Bacterial Foot Rot of Rice Caused by a Strain of *Erwinia chrysanthemi*

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ABSTRACT

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A bacterial disease of rice, "bacterial foot rot," was found in Japan in July 1977. It was characterized by a dark brown decay of the tillers. In the early stages of the disease, brown sheath rot appeared that seemed to spread from the ligule regions. The lesions quickly extended down to nodes, culms, and finally to crowns. Neighboring tillers of the same crown were invaded systemically, causing foot rot symptoms. A soft rot, with an unpleasant odor, developed in young tissues of infected tillers. In the advanced stage, many tillers decayed so that entire diseased plants could be removed easily with a slight pull. The syndrome was similar to the damage caused by the rice stem borer or the "kresek" symptom of bacterial leaf blight. The phenotypic characters of the rice strains were very similar to those of the corn pathovar of *Erwinia chrysanthemi*. The rice strain produced top soft rot or stalk rot on corn seedlings within 1 wk. The corn strain was less virulent on rice, however, suggesting that the rice strain may be a distinct pathovar of the bacterium.

A bacterial disease of rice, not described previously, was first noted in the experimental fields of the National Institute of Genetics, Mishima, Shizuoka, Japan, in July 1977. The early stage of the disease was characterized by deep brown discoloration of the leaf sheaths and yellowing of the leaves extending from these sheaths. The appearance of the discolored sheaths was similar to that of bacterial sheath rot, which can be caused by several different bacteria. However, the new disease differed from bacterial sheath rot in that the causal organism primarily attacked the base of the culm and invaded the crown systemically, resulting in foot rot. The pathogenic bacterium was identical in many respects with *Erwinia chrysanthemi* Burkh., McFadden and Dimock.

This article reports observations on the disease and on the characteristics of the causal organism.

MATERIALS AND METHODS

Bacteria. The bacterial strains used in the study are listed in Table 1.

Media. Unless otherwise mentioned, yeast extract-peptone agar medium was used. All in vitro experiments were done at 28 C, but cultures were maintained at 5 C. For preservation, cultures were lyophilized in 10% skim milk or frozen on yeast extract-dextrose-calcium carbonate agar medium (YDC).

Physiological characteristics. Dye's methods (2,3) were used for the tests of anaerobic growth, utilization of carbohydrates as a sole source of carbon, production of reducing substances from sucrose, effect of NaCl concentration, growth in KCN, growth factor requirement, utilization of asparagine, amino acid decarboxylases, phenylalanine deaminase, catalase, oxidase, lipase, acetoin and urease production, nitrate respiration, H_2S production, hydrolysis of casein and starch, cellulolytic activity, and levan formation. Methyl red and gluconate tests, and tests for mode of utilization of carbon compounds, gelatin liquefaction, indole and tyrosinase production, nitrate reduction, and action on litmus milk were as described by Cowan and Steel (1). Thornley's method (10) was used for the arginine dihydrolase test. Pectate liquefaction was tested in pectate gel medium (11). Temperature relations were analyzed with a temperature-gradient biophotorecorder (Toyo Kagaku Sangyo Co. Ltd., Tokyo). Table 2 shows the results of these studies.

Inoculation tests. The field inoculation tests were made in early August and the greenhouse tests in early August through September.

Rice plants, cv. Kimmaze, in the tillering to booting stages, were used for inoculation. The plants were grown in pots in a greenhouse and in an experimental plot that was irrigated with well water. Bacterial suspensions (10^8 cells/ml), at 0.2 ml per tiller, were injected into leaf sheaths about half way up the plant above water level. Inoculations also were made by spraying the bacterial suspensions over the plants or by puncturing a healthy leaf sheath with a needle through a drop of bacterial suspension. The inoculated plants were left uncovered.

Iris ensata Thunb. var. *hortensis* Makino et Nemoto rhizomes were planted in pots and grown in a greenhouse. The new unfolded leaves were inoculated by puncturing the leaf blades and sheaths.

Corn plants, cv. Golden Cross Bantam T51, were grown in a greenhouse and 30 day old seedlings were inoculated by the injection method described for rice or by pouring a few drops of bacterial suspensions into whorls.

When 30 days old, sorghum seedlings of unknown cultivar were inoculated by the same methods applied for corn.

Slices of bulb onion, potato tubers, and green tomatoes were inoculated and placed in petri dishes to test capabilities of strains to cause soft rot of these tissues.

RESULTS

Symptoms on naturally infected plants. On rice the disease often started from the ligules. A dark brown sheath rot and drooping of the dead leaves were characteristic symptoms. The nodes, culms,

TABLE 1. Hosts and sources of the isolates of *Erwinia* spp.

Strain number	Host	Source and designation
ER1 - ER10	Rice	Isolated by Goto
EI1	Iris	Isolated by Goto
EC1	Chinese cabbage	*E. carotovora* isolated by Goto
Ea	Radish	*E. carotovora* isolated by Goto
ECH1 - ECH3	Chrysanthemum	*E. chrysanthemi* isolated by Goto
SR32[a]		Cornell, NY, *E. chrysanthemi*
SR56[a]	Corn	Egypt
SR61[a]	Corn	Lincoln Co, Wisconsin
SR90[a]	Corn	Pantnagar, India
SR120[a]	Corn	Hawaii
SR140[a]	Corn	Bologna, Italy
SR149[a]	Sugar cane	Australia
SR171[a]	Corn	Colombia
SR260[a]	Corn	South Africa
SR261[a]	Corn	Costa Rica

[a]Provided by A. Kelman.

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TABLE 2. Comparison of physiologic properties of strains of *Erwinia chrysanthemi* and *Erwinia carotovora*

Characteristics	*E. chrysanthemi* from:[a]			*E. carotovora*[a]	
	Rice	Corn	Chrysanthemum	Ea	EC1
Flagellation	P	P	P	P	P
Anaerobic growth	+	+	+	+	−
Capsule	−	−	−	−	−
Gram stain	−	−	−	−	−
Gas from glucose	+	+	+	−	−
Hugh-Leifson test	F	F	F	F	F
H_2S production	+	+(5/10)[b]	−	−	−
Gelatin liquefaction	+	+	+	+	+
Nitrate reduction	+	+	+	+	+
Denitrification	−	−	−	−	−
Red. sub. from sucrose	+	+	+	+	−
Gluconate test	−	−	−	+	−
Kovacs' oxidase	−	−	−	−	−
Catalase	+	+	+	+	−
Litmus milk	RCPD	RCPD	RCPD	RCPD	RCPD
Hydrolysis of starch	−	−	−	−	−
Hydrolysis of casein	+	+	+	+	+
Growth in 5% NaCl	−	−	−	+	+
Growth at 40 C	+	+	−	−	−
Growth in KCN	+	+(7/10)	+	−	−
Arginine dihydrolase	−	−	−	−	−
Decarboxylase:					
Arginine	−	+(3/10)	+	+	−
Lysine	−	−	−	−	−
Glutamic acid	−	−	−	−	−
Ornithine	−	−	−	−	−
Phenylalaine deaminase	−	−	−	−	−
Urease	−	−	−	−	−
Blue pigment on YDC[c]	−	+(2/10)	+	−	−
Brown pigment on PSA[d]	+	+(5/10)	−	−	−
Methyl red test	+	+(4/10)	+	+	+
Acetoin production	+	+	+	−	+
CMC[e] liquefaction	+	+	+	+	+
Phosphatase	+	+	+	+	−
Pectate liquefaction	+	+	+	+	+
Hydrolysis of:					
Tween 80	+	+(5/10)	−	−	−
Cottonseed oil	−	−	−	−	−
Polygalacturonate utilization	+	+	+	+	+
Potato soft rot	+	+(9/10)	+	+	+
Tobacco hypersensitivity	+	+(6/10)	−	+	−
Utilization of:					
Xylose	+	+	+	+	+
Ribose	+	+	+	+	+
Rhamnose	+	+	+	+	+
Arabinose	+	+	+	+	+
Mannose	+	+	+	+	+
Galactose	+	+	+	+	+
Fructose	+	+	+	+	+
Glucose	+	+	+	+	+
Lactose	+d[f]	+d[f]	+d[f]	+	+
Melibiose	+	+	+	+	+
Cellobiose	+	+	+	+	+
Trehalose	−	−	−	+	+
Sucrose	+	+	+	+	+
Maltose	−	−	−	−	−
Raffinose	+	+	+	+	+
Melezitose	−	−	−	−	−
Starch	−	−	−	−	−
Inulin	−	−	−	−	−
Glycogen	−	−	−	−	−
Xylan	−	−	−	−	−
Dextrin	−	−	−	−	−
Glycerol	+	+	+	+	−
Mannitol	+	+	+	+	+
Inositol	+	+(7/10)	+	+	−
Sorbitol	−	−	−	+	−
Dulcitol	−	−	−	−	−
Aesculin	+	+	+	+	+
Salicin	+	+	+	+	+
α-Methyl glucoside	−	−	−	−	−
Fumarate	+	+	+	+	+
Acetate	+	+	+	+	−
Lactate	+	+	+	+	+

(continued)

TABLE 2. (continued)

Characteristics	*E. chrysanthemi* from:[a]			*E. carotovora*[a]	
	Rice	Corn	Chrysanthemum	Ea	EC1
Citrate	+	+	+	−	+
Formate	+	+	+	+	+
Malate	+	+	+	+	+
Succinate	+	+	+	+	+
Propionate	−	−	−	−	−
Oxalate	−	−	−	−	−
Malonate	−	+(9/10)	+	+	−
Benzoate	−	−	−	−	−
Tartrate	−	+(7/10)	−	−	−
Maximum growth temperature (C)	43	43	38	37	37
Optimum growth temperature (C)	36	36	30	30	31
Pathogenicity on:					
Rice	+	+(3/10)	−	−	−
Corn	+	+(3/10)[g]	−	−	−
Chrysanthemum	−	−	+	−	−

[a]P = peritrichous, F = fermentative, RCPD = red, curd formation, peptonized and discolored.
[b](Number of positive reactions/10 isolates).
[c]YDC = yeast extract dextrose calcium carbonate agar.
[d]PSA = potato sucrose agar.
[e]CMC = carboxymethyl cellulose.
[f]Delayed utilization.
[g]Stalk rot.

and crowns also were decayed so that the diseased tillers pulled out easily. Infected nodes turned black. Longitudinal sections of infected culms showed dark brown decay with bacterial ooze on the inner surface. A strong, unpleasant odor was produced. The tissues of the upper nodes and the folded younger leaves were soft-rotted and collapsed. The leaf blades yellowed and finally died as the lesions of the leaf sheaths extended upward. When the crown was invaded systemically, the younger leaves often showed wilting with slight discoloration, and only a few tillers were green in the advanced stage. However, even the healthy-looking tillers were already attacked in the basal parts of the culms connected to the crown. The roots attached to the diseased nodes decayed, became dark brown, and could easily be pulled off. The most severe infection occurred on a breeding line of cv. Taichung 65. From the diseased leaf sheaths, culms, and crowns, one type of *Erwinia* was isolated consistently on nutrient agar plates.

Iris plants growing in the vicinity of the experimental plots showed a reddish brown to dark brown decay at the foot of leaves, leaf sheaths, and flower stalks. In advanced stages of the disease, the vascular bundles of the basal parts of the plants became exposed, but infections of the rhizome were less extensive, causing brown discoloration restricted to the surface layer. Two kinds of pathogenic bacteria, an *Erwinia* and a fluorescent pseudomonad, were isolated from the diseased tissues.

Symptoms on artificially inoculated plants. *Rice.* Symptoms identical to those described were produced after inoculation with strains ER1 to ER10 and EI1. When bacterial suspensions were injected into leaf sheaths, the initial symptoms appeared within 20 hr as water-soaked lesions around inoculation points. The youngest folded leaves started to wilt in 2 days. Within 3–4 days, lesions extended to the whole length of the leaf sheath, causing a dark brown decay and wilting of a few younger leaves. The wilted younger leaves showed soft rot in the basal area near the growing points. The nodes, culms, and crowns then became infected through the diseased leaf sheaths and decayed dark brown. As the disease advanced, the number of dead tillers increased. When the plants were sprayed with bacterial suspensions, dark brown lesions started to develop from the site of the ligule 2–3 days after inoculation. Strains SR120, SR61, and SR56 from corn induced brown sheath rot on rice seedlings. Symptoms were mild, however, and systemic decay of the tillers did not develop. Other isolates from corn showed no pathogenicity on rice either by the injection method or the puncturing method.

Corn. Isolates ER1 to ER10, EI1, SR61, SR171, and SR120 produced water-soaked, greenish brown to dark brown lesions on the leaf sheaths around the punctures. The youngest leaf in the whorl started to wilt in 48 hr. The disease progressed quickly, causing wilt of the upper leaves and soft rot of the stalk. Such plants fell over within 48–72 hr. The base of the stalk became so soft that the tops of the plants pulled out easily. This syndrome was exactly as described by Hoppe and Kelman (7). The incubation period to reach the stalk rot symptom was about 48 hr for 2 wk old seedlings and 3–5 days for 4 wk old seedlings. Stalk rot also was induced by pouring a few drops of bacterial suspensions into the whorl. The symptoms developed 7–10 days after inoculation of 30 day old seedlings. Strains SR140, SR149, SR260, and SR261 produced water-soaked stripes on young unfolding leaves, but these lesions did not become soft-rotted. Isolates SR32, SR56, and SR90 were avirulent on corn. Isolates ECH1, ECH2, ECH3, Ea, and EC1 produced yellowish chlorotic spots around punctures on new leaves expanding from the whorl. The lesions did not enlarge, however, and the chlorotic symptoms soon disappeared.

Strains ER1 to ER10 and EI1 also were strongly virulent on sorghum and iris causing top rot or leaf blight. These strains also caused soft rot on slices of potato, bulb onion, and green tomato within 24 hr, but not on the young shoots of chrysanthemum. Strains of *E. carotovora* and *E. chrysanthemi* were not virulent on sorghum and iris plants. All strains except SR32, SR149, SR171, SR261, and EC1 induced the hypersensitive reaction on tobacco leaves (cv. Bright Yellow) 6–8 hr after infiltrating the bacterial suspensions at 10^8 cells/ml.

Physiological characteristics. The bacterial cultures isolated from rice and iris plants were all identical in morphological, cultural, and biochemical properties. The bacterial cells were motile with four to six peritrichous flagella. Grayish white, circular, amoeboid, or sometimes rhizoid colonies formed in 24 hr on yeast extract peptone agar plates. Variants forming small mucoid colony types appeared in old cultures, particularly in liquid media. On YDC medium, colonies turned brown to dark brown without discoloring the medium. On potato-sucrose agar, however, a diffusible dark brown pigment was produced in 1 wk. The physiological characteristics of these strains were compared with those of two isolates of *E. carotovora*, three of *E. chrysanthemi*, and 10 of the corn pathovar of *E. chrysanthemi* (Table 2).

DISCUSSION

Symptoms on the rice leaf sheaths resembled those of the various bacterial sheath rots that have been attributed to *Pseudomonas oryzicola* (8), *P. fuscovaginae* (9), and *P. panici* (4). Several cultures

of a fluorescent pseudomonad, which was isolated from iris plants, also were pathogenic on rice and caused discoloration of leaf sheaths; but the lesions usually were restricted to leaf sheaths and did not extend to culms and crowns. In contrast, our *Erwinia* infected the rice root crown systemically, resulting in severe decay at the foot of the plant.

The *Erwinia* from rice seemed closely related to *E. chrysanthemi* isolated from corn (6,7, R. S. Dickey, *personal communication*), because it was virulent on corn, causing top or stalk rot or both. However, the corn pathovar of *E. chrysanthemi* differed from the rice pathogen; only a few of the 10 isolates from corn could induce mild symptoms on rice. Although the disease severity varied depending on the cultivars used and the conditions after inoculation, the virulence of the corn pathovar on rice was clearly less than that of the rice strain. This suggests that the rice strain is a distinct pathovar of the bacterium, although there is a close similarity between the corn pathovar and the rice strains in their pathogenicity to corn.

The phenotypic characteristics of the rice bacterium were similar to those of *E. chrysanthemi* isolated from either chrysanthemum or corn. The rice strain differed from the strains isolated from corn or chrysanthemum in characteristics such as pigmentation, H_2S production, growth in KCN, methyl red test, utilization of inositol, malonate, tartrate, and pathogenicity on rice. Such differences are not significant because *E. chrysanthemi* is a collective species that includes a number of strains with diverse characteristics (R. S. Dickey, *personal communication*). Furthermore, the rice and corn isolates had the same optimum and maximum growth temperatures of 36 and 43 C, respectively. For other isolates of *E. chrysanthemi* and *E. carotovora*, however, these temperatures were 30 and 37 C, respectively. Thus, the rice and corn isolates were clearly separated from other strains of *E. chrysanthemi*. Because of the diveristy in the corn pathovar, the present *Erwinia* from rice can be considered to belong to the same biovar as the corn strain of *E. chrysanthemi*.

The optimum and maximum growth temperatures of the corn pathovar were considerably higher than those of the other plant pathogenic bacteria. Although this does not necessarily mean that the disease occurs only at high temperatures, it is likely it also occurs on rice in the tropics. The syndrome of bacterial foot rot is similar to that of the "kresek" symptom of bacterial leaf blight caused by *Xanthomonas oryzae*. The kresek symptom may or may not follow the leaf blight phase under field conditions. Bacterial foot rot and the kresek phase of bacterial leaf blight may have been confused in the past.

A bacterial disease of rice similar to the present one was observed in Indonesia in 1964 (5). It was restricted to the leaf sheaths of a few tillers. Although the bacteriological characteristics indicated a relationship of the causal organism with *E. chrysanthemi*, it was identified as a strain of *E. carotovora*.

LITERATURE CITED

1. COWAN, S. T., and K. J. STEEL. 1965. Manual for the Identification of Medical Bacteria. Cambridge University Press, London. 217 pp.
2. DYE, D. W. 1968. A taxonomic study of the genus Erwinia. I. The "amylovora" group. N.Z. J. Sci. 11:590-607.
3. DYE, D. W. 1969. A taxonomic study of the genus Erwinia. II. The "carotovora" group. N.Z. J. Sci. 12:81-97.
4. GOTO, M. 1964. Nomenclature of the bacteria causing bacterial leaf streak and bacterial stripe of rice. Rep. Fac. Agric. Shizuoka Univ. 14:3-10.
5. GOTO, M., 1965. A comparative study of the sheath rot bacteria of rice. Ann. Phytopathol. Soc. Jpn. 30:42-45.
6. HARTMAN, J. R., and A. KELMAN. 1973. An improved method for the inoculation of corn with Erwinia spp. Phytopathology 63:658-663.
7. HOPPE, P. E., and A. KELMAN. 1969. Bacterial top and stalk rot disease of corn in Wisconsin. Plant Dis. Rep. 53:66-70.
8. KLEMENT, Z. 1955. A new bacterial disease of rice caused by Pseudomonas oryzicola n. sp. Acta Microbiol. Acad. Sci. Hung. 2:265-274.
9. TANII, A., K. MIYAJIMA, and T. AKITA. 1976. The sheath brown rot disease of rice plant and its causal bacterium, Pseudomonas fuscovaginae A. Tanii, K. Miyajima et T. Akita sp. nov. Ann. Phytopathol. Soc. Jpn. 42:540-548.
10. THORNLEY, M. J. 1960. The differentiation of Pseudomonas from other gram negative bacteria on the basis of arginine metabolism. J. Appl. Bacteriol. 23:37-52.
11. TUITE, J. 1969. Plant Pathological methods Fungi and Bacteria. Burgess Publishing Company, Minneapolis, MN. 239 pp.