First Detection of *Pseudomonas fuscovaginae* on Maize and Sorghum in Burundi

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ABSTRACT

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Pseudomonas fuscovaginae was isolated from brown rot lesions on leaf sheaths and husks of maize at the silking stage and on flag leaf sheaths of sorghum at the booting stage in smallholders' fields between 1,450 and 2,100 meters above sea level in Burundi. In comparative biochemical, serological, and pathogenicity tests, the strains from maize and sorghum were similar to those previously isolated from rice.

Pseudomonas fuscovaginae Miyajima, Tanii, and Akita nom. rev. 1983 was identified as the causal agent of bacterial sheath brown rot of rice (*Oryza sativa* L.) in Japan (8,9,12). In 1982, this pathogen was isolated for the first time out of Japan from lesions on the Chinese rice cultivar Yunnan 3, recently introduced into highland swamps between 1,450 and 1,600 meters above sea level (masl) in Burundi (2,4). The disease is a major constraint for rice cultivation in this area. *P. fuscovaginae* also was detected on rice in similar ecological locations in Zaire, Rwanda, and Madagascar (*unpublished*), as well as in Latin America (14). Except for the latter, the strains from various origins have similar biochemical, serological, and pathogenic characteristics (4).

Early signs of disease can be detected as dark brown stripes on leaves of rice seedlings, but the most obvious symptoms develop at booting. Irregular water-soaked patches appear on the flag leaf sheath and evolve into gray and, later, dark brown lesions a few millimeters to 20 cm long. Lesions are limited by a black-purple border and at a later stage have grayish, withered centers. Conspicuous lesions on the leaf sheath often are associated with a reduction in elongation of the peduncle. In addition, panicle emergence can be affected, which leads to browning of the glumes and sterility of the lower part of the panicle.

Surveys in Burundi detected large, irregular, purple-brown lesions on sheaths and husks of maize (*Zea mays* L.) resembling purple leaf sheath, previously attributed to saprophytic

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fungi and bacteria growing on pollen and particulate matter lodged between the stalk and the leaf sheath and considered a harmless discoloration (11). This symptom also has been frequently found associated in Burundi with abundant colonization by *Fusarium moniliforme* var. *subglutinans* Wr. & Reink. (teleomorph: *Gibberella fujikuroi* var. *subglutinans* Edwards) (1). Isolations of bacteria similar to *P. fuscovaginae* plus observations of similar symptoms on sorghum (*Sorghum bicolor* (L.) Moench) prompted us to analyze further the association of *P. fuscovaginae* with brown sheath rot symptoms and to compare strains from these plants with those from rice.

MATERIALS AND METHODS

Isolation. Maize and sorghum plants with sheath rot symptoms were collected between January and June 1986 in smallholders' fields in Burundi and assayed within 2 days at ISABU, Bujumbura. A loopful of each sample of 0.4 cm^2 of diseased tissue, macerated in sterile water, was streaked onto King's medium B (KB) (10). Isolation plates were viewed under UV light after incubation at 28 C for 1–3 days. Single colonies of fluorescent pseudomonads, suspected of being *P. fuscovaginae*, and colonies of the most frequently observed other bacteria were streaked on fresh KB plates for purity check. The resulting subcultures were transferred to agar slants of KB and maintained at 4 C until further characterization.

Serological tests. Bacteria were washed with sterile distilled water from 24-hr-old cultures on KB slants, and the concentration was adjusted to 1×10^9 cells per milliliter using a Petroff-Hausser counting chamber. The slide agglutination test was performed by mixing a drop of the bacterial suspension with a drop of a 1/80 dilution of an antiserum against strain HMB266 of *P. fuscovaginae* from Burundi (4). The reaction was considered positive if agglutination was visible to the unaided eye.

Biochemical tests. Seven strains from maize and two strains from sorghum suspected of being *P. fuscovaginae* were compared with the reference strains HMB264 and HMB266 of *P. fuscovaginae* (originally from rice in Burundi) (2,4) in the following biochemical tests: glucose metabolism (3), acid production from inositol and trehalose (5), Kovac's oxidase (3), arginine dihydrolase (13), esculin hydrolysis (7), 2-ketogluconate production (6), and nitrate reduction (3).

Pathogenicity tests. Maize (cv. GPS5) and rice (cv. Yunnan 3) seedlings were grown in pots outside under shade at Bujumbura (800 masl, 20–32 C). For routine pathogenicity testing, maize plants were inoculated at the four- to five-leaf stage by injecting 5 ml of a bacterial suspension (1×10^9 cells per milliliter) between the unfolded leaves. Rice tillers were inoculated at booting by injecting the bacterial suspension into the boot. The effect of saprophytic bacteria and of *F. m.* var. *subglutinans* on symptom expression by *P. fuscovaginae* was also assessed. Maize plants were inoculated with mixtures of cell suspensions of *P. fuscovaginae* strain EPFM1 from maize with four strains of saprophytic bacteria or with a 1×10^6 conidia per milliliter suspension of *F. m.* var. *subglutinans*. Conidia of *F. m.* var. *subglutinans* were collected from a 7-day-old culture on PDA slants of an isolate from maize in Burundi.

Strains of *P. fuscovaginae* from maize (EPFM3) and from sorghum (E139) were compared with strains from rice: 6801 (type strain from Japan, = NCPPB3085, NIAS1177, PDDCC5140), E37 (from seeds supplied by IRRI, Philippines), E124 (from Madagascar), and HMB266 (from Burundi). Strain E13, a fluorescent pseudomonad isolated from rice but not pathogenic on this host (4), and sterile water were used as controls. Strains were cross-inoculated under controlled environmental conditions to maize (cv. GPS5), sorghum (Burundian cv. S90), and rice (cv. Yunnan 3). Seedlings were grown in pots to the four-leaf stage in a greenhouse (20–25 C). Inoculation was by injection of bacterial suspensions (1×10^8 cells per milliliter) between the leaf sheaths at 5 cm above soil level. The injection point on maize and sorghum was covered with petroleum jelly (Vaseline). Plants were then transferred to a moist chamber (17–20 C). Maize plants were transferred to the greenhouse after 5 days of incubation, whereas rice and sorghum plants were kept in the moist chamber. Disease levels were evaluated and recorded 10 days after inoculation.

RESULTS

Symptoms and distribution of the disease. In January 1986, sheath or husk rot symptoms were noticed on scattered maize plants at the silking stage in smallholders' fields at various highland locations in Burundi: Gisha (1,550 masl), Kayanza (1,850 masl), Kizozi (2,100 masl), and Munanira (2,100 masl). The field at Gisha was near rice plots with plants infected by *P. fuscovaginae*, whereas the one at Kizozi was more than 50 km from any rice cultivation. Initial symptoms were glossy, brown-black, water-soaked spots on the adaxial side of leaf sheaths. Lesions were distinct and about 2 mm in diameter but later coalesced to continuous areas up to 20 cm long. Bacteria colonized inside the sheath, with symptoms on the abaxial side visible as patches with a light brown, withered center and a sharp, dark purplish brown border that faded out progressively toward the healthy tissues. The stalk under the infected sheaths was neither discolored nor altered in its growth. Symptoms occurred on ears between layers of the outer husks and extended to the inner layer without visibly affecting grain formation.

In May 1986, similar leaf sheath rot lesions, but with a reddish brown border, were detected on several sorghum plants at booting stage in farmers' fields at Kayanza and Munanira. Occasionally, inhibition of panicle emergence and large grayish, water-soaked lesions on the flag leaf sheath were observed as for *P. fuscovaginae* infections on rice.

Association of *P. fuscovaginae* with the observed symptoms. In transverse sections through samples taken at the margin of the lesions on maize or sorghum sheaths and examined under the microscope, browning of the cells was always visible on the adaxial side of the sheaths, whereas the abaxial side appeared healthy. This suggests that infection progresses from the inside to the outside of the sheaths. Bacterial ooze was noted for sections from all analyzed lesions. *F. m.* var. *subglutinans* was found associated with the lesions on maize but not with those on sorghum.

Bacterial isolations were done from

Table 1. Comparison of reference strains HMB264 and HMB266 of *Pseudomonas fuscovaginae* from rice with isolates from sheath brown rot on maize and sorghum in Burundi

Characteristics	HMB264 and HMB266	Maize (7 strains)	Sorghum (2 strains)
Yellow-brown fluorescence on King's medium B	+	+	+
Agglutination with HMB266 antiserum	+	+	+
Oxidative metabolism of glucose	+	+	+
Acid production from inositol	−	−	−
trehalose	+	+	+
Arginine dihydrolase	+	+	+
Esculin hydrolysis	−	−	−
2-Ketogluconate production	−	−	−
Kovac's oxidase	+	+	+
Nitrate reduction	−	−	−
Pathogenicity on maize	+	+	+
rice	+	+	+
sorghum	+	+	+

lesions of nine maize plants collected at four locations and from two sorghum plants from Munanira. Fluorescent pseudomonads were found in 19 of 27 isolations from maize and in seven of 16 isolations from sorghum. Under near-UV light, small (about 2 mm in diameter), smooth, translucent colonies with a yellow-brown fluorescence could be detected in 12 isolations from seven maize plants and in three isolations from both sorghum plants. This type of colony, similar to colonies of *P. fuscovaginae* from rice, was clearly distinguishable from the whitish colonies with greenish fluorescence of the faster growing, often saprophytic fluorescent pseudomonads. *P. fuscovaginae*-type colonies sometimes accounted for less than 5% of the colonies in the isolation plates.

All subcultures of colonies of the *P. fuscovaginae* type from both maize and sorghum, but none of the other fluorescent pseudomonads, reacted positively with *P. fuscovaginae* antiserum in the slide agglutination tests. They also had the same characteristics as the HMB264 and HMB266 reference strains in the basic biochemical tests (Table 1). They were strictly aerobic, positive for arginine dihydrolase and Kovac's oxidase, but negative for esculin hydrolysis and nitrate reduction. All showed in particular the simultaneous occurrence of no 2-ketogluconate production and of acid production from trehalose but not from inositol. No other fluorescent pseudomonad showed these characteristics.

Pathogenicity. In routine pathogenicity tests on maize, all *P. fuscovaginae*-type colonies from maize and sorghum and the *P. fuscovaginae* reference strains from rice induced a dark brown, watery rot extending after 5 days to 10 cm from the inoculation point on the pseudostem to the leaves and often causing a complete rot of the younger leaf. *P. fuscovaginae* was easily reisolated from the edges of the lesions. None of the other bacteria in the isolation plates induced necrotic lesions on maize extending more than 1 cm from the inoculation point. On rice, typical brown rot lesions extended to 20 cm on the flag leaf sheath 5 days after inoculation; after 8 days, the lesions showed a grayish center, similar to lesions resulting from natural infections or from the type strain of *P. fuscovaginae*.

Mixing *P. fuscovaginae* with other bacteria or conidia of *F. m.* var. *subglutinans* did not increase disease severity on maize; the saprophytic bacterial isolates S1 and S2 even strongly reduced it (Fig. 1). *F. m.* var. *subglutinans* induced browning and distortion of the youngest leaf but no extensive necrosis of the older leaf sheaths.

In inoculations done on maize under controlled environmental conditions in Belgium, rotting and browning of the outer leaf sheath were less conspicuous than in Burundi. Symptoms on the youngest leaf were, nevertheless, well expressed and similar for the various *P. fuscovaginae* strains (Table 2). The cross-inoculation experiments did not reveal any physiological specialization. No symptom was observed on any of the plants after injection of sterile water or inoculation with the saprophytic fluorescent pseudomonad E13.

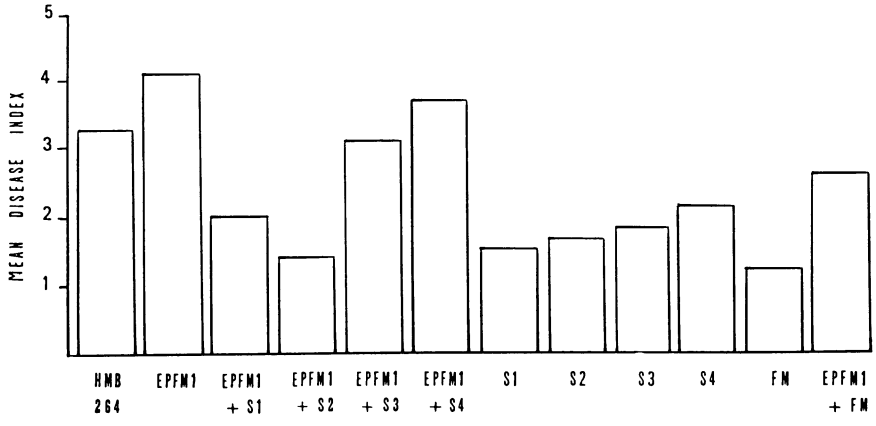

Fig. 1. Disease rating of maize plants 5 days after inoculation with *Pseudomonas fuscovaginae* strains HMB264 from rice or EPFM1 from maize, saprophytic bacterial isolates (S1, S2, S3, S4), and *Fusarium moniliforme* var. *subglutinans* (FM) from maize, alone or in mixture. Disease rating scale: 1 = no symptom, 2 = necrotic lesion less than 1 cm around the inoculation on the outermost leaf sheath, 3 = 1- to 2-cm lesion, 4 = 2- to 5-cm lesion, 5 = lesion larger than 5 cm. Values are based on the means of 10 plants.

Table 2. Disease severity on maize, rice, and sorghum plants after cross-inoculations with strains of *Pseudomonas fuscovaginae* originally isolated from those hosts

Strain	Plant	Country of origin	Disease rating on maize[a]	Length of sheath lesion (mm)[b]	
				Rice	Sorghum
EPFM3	Maize	Burundi	4.2 ± 0.8	2.7 ± 2.0	3.8 ± 1.5
6801	Rice	Japan	3.7 ± 0.8	3.1 ± 2.9	3.3 ± 2.6
E37	Rice	Philippines	4.3 ± 0.8	3.6 ± 2.7	4.1 ± 2.6
E124	Rice	Madagascar	4.5 ± 0.5	3.4 ± 2.5	3.6 ± 1.7
HMB266	Rice	Burundi	4.1 ± 0.5	1.8 ± 1.5	4.0 ± 1.4
E139	Sorghum	Burundi	3.3 ± 0.8	3.0 ± 1.7	3.8 ± 1.8
E13[c]	Rice	Burundi	0	0	0
Water	...	...	0	0	0

[a]Disease rating scale of last unfolded leaf after inoculation: 0 = no symptom around puncture, 1 = humid rot or necrosis less than 1 mm around puncture, 2 = necrosis surrounded by yellow halo less than 1 cm, 3 = necrotic stripe extending 1–2 cm around puncture, 4 = large chlorotic areas on lamina, 5 = large necrotic areas and abnormal unfolding.
[b]Means and standard deviations calculated on 20 seedlings.
[c]Saprophytic fluorescent pseudomonad.

DISCUSSION

The biochemical characteristics of the fluorescent pseudomonads, identified in isolation plates from sheath rot lesions on maize and sorghum by their particular fluorescence under near-UV light, are identical with those of *P. fuscovaginae* isolates from rice. These characteristics were found in previous work (4) to be useful for distinguishing *P. fuscovaginae* from saprophytic fluorescent pseudomonads occurring in rice lesions. The identification of the maize and sorghum isolates as *P. fuscovaginae* is supported by the serological and pathogenicity tests.

In inoculation tests, Miyajima (8) demonstrated pathogenicity of *P. fuscovaginae* on maize and various other grasses. He also isolated epiphytic bacteria from *Agrostis clavata* Trin., *Dactylis glomerata* L., and *Phleum pratense* L. growing near paddy fields. This is the first report of *P. fuscovaginae* in field symptoms on a crop other than rice, however. Without our experience with this bacterium, detection would have been difficult because of the relatively low frequency of *P. fuscovaginae* colonies on the isolation plates. In the test performed with bacteria and *F. m.* var. *subglutinans* from these isolations, only *P. fuscovaginae* was able to reproduce the observed symptoms. Symptom expression was hampered rather than stimulated by the other organisms. These data suggest that the etiology of purple leaf sheath (11) and of leaf sheath rot on Gramineae in other

parts of the world should be reinvestigated.

Isolation of *P. fuscovaginae* distant from locations where rice was recently grown on a large scale suggests a widespread occurrence without direct relation to rice. Its scattered detection in fields and restriction to leaf sheath and husk expansion because of booting or growth of the ear indicate that *P. fuscovaginae* is an opportunistic pathogen whose development and/or symptom induction is favored by certain particular conditions. The previous detection of this pathogen only at the northern limit of rice cultivation in Japan and in the tropics at altitudes exceeding 1,300 masl points out the favorable influence of low temperatures on symptom development. Further surveys are needed to confirm this hypothesis as well as to determine what are the specific stimuli for *P. fuscovaginae* on expanding leaf sheaths.

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