolates from cabbage and potatoes were pathogenic to corn. The corn isolates were weakly pathogenic to the two susceptible cultivars of pineapple tested as shown by the limited water-soaked lesions with a mean length of 4.5 mm and 5 mm on *Nangka* and Red Spanish respectively.

CONCLUSION

Based on the symptoms, pathogenicity, cultural, biochemical and physiological studies above, the pathogen of bacterial stalk rot of corn in Malaysia was found to be similar with *Erwinia chrysanthemi* Burk., McFadden and Dimock and is therefore identified as such. The corn stains were only weakly pathogenic to pineapple, suggesting that it may be a distinct pathovar of the bacterium. The *E. chrysanthemi* was found to be highly pathogenic to 3-wk and 5-wk old corn plants of a popular corn variety, Metro, and a recent swet corn variety, 'Bakti', grown in Malaysia.

ACKNOWLEDGEMENTS

The author wishes to express her appreciation to Commonwealth Mycological Institute, United Kingdom, for the confirmation of the isolates; to Dr. Yap Thoo Chai, Department of Agronomy and Horticulture, Faculty of Agriculture, Universiti Pertanian Malaysia, for providing the corn seeds and to Dr. W.H. Lim, Mardi, Jalan Kebun, for providing the pineapple leaves used in this study.

REFERENCES

BRADBURY, J.F. (1970): Isolation and preliminary study of bacteria from plants. *Rev. Pl. Path.* **49(5)**: 213-218.

BRADSHAW, L.J. (1963): Laboratory microbiology. W.B. Saunders Co. Philadelphia.

DICKEY, R.S. (1979): *Erwinia chrysanthemi:* A comparative study of Phenotypic properties of strains from several hosts and other *Erwinia* species. *Phytopath.* **69:** 324-329.

DYE, D.W. (1968): A taxonomic study of the genus *Erwinia.* I. The "amylovora" group. *N.Z. J. Sci.* **11:** 590-607.

DYE, D.W. (1969): A taxonomic study of the genus *Erwinia* II. The "Caratovora" group. *N.Z. J. Sci.* **12:** 81-97.

GRAHAM, D.C. (1964): Taxonomy of the soft rot coliform bacteria. *Annu. Rev. Phytopathol.* **2:** 13-42.

HARTMAN, J.R. and KELMAN, A. (1973): An improved method for the inoculation of corn with *Erwinia* spp. *Phytopath.* **63:** 658-663.

HAYWARD, A.C. (1960): A method for characterizing *Pseudomonas solanacearum. Nature.* **186:** 405.

HINGORANI, M.K., GRANT, V.J. and SINGH, N.J. (1959): *Erwinia caratovora* f. sp. *zeae,* a destructive pathogen of maize in India. *Indian Phytopath.* **12:** 151-157.

HOPPE, P.E. and KELMAN, A. (1969): Bacterial top and stalk rot disease of corn in Wisconsin. *Pl. Dis. Reptr.* **53:** 66-70.

LELLIOTT, R.A. (1957): Description of the species of genus *Erwinia.* Pages 332-340 in R.I. Buchanan and N.E. Gibbons, (eds.) Bergey's Manual of Determinative Bacteriology. 8th ed. The Williams and Wilkins Co. Baltimore.

LIM, W.H. (1971): Evaluating the susceptibility of pineapple cultivars to bacterial heart rot. *Malaya Pineapple* **1:** 23-27.

LUDBROOK, W.V. (1942): Top rot of maize, sweet corn and sorghum. *Australian J. Council Sci. and Ind. Res.* **15:** 213-216.

PAUER, G.D. (1964): *Erwinia caratovora* f. sp. *zeae,* the bacterium causing stalk rot of maize in the Republic of South Africa. *So. African J. Agr. Sci.* **7:** 581-582.

PRASAD, H.H. (1930): A bacterial stalk rot of maize. *Agr. J. India.* **25:** 72.

SABET, K.A. (1954): A new bacterial disease of maize in Egypt. *Emp. J. Exp. Agr.* **85:** 65-67.

SABET, K.A. (1957): A note on the identity of a bacterium causing stalk rot and leaf blight of maize in Southern Rhodesia. *Emp. J. Exp. Agr.* **98:** 165-166.

SABET, K.A., SAMRA, A.S. and EL-SHAFEY, H.A. (1964): On the systematic position of the coliform bacteria responsible for stalk rot of maize. *Ann. Appl. Biol.* **53:** 267.

STARR, M.P. and M. MANDEL (1969): DNA base composition and taxonomy of phytopathogenic and other enterobacteria. *J. Gen. Microbiol.* **56:** 113-123.

VOLCANI, Z. (1961): A maize stalk disease caused by a strain of *Erwinia caratovora* in Israel. *J. Agr. Research.* **11:** 179-183.

ZACHOS, D.G., PANAGOPOULOS, G.G. and S.A. MAKRIS. (1963): A disease of maize in Greece caused by *Erwinia caratovora* (Jones) Holland. *Ann. Inst. Phytopath. Benaki,* **5:** 288-293.

(Received 11 January 1982)