New Bacterial Pathogen Causing Blight of Cassava in Southern Africa

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ABSTRACT

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Cultures from lesions similar to bacterial blight from cassava plantings in Natal and Swaziland yielded a yellow-pigmented organism showing characteristics of the genus *Erwinia*. Pathogenicity was established by induction of similar lesions in experimental plants by leaf inoculation. Field and laboratory symptoms were similar. Symptoms progressed from angular chlorotic lesions to tip and margin necrosis followed by wilting, and finally, abscission. Plant death occurred 5 wk after inoculation in the laboratory. Taxonomic profiles of isolates suggest similarity to the *Erwinia herbicola-Enterobacter agglomerans* complex.

Cassava (*Manihot esculenta* Crantz) has been grown on a small scale in southern Africa for many years. In the past decade, this crop has expanded markedly as a commercial source of both human and animal foods. Cassava bacterial blight (CBB), caused by *Xanthomonas campestris* pv. *manihotis* (Berthet & Bondar) Dye, can be a limiting factor in successful cultivation of cassava throughout the subtropics (5) but has only recently been reported in South Africa from large commercial plantations (7). Of lesser economic importance is an infection by another xanthomonad, *X. campestris* pv. *cassavae* (Wiehe & Dowson) Maraite & Weyns, which causes leaf necrosis of cassava in eastern Africa (11). Sporadic reports of crop losses from a blight disease similar in field symptoms to those of CBB necessitated this investigation.

The initial symptoms are small, often angular chlorotic lesions followed by marginal curling and tip necrosis. After this stage, leaves rapidly wilt and abscise. Tips of young shoots can also become necrotic in severe cases. B. Beck (*personal communication*) has recorded large numbers of plants in commercial plantations dying within 8 wk after leaf symptoms appear. The disease occurs sporadically in southern Africa and has been recorded in areas as far apart as the Transvaal Lowveld and Swaziland. This paper reports a blight disease similar to CBB caused by a species in the *Erwinia herbicola* (Lohnis) Dye-*Enterobacter agglomerans* (Ewing & Fife) complex.

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MATERIALS AND METHODS

Isolation and characterization of pathogen. Wilted and dead leaves, and stems with tip necrosis, were collected from cassava plants in Natal (South Africa) and Swaziland. Tissue was surface-sterilized with 2.5% (v/v) sodium hypochlorite, surface-dried, and aseptically cut into 1-cm^2 pieces that were placed into nutrient broth (Oxoid) containing 5% D-glucose. After 18 hr of incubation at 28 C, the broth was streaked onto the same medium containing 1% agar and incubated for 48 hr. Discrete colonies were restreaked onto agar plates twice before being used for biochemical characterization and pathogenicity tests. Initial morphological characterization was made using Gram's stain and from ultramicrographs of preparations negatively stained with 3% phosphotungstic acid to demonstrate flagellation. Biochemical characterization was done using the API-20E system (API System S.A., La Balme Les Grottes 38390, Montalieu Vercieu, France), whereas alkaline and acid phosphatase were tested using these galleries in the API-ZYM test system. Anaerobic growth was determined in an anaerobic cabinet on prereduced glucose nutrient agar with a gas phase of 80% nitrogen, 13% carbon dioxide, and 7% hydrogen. Starch hydrolysis, phenylalanine deaminase activity, glucose fermentation, and hydrogen sulphide production were tested as described by MacFaddin (6). Acid production from cellobiose and maltose was tested in fermentation medium (6) containing these sugars at a concentration of 1%. Salt tolerance was tested in nutrient broth containing 10% sodium chloride. Pectate degradation was determined by the method of Starr (10) with sodium polypectate (Raltech). Yeast extract-glucose-calcium carbonate agar (YGCA) was used to define pigment production and maintain stock cultures. Biochemical profiles were compared with those obtained from reference cultures of selected strains of *Erwinia herbicola* (EC11 [ATCC 23372], EH116 [ATCC 14537], EM101 [ATCC 23374], and EH101]), and strain XM4 (ATCC 23380) of *X. campestris* pv. *manihotis*, all donated by M. P. Starr.

Serology. An overnight culture grown in 5% glucose nutrient broth was washed in saline and heat-treated for 30 min at 60 C. A hyperimmune rabbit serum was obtained by intravenous injection of 1 ml of newly prepared bacterial suspension on alternate days for 14 days and bleeding 7 days later. After decomplementation, this serum was used in tube agglutination tests.

Inoculation procedures. Ten-week-old rooted cuttings from symptom-free plants were used as test plants. Four inoculation procedures using about 10^8 bacteria per milliliter (Macfarland tube no. 5) in nutrient broth were carried out: 1) mixed celite and culture rubbed on the leaf surface and left in situ, 2) culture without abrasive rubbed on leaf surface, 3) injection of 1 ml of culture with a 26-gauge needle into stem or leaf vein, and 4) root inoculation effected by pouring 100 ml of culture into the soil and wounding roots by repeated insertion of a knife into the soil. Fifteen test plants and five controls were used per treatment. Controls received sterile broth instead of culture. All plants were kept at ambient temperatures (15–26 C) in a 14-hr daylight cycle in a glasshouse. Pure cultures, isolated as described previously from infected plants in this experiment, were used to inoculate a second batch of plants by inoculation method 2.

RESULTS

Primary cultures were yellow, mucoid colonies containing gram-negative, motile, cocco-bacilli 0.8 × 1.9–2.1 μm with variable peritrichous flagella. Growth under anaerobic conditions was similar but slower, requiring 48 hr to produce visible colonies. The temperature optimum lay between 25 and 35 C, although growth occurred at both 11 and

40 C. Results of biochemical tests are compared with reference strains of *E. herbicola* and *X. campestris* pv. *manihotis* in Table 1. In addition, all strains showed catalase but not oxidase activity, and all were positive for alkaline and acid phosphatase.

Root and stem inoculations failed to induce lesions, even after 93 days; leaf vein injection only induced symptoms after 42 days. Ten days after leaf inoculation by each technique, leaves showed marginal curling and small angular chlorotic lesions that became necrotic by the 14th day (Fig. 1). Rapid wilt proceeded from distal to proximal portions of leaves by 17 days, abscission occurred by 30 days, and all infected plants were dead by 5 wk after inoculation. Leaves showing necrotic lesions yielded organisms identical to the original isolate for all biochemical parameters tested. They also reproduced identical lesions on additional cassava plants inoculated by the leaf-rubbing technique. Cross-agglutination showed serological identity of all cassava isolates with *E. herbicola* strains EH101 and EC11. None of the other type cultures reacted with this antiserum.

Table 1. Comparative biochemical reactions of bacteria associated with blighted cassava

Characteristic	Cassava isolate	*Erwinia herbicola* (EC11, EH101, EH116, EM101)	*Xanthomonas campestris* pv. *manihotis* (XM4)
Urease	–[a]	–	–
H_2S production	–	–	+
Nitrate reduction	+	+	–
Indole production	–	–	–
Acetoin production	+	+	–
Glucose (acid)	+	+	+
Glucose (gas)	+	–	–
Fermentative	+	+	–
Arabinose (acid)	+	+	+
Sucrose (acid)	+	+	+
Mannitol (acid)	+	+	–
Inositol (acid)	–	–	–
Sorbitol (acid)	+	+	–
Maltose (acid)	+	+	–
Rhamnose (acid)	+	+	–
Melibiose (acid)	+	–	+
Cellobiose (acid)	+	–	+
Starch hydrolysis	–	–	+
Phenylalanine deaminse	–	+	–
Citrate utilization	+	–	+
Pectate degradation	–	–	+
Gelatin hydrolysis	–	–	+
Arginine dihydrolase	–	–	–
Lysine decarboxylase	–	–	–
Salt tolerance (10% NaCl)	+	+	–

[a] + = Positive result and – = negative result for biochemical characteristic.

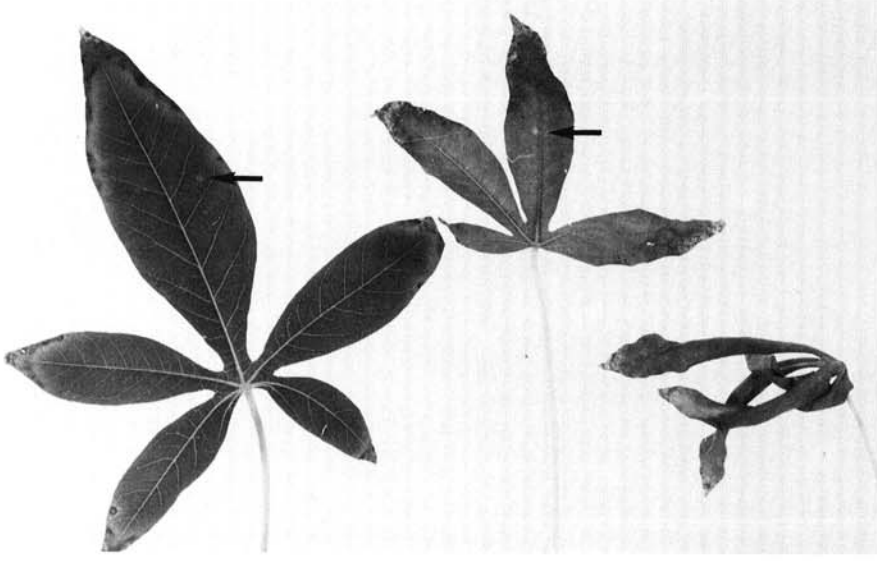

Fig. 1. Cassava leaves 10, 18, and 30 days after inoculation, showing chlorotic lesions (arrows), advancing tip necrosis, and marginal curling.

DISCUSSION

Although the symptomatology of this disease is sufficiently similar to CBB to cause confusion in the field, important differences were revealed. An *Erwinia* species was isolated in pure culture from affected leaves and reproduced the field symptoms in experimental plants infected by leaf inoculation. Symptoms in experimental plants differed from those of CBB in that necrotic lesions appeared later, were never water-soaked, and only 40% exuded gum (7). Root, stem, and leaf vein injections were not effective, whereas leaf-surface abrasion was always effective. Because this disease affects the foliage, we are not surprised that inoculations via routes other than leaves were unsuccessful. The collapse of leaves suggests systemic infection or production of a toxin. The general course of this disease, together with the high mortality rate and ease of transmission, indicate that this pathogenic *Erwinia* species is highly virulent.

Extensive characterization of the organisms isolated from affected cassava plants has failed to define their exact taxonomic status. Peritrichous flagellation, fermentative metabolism, and salt tolerance clearly differentiate them from *X. campestris* pv. *manihotis* (1) and place them in the *E. herbicola* group of Dye's classification (3). Recent extensive studies of this group, however, have shown it to be a heterogeneous collection of strains including some identified as *Enterobacter agglomerans*. Brenner et al (1) demonstrated seven distinct clusters defined by DNA homology encompassing *Erwinia herbicola* strains on the one hand and *Enterobacter agglomerans* on the other. Comprehensive phenotypic characterization of the *Erwinia-Enterobacter* group by Mergaert et at (9) revealed strains of *Erwinia herbicola, Enterobacter agglomerans,* and *Erwinia milletiae* in five subphenons with the respective type strains falling within a single phenon. In an earlier study (8), the same workers showed that a 60-character phenotypic profile of 22 strains of this group failed to yield a single differentiating feature. Unfortunately, phenotypic characterization of the cassava isolate and a selection of type cultures merely added to the confusion. The biochemical profiles of *Erwinia herbicola* strains EH101, EH116, EM101, and EC11 were all similar and in keeping with published data (4). The strain EC11 (ATCC 23372) was included in this study because it was originally isolated from cassava with leaf spot and named *Erwinia cassavae* by Burkholder (2). In producing gas from glucose, the strains that are the subject of this report showed biochemical profiles more similar to the *Enterobacter* group than to typical *Erwinia herbicola*. The API-20E seven-digit code (1205573) derived for these organisms is identical to that of a strain of *Enterobacter agglomerans* isolated from fermenting

wort in South Africa (9). Therefore, until the taxonomy of the *Erwinia herbicola-Enterobacter agglomerans* complex is revised, the full identity of this new cassava pathogen cannot be fully established.

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