Bacterial Sheath Rot of Wheat Caused by *Pseudomonas fuscovaginae* in the Highlands of Mexico

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ABSTRACT

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Angular, blackish brown lesions, 10 or 20 cm in length, bordered with a purple-black angular area 1–2 mm wide and showing a grayish center, were frequently observed on leaf sheaths of bread wheat, durum wheat, and triticale at the booting stage in the central highlands of Mexico, 2,249–2,640 m above sea level. Fluorescent, strictly aerobic bacteria identified as *Pseudomonas fuscovaginae* (based on their positive reaction for Kovac's oxidase and arginine dihydrolase but negative for esculin hydrolysis and nitrate reduction) were isolated from these lesions. The pathogenic strains from wheat behaved similarly to those isolated from rice in other countries with regard to nonproduction of 2-ketogluconate, acid production from trehalose but not from inositol, agglutination with antiserum against a reference strain, and pathogenicity on rice and wheat.

A disease of unknown etiology was frequently observed on bread wheat (*Triticum aestivum* L.) tillers at the booting stage at Centro de Organisación y Desarollo Agricola en el Estado de Mexico (CODAGEM) and Atizapan Experiment Stations near Toluca, Mexico, (2,640 m above sea level; masl) in August 1987. The symptoms, characterized by irregular, angular, blackish brown lesions bordered by a purple-black water-soaked area, were similar to those of bacterial sheath brown rot caused by *Pseudomonas fuscovaginae* Miyajima, Tanii and Akita on rice (*Oryza sativa* L.; 1,4,12,13).

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Wheat's susceptibility to *P. fuscovaginae* has been demonstrated by artificial inoculations (12). Natural infection of other graminaceae, such as maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* [L.] Moench), has been reported (7). This pathogen was recently found on rice in various Latin American countries including Mexico (19,20), confirming the bacterium's wide geographical distribution (4–6,15). Because the pathogen causing the symptoms in wheat described above had not been identified and because of the water-soaked appearance of young lesions, this study was conducted to check the possible bacterial origin of the disease. The aim of this work was to identify and characterize the causal agent of the sheath rot of wheat in the highlands of Mexico and to assess its distribution and importance.

MATERIALS AND METHODS

The frequency of diseased tillers in 3 m × 0.5 m rows was determined. Samples were collected at CODAGEM Experiment Station in 1987 and in commercial fields around Chalco and Patzcuaro in 1988. On the same day of collection, lesions were examined for bacterial oozing under a dark field at ×100. Loopfuls of lesions that had been crushed in sterile water were streaked on King's medium B (KB; 10) and the plates were incubated at 28 C. After 2 days, single fluorescent colonies were subcultured to fresh plates of KB. Pure colonies were transferred to glucose-yeast-chalk agar slants (16) for conservation at 4 C. Greenish brown fluorescent pigmentation on KB was considered indicative of *P. fuscovaginae*. Isolations were repeated in 1988 from wheat samples collected around Chalco and wheat and triticale (× *triticosecale* Wittmack) samples collected around Patzcuaro in commercial fields.

Pathogenicity of all fluorescent strains was tested on wheat (cv. Seri 82) and triticale (genotype Mus "S") plants grown in the greenhouse in pots up to the five-leaf stage. Plants were pricked at 5 cm above soil level between leaf sheaths with a sterilized needle that had been dipped in a 24-hr-old pure culture on KB agar. The inoculated plants were covered with a plastic bag for 5 days. When symptoms were produced, reisolation was done on KBC agar (KB +

1.5 g of boric acid/l + cephalexin at 0.08 g/l + cycloheximide at 0.2 g/l; 14). Pathogenicity tests were repeated on wheat and triticale plants at the booting stage. A 0.5-ml sample of concentrated bacterial suspension diluted to about 1×10^9 cells/ml as determined in a Petroff-Hausser counting chamber was injected into the boot. The plants were incubated for 1 wk in a humid chamber. Sterile, distilled water was injected in control plants. Pathogenicity on rice was tested at Laboratoire de Phytopathologie, Université Catholique de Louvain (UCL), Belgium, on plants of cv. Yunnan 3 grown in the greenhouse up to the three- to four-leaf stage. Leaf sheaths at 5 cm above the soil level were pricked with a needle that had been dipped as just described. Control plants were inoculated with reference strains of *P. fuscovaginae* (HMB266 from Burundi and 6801 [= NCPPB 3085, PDDCC 5940 type strain] from Japan, the latter provided by K. Miyajima).

Characteristics of the bacterial strains (Tables 1 and 2) were determined by standard biochemical tests (2,8,9,11, 17,18) selected to distinguish *P. fuscovaginae* from other fluorescent pseudomonads (4). Serological tests were performed at UCL. For the slide agglutination test, bacteria from 24-hr-old cultures on KB slants were suspended in sterile, distilled water and diluted to 1×10^9 cells/ml as determined in a Petroff-Hausser counting chamber. A drop of the bacterial suspension was mixed with a drop of a 1/40 dilution of antiserum 334X against *P. fuscovaginae* strain HMB266 (4). The reaction of strains was considered positive if agglutination was visible to the unaided eye and compared with reactions of *P. fuscovaginae* reference strains HMB266 and 6801.

RESULTS

In the field, symptoms included angular, blackish brown lesions on leaf sheaths which were most obvious on flag leaf sheaths. The most severe cases resulted in poor ear emergence and sterility. Lesions were irregular, necrotic, 10 or 20 cm in length, and bordered with a purple-black area measuring 1–2 mm. The center of the lesion appeared grayish and dead (Fig. 1). Under a dark field, bacterial streaming was observed from the border of the lesions. Infection appeared to begin on the adaxial side of the sheath where water was retained in the boot. Stems and leaf laminae were not affected. As lesions aged, they often became infected with secondary fungi.

In counts taken in September 1987 at CODAGEM Experiment Station, the wheat cultivars Anahuac and Seri 82 were the most severely attacked, with 18% and 20% of the tillers affected, respectively. Similar symptoms were also observed on triticale and durum wheat (*Triticum turgidum* L. var. *durum*). In August through September 1988, the same disease was observed in several commercial fields near Chalco and Amecameca (2,300 masl) and around Patzcuaro (2,350 masl). The disease was observed randomly among farmers' fields. The incidence of diseased tillers was less than 0.1% in the areas around Chalco, Amecameca, and Patzcuaro. Symptoms were occasionally observed in the slightly drier Texcoco area (2,249 masl).

In 1987, fluorescent *Pseudomonas* were recovered on KB from 16 of 20 samples of bread wheat and durum wheat from the CODAGEM and Atizapan Experiment Stations. The 24-hr-old cultures obtained from single colonies were white to light brown, smooth colonies measuring 1–2 mm in diameter, with the exception of PF8.2. All except PF8.2 produced a yellow-orange, slightly diffuse pigment visible to the unaided eye which fluoresced under UV light. Older cultures turned brown on KB medium. Strain PF8.2 grew more quickly, producing whitish colonies 2 mm in diameter within 24 hr. A very diffuse greenish fluorescent pigment was produced.

Strains isolated in 1987 from 12 samples were pathogenic on wheat and triticale (Table 1). On young plants, the whole stem was diseased after 5 days and plants collapsed. New tillering from the stem bases occurred as previously observed on wheat inoculated with *P. fuscovaginae* in Belgium (Duveiller et al,

Table 1. References of the identified *Pseudomonas fuscovaginae* from Mexico and type strains from Japan and Burundi

Origin	Year	Host	Strain number
CODAGEM	1987	Bread wheat	PF1.1, PF10.1, PF11.1, PF12.1, PF13.1, PF14.1, PF15.1, PF16.1, PF19.1, PF20.1, PF21.1
Chalco	1988	Bread wheat	PF25.2, PF26.2, PF27.1, PF28.1, PF29.1, PF31.1
Patzcuaro	1988	Bread wheat	PF35.1, PF36.3, PF37.1, PF39.1, PF40.1, PF43.1
Atizapan	1987	Durum wheat	PF8.1
Patzcuaro	1988	Triticale	PF44.1, PF45.1, PF46.1
Japan		Rice	6801
Burundi	1982	Rice	HMB264, HMB266

Table 2. Characteristics of pathogenic fluorescent pseudomonads isolated from brown sheath rot on wheat and triticale in Mexico, compared to *Pseudomonas fuscovaginae* reference strains from Japan (6801) and Burundi (HMB264, HMB266) and to nonpathogenic fluorescent pseudomonads isolated in association with brown sheath rot symptoms

	Pseudomonas fuscovaginae		Nonpathogenic fluorescent pseudomonads	
Characteristics[a]	Mexican strains	HMB264, HMB266 6801	PF8.2[b]	PF38.1, PF41.1[c] PF42.2, PF43.1
Pathogenicity				
Wheat	+	+	−	−
Rice	+	+	ND	ND
Triticale	+	+	−	−
Gram	−	−	−	−
Fluorescence on KB				
Yellow-brown	+	+	−	−
Green, very diffuse	−	−	+	+
Kovac's oxidase	+	+	+	+
Arginine dihydrolase	+	+	+	+
Acid production from:				
Glucose aerobically	+	+	+	+
Glucose anaerobically	−	−	−	−
Trehalose	+	+	+	+
Inositol	−	−	+	+
Sorbitol	−	−	−	ND
Saccharose	−	−	+	ND
Nitrate reduction	−	−	−	−
Levan production	−	−	−	−
Esculin hydrolysis	−	−	−	+
Production of 2-ketogluconate	−[d]	−	+	+
Slide agglutination with *P. fuscovaginae*-HMB266 antiserum	+[d]	+	−	ND

[a] + = Positive reaction, − = Negative reaction, ND = not done.
[b] Strain isolated from durum wheat (Atizapan)
[c] Strains isolated from bread wheat (Chalco)
[d] Not done for PF8.1 isolated from durum wheat in Atizapan

unpublished data). The bacterium was easily reisolated on KBC medium in nearly pure culture. Plants inoculated at the booting stage showed symptoms similar to those observed in the field. Lesions extended more than 10 cm from the point of inoculation. Strain PF8.2 was not pathogenic. The symptoms induced on rice by the wheat strains were similar to those induced by the reference strains of *P. fuscovaginae* from rice. In 1988, pathogenic fluorescent pseudomonads were isolated from six of seven samples of wheat from Chalco, from six of nine from Patzcuaro, and from three of three samples of triticale from Patzcuaro (Table 1). The strains showed no pathogenic specialization.

All strains were gram-negative, strictly aerobic, and positive for arginine dihydrolase and Kovac's oxidase but negative for nitrate reduction (Table 2). Only the pathogenic ones produced acid from trehalose but not from inositol, sorbitol, or saccharose and were negative for levan production, 2-ketogluconate production, and esculin hydrolysis. All pathogenic strains from wheat and triticale reacted positively with antiserum to *P. fuscovaginae* as did the reference strains from Burundi and Japan (Table 2). The nonpathogenic, fluorescent bacteria, such as PF8.2, PF38.1, PF41.1, PF42.2, and PF43.1, frequently isolated from brown sheath rot symptoms in wheat in association with pathogenic strains, always produced 2-ketogluconate and acid from inositol (Table 2).

DISCUSSION

A pathogenic bacterium was isolated from a sheath rot on wheat in the central highlands of Mexico. The bacterium was identified as *P. fuscovaginae*, the causal agent of the bacterial sheath brown rot of rice (4,12), sorghum, and maize (7). The identification was based on phenotypical (13), serological, and pathogenic characteristics (4). This pathogen is known to be pathogenic by inoculation on other cereal crops and was isolated from various grasses growing near paddy fields in Japan (12). In previous studies, *P. fuscovaginae* was distinguished from other fluorescent pseudomonads that were oxidase-positive and arginine dihydrolase-positive by the absence of production of 2-ketogluconate, and by the acid production from trehalose but not from inositol (4,6). The interest of these basic identification tests is thus confirmed.

Fig. 1. Bacterial sheath rot of wheat observed in August–September 1987 at CODAGEM Experiment Station near Toluca, Mexico.

This is the first report of *P. fuscovaginae* as causal agent of sheath rot on wheat under natural conditions. Previously, wheat susceptibility was demonstrated by artificial inoculation (12). These inoculations as well as the recent isolations from maize and sorghum (7) and from the graminaceous weed *Leersia hexandra* Sw. in Burundi (Maraite, *unpublished data*) suggest a wide host range for *P. fuscovaginae* among the graminaceae.

In all analyzed cases up until now, *P. fuscovaginae* has been found associated with lesions on expanding leaf sheaths or husks, as well as with discolored grains (4,19). On rice, where seed transmission was demonstrated (4,19,20), severe symptoms develop only on flag leaf sheaths. The particular susceptibility of the booting stage is confirmed in wheat. The mechanism, enhanced multiplication of bacteria caused by accumulation of water in the boot and release of nutrients, or increased sensitivity of the leaf sheath tissues, is not yet known.

Low temperatures and high humidity at booting-heading stage are considered favorable to bacterial sheath brown rot of rice in Japan (12) and Africa (1). The area where *P. fuscovaginae* was detected in Mexico is also characterized by a humid tropical highland environment with cool night temperatures and high rainfall. At Atizapan during the second half of July 1988, the average maximum and minimum temperatures were 19 C and 9 C, respectively. Almost 60% of the total annual rainfall (an annual average of 899 mm in 1975–82) occurs from June to August.

CIAT reported isolation of *P. fuscovaginae* from samples of discolored rice seed and/or leaf sheaths collected in 20 countries (3). Zeigler reported the isolation of the bacterium from Colombia and Surinam (19), but the precise field locations were not given. On the other hand, strains identified as *P. fuscovaginae* by Zeigler in Latin America did not react with the HMB266 antiserum, which did agglutinate with the strains of *P. fuscovaginae* from Japan, Burundi, Rwanda, Madagascar, and Mexico (Maraite, *unpublished data*). A more precise characterization of the ecological requirements of the various strains identified as *P. fuscovaginae* is needed.

Symptoms on wheat similar to those now known to be caused by *P. fuscovaginae* have been observed in the humid highlands of Mexico since the 1970s (H. J. Dubin and L. I. Gilchrist S., *personal communication*). In 1987, the presence of *P. fuscovaginae* on rice in Mexico was suggested but not proven (19). These previous reports and the widespread occurrence of *P. fuscovaginae* in this study are evidence that this pathogen is not new to Mexico. Its recent identification in places as different as Japan, Central Africa, Madagascar, and various Latin American countries indicates that it is an ubiquitous, opportunistic pathogen requiring the conjunction of a crop at the susceptible growth stage, a sufficient amount of inoculum, and favorable environment for disease development.

The overall low incidence, despite its widespread and most likely ancient origin in the Mexican highlands, is suggestive that bacterial sheath brown rot will probably not be a major constraint to wheat production. However, severe attack observed at CODAGEM on some cultivars is evidence that the disease should not be ignored. The conditions conducive to its development should be analyzed in order to avoid a situation where, through changed farming practices or release of particularly susceptible genotypes to disease-prone areas, *P. fuscovaginae* evolves, as for rice, from a minor pathogen to a major constraint.

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