# Bacterial Diseases of Rice. I. Pathogenic Bacteria Associated with Sheath Rot Complex and Grain Discoloration of Rice in the Philippines

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**ABSTRACT**

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Surveys were made to determine if bacterial pathogens were associated with grain discoloration and leaf sheath rot of rice in the major rice-growing districts in the Philippines. In 1988 and 1989, 304 diseased plant and grain samples were collected from 16 different provinces, and bacterial pathogens were found in 64 samples representing 12 of the provinces. In other cases, sheath rot or grain discoloration was attributed to fungal disease, insects, or abiotic factors. Pathogenic bacteria (204 strains) represented 3.6% of the total number of strains isolated and tested. Inoculations into the leaf sheaths of seedlings or the flag leaf sheaths produced symptoms characteristic of infection by *Burkholderia glumae* (formerly *Pseudomonas glumae*) and *Pseudomonas fuscovaginae*. None of the pathogens could be associated with distinctive symptoms. Strains of putative *P. fuscovaginae* were isolated from samples collected from the tropical lowland provinces of Laguna, Palawan, and Davao, and from the tropical highland (above 950 m) province of Ifugao. Since bacterial pathogens were isolated from 21% of the collections with sheath rot or grain discoloration, bacteria appear important in these disease complexes in the Philippines.

Sheath rot complex and grain discoloration describe the disease of rice (*Oryza sativa* L.) that appears as brown discoloration or rot of the flag leaf sheath and discoloration of the grain. The disease seems widespread and more prevalent in tropical Asia since the introduction of modern semidwarf and photoperiod-insensitive cultivars (5). The disease is especially apparent during the rainy season, and the intensity of infection varies from mild to severe (8).

The causal agent(s) of this disease have not been determined with certainty. Grain discoloration has been ascribed to rice bugs in West Africa and to adverse abiotic factors (21). Recently, several fluorescent and nonfluorescent pseudomonads, including *Pseudomonas glumae*, reclassified as *Burkholderia glumae* (20), and *Pseudomonas fuscovaginae,* have been associated with sheath rot and grain discoloration in the tropics (3,15,23). *B. glumae*, which causes both seedling rot and grain rot, also known as glume blight (grayish discoloration of the grain), was first reported in Japan and later in other Asian countries (4,19). Seedling rot on seedlings raised in nursery boxes for mechanical transplanting is common in northeast Japan; while grain rot, occurring at the flowering stage under high temperature and high humidity, is common in southwest Japan (S. Takaya, *personal communication*). *P. fuscovaginae*, which causes sheath brown rot, was first reported by Tanii et al. (17) in Hokkaido, the northern part of Japan. Initially, the disease was related to cold stress in temperate rice growing environments until *P. fuscovaginae* was found in Columbia, Latin America (23); and in Burundi and Madagascar, Africa (3,15). It was also detected from seeds sent to Burundi from the Philippines (3).

Sheath rot is a fungal disease of rice caused by *Sarocladium oryzae* (Sawada) W. Gams & D. Hawksworth, described here for convenience as Sarocladium sheath rot, which is well documented in the literature (13). *S. oryzae* and other fungal pathogens, such as *Bipolaris oryzae* (Breda de Haan) Shoemaker and *Fusarium* spp., were also isolated from discolored seeds (9). The frequency of isolating *S. oryzae* from discolored seeds was lower than 10% (7). In the tropics, the symptoms of sheath rot complex, bacterial sheath brown rot, and Sarocladium sheath rot are difficult to differentiate based on descriptions in the literature. The typical bacterial sheath brown rot symptoms described by Tanii et al. (17) have not been observed on tropical rice.

At a workshop held at IRRI, scientists experienced with bacterial sheath brown rot found plants with symptoms of the disease. Both *Pseudomonas* spp. and *S. oryzae* were readily isolated from sheath tissues showing brown to dark discoloration and rotting on plants with Sarocladium sheath rot.

*B. glumae* and *P. fuscovaginae* appear to cause similar symptoms in the tropics. *Pseudomonas avenae*, reclassified as *Acidovorax avenae* subsp. *avenae* (22), causes bacterial stripe and has long been recorded in the tropics (13,18). Although the abovementioned bacterial pathogens are described in the literature as causing distinct symptoms, the differentiation between these symptoms is not clear. Since pathogens cannot be uniquely associated with symptoms, the present study was designed to investigate the occurrence of pathogenic bacteria involved in the sheath rot complex and in grain discoloration. The specific objective was to determine if *P. fuscovaginae* and other pathogenic pseudomonads were indeed present in the Philippines.

## MATERIALS AND METHODS

**Isolation of bacteria from plant material.** Plants in the ripening stage with sheath rot and grain discoloration were collected throughout the Philippines during the wet seasons of 1988 and 1989. The number of samples collected in a particular province corresponded with the availability of sampling sites at that location. Sheath and grain material collected from 10 hills per field composed one sample. The collected samples contained a wide range of sheath and/or grain symptoms, varying from translucent to brown dots to brown blotches to brown streaks to a completely brown sheath, and/or clear to brown spots to brown blotches to completely dark discolored seeds. A total of 304 samples were collected from rice grown in 16 different provinces. The discolored sheaths and grains were examined under a stereomicroscope, and material infested with fungi was discarded, as those discolorations were attributed to *S. oryzae*. (Only seven samples were excluded for that reason.) Small segments of sheath from the region between discolored and

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adjacent healthy tissue were plated directly on King's medium B (6) and incubated at 28°C for 1 to 2 days. Any bacterial growth was restreaked to separate colonies.

To provide additional assays of grain in field samples, about 20 seeds from each sample were crushed in 10 ml of sterile water. The suspension was shaken for 5 min at room temperature. Loopfuls of the suspension were streaked onto King's medium B. Two grain samples of 1 kg each of the varieties IR54 (Los Baños, dry season 1989) and IR8866 (Banaue, same season) were sorted into discolored and symptomless seeds. Samples (100 g) of each were macerated and thoroughly mixed in 500 ml of sterile distilled water containing 0.025% Tween 20 and shaken for 2 h at room temperature. Portions (100 µl) from decimally diluted seed-soak were streaked on nutrient agar and incubated at 28°C for 2 to 3 days.

Three to five predominant colony types and all fluorescent colony types were purified by repeated streaking and kept on nutrient agar (Difco) slants. For long-term storage, the strains were suspended in 10% skimmed milk at 4°C or in nutrient broth with 30% glycerol at –70°C. All pathogenic strains were also lyophilized.

**Biochemical characteristics.** The pathogenic strains were divided into fluorescent and nonfluorescent groups based on fluorescent pigment production on King's medium B. One-day-old nutrient agar cultures were used for the Gram staining, Kovac's oxidase reaction, and for the inoculation of Hugh and Leifson's OF medium (2).

**Pathogenicity test.** Inoculations were made on greenhouse-grown rice seedlings. Seeds of TN1 were washed for 30 s in 70% ethanol and then rinsed three times in sterile distilled water. Seeds were germinated on wet paper towels in a growth chamber at 28°C under artificial light (16 h/day). After 1 week, the seedlings were transplanted into plastic containers filled with sterilized soil. After transplanting, plants were grown in the greenhouse under natural light, with day/night temperatures of about 30/20°C and a relative humidity of 40 to 65%.

The first screening of the strains consisted of injecting an overnight-grown nutrient broth culture into the leaf sheath of 21-day-old seedlings. In later screenings, bacterial cells collected by centrifugation of a nutrient broth culture were resuspended in sterile water to a concentration of ca. $10^8$ CFU/ml. For the control, sterile water was injected into the plant sheaths. Symptoms were observed 1 week after inoculation. Only strains that produced symptoms on two of three seedlings in a first trial and four of four to eight seedlings in a second trial were retained and tested in three subsequent pathogenicity tests.

The pathogenic strains were also tested on booting cultivar TN1 about 65 days after sowing. Bacteria from an 18-h nutrient broth culture suspended in nutrient broth in sterile water to a concentration of ca. $10^8$ CFU/ml were injected in the flag leaf sheath, and sterile water was injected as a control. Symptoms were observed 2 weeks after inoculation. *A. a.* subsp. *avenae* was detected by the method of Shakya and Chung (16); 5 g of TN1 seeds were added to 50 ml of nutrient broth inoculated with a loopful of freshly grown bacteria scraped from nutrient agar plates of each nonfluorescent strain. The suspension was shaken for 18 h at 28°C. The seeds were then germinated in petri dishes on three layers of filter paper saturated with 230 ppm urea solution. One set was watered afterwards with 225 ppm urea solution; an equivalent set was watered with distilled water only. The seedlings were observed 10 days after sowing.

Further characterization of the pathogenic strains identified by the Biolog system (1) as related to *P. fuscovaginae*, *B. glumae*, and *A. a.* subsp. *avenae* was made by seed-soak inoculation of rice seedlings of cultivars TN1 and IR24. The rice seeds, which had been washed for 30 s in 70% ethanol and rinsed three times in sterile distilled water, were soaked in inoculum ($10^8$ CFU/ml) at 25°C for 2 h. For the

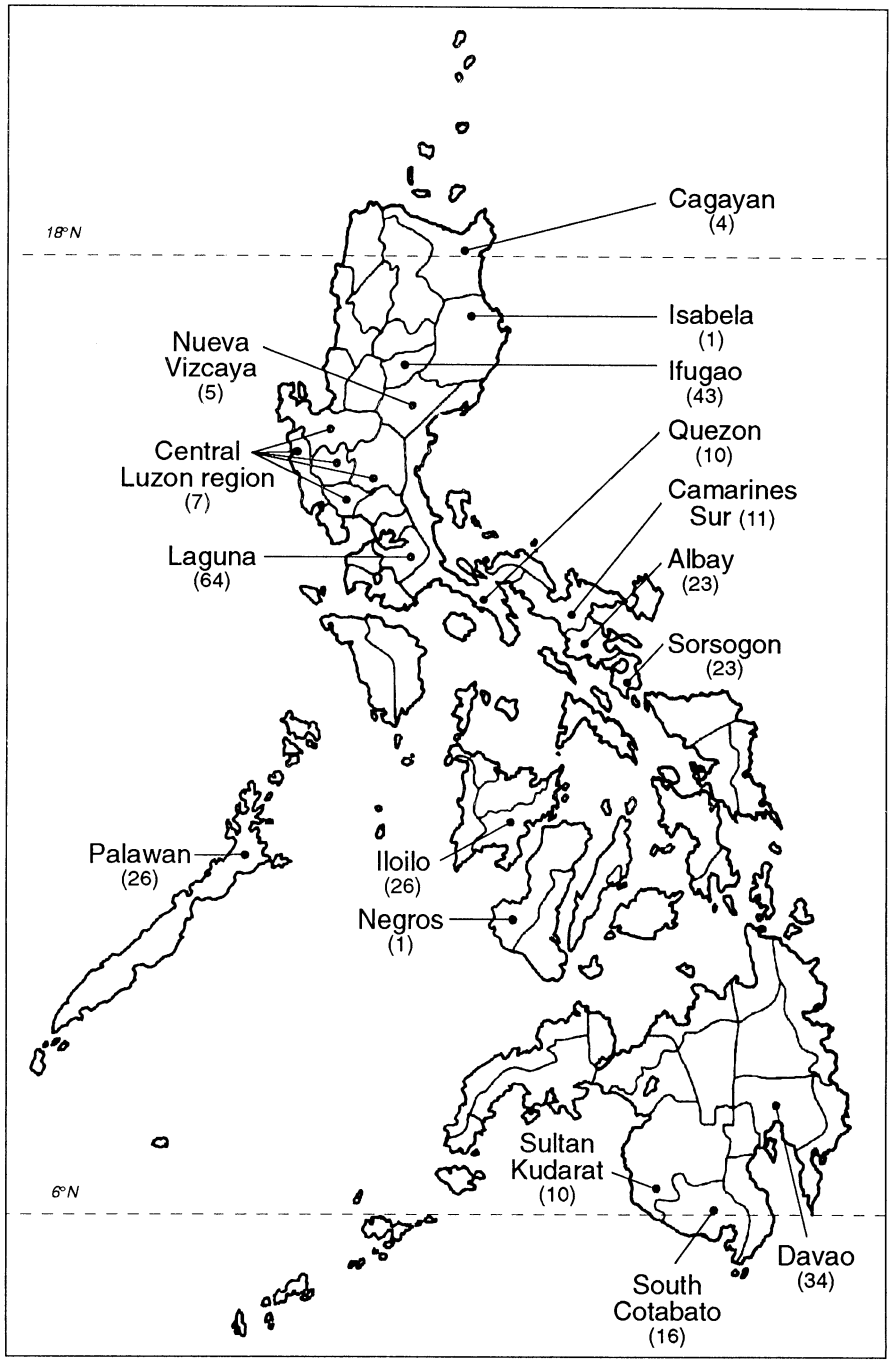


**Fig. 1.** Sampling sites and respective number of samples collected over a period of 2 years in the major rice growing provinces of the Philippines.

control, surface sterilized seeds were soaked in sterile distilled water. The seeds were then sown on autoclaved garden soil in plastic containers (14 × 17 × 4.5 cm). Two trays with two rows per tray and 25 seeds per row were used to test each strain. Inoculated plants were incubated in a dew chamber (near 100% RH, 28°C) for 24 h and were transferred to the greenhouse. They were observed 2 weeks after sowing. Strains identified by Biolog (1) as *P. fuscovaginae* and *B. glumae* were injected until runoff into the flag leaf sheath of cultivars IR24 and IR64 at maximum tillering stage (about 50 days after sowing) and at booting stage (about 70 days after sowing), respectively. At maximum tillering, five hills with four tillers per hill were inoculated with each strain. At booting, two hills with two tillers per hill were inoculated with each strain. The inoculum and controls were prepared as before. Symptoms were observed until grain maturity.

## RESULTS

Over 5,600 bacterial strains were isolated from 304 samples collected in 16 provinces (Fig. 1) and tested for pathogenicity on 21-day-old TN1 seedlings. Out of 64 samples distributed over 12 provinces, 204 pathogenic strains were obtained, comprising 150 strains from plants showing symptoms of sheath rot and grain discoloration, 14 strains from diseased seedlings, and 40 strains from two grain samples of 1 kg. The seed of cultivar IR54 contained 227 g of discolored seed and yielded eight pathogens, four of them from discolored seed. Thirty-two pathogens were obtained from the IR8866 seed. Eight of these strains came from discolored seed, which weighed 223 g. Distribution and isolation frequency of the 204 strains, grouped by subsequent Biolog clustering (1), are presented in Table 1. From four provinces (Negros, Quezon, Sorsogon, and Central Luzon region), no pathogenic forms were obtained from the 525 strains isolated from 41 rice collections. Some strains were related to *Erwinia herbicola* (14 strains) and *Stenotrophomonas maltophilia* (four strains), but these were not included in the present study.

**Biochemical characteristics.** The 204 pathogenic strains isolated from Philippine-grown rice were oxidative, Gram-negative rods. The nonfluorescent group contained 19 strains, and 185 strains produced fluorescent pigment on King's medium B. Kovac's oxidase reaction was variable among strains.

**Pathogenicity and symptoms.** Initial pathogenicity of the 204 strains was based on seedling symptoms ranging from localized browning around the inoculation point to brown streaks all over the sheath and extending to the midrib of the youngest leaf. More severely infected seedlings wilted. All strains were tested on 24 to 33 TN1 seedlings (discrepancy due to nongerminated seeds) over five trials (Table 2). Most of the 204 strains did not cause consistent symptoms. Although the observed symptoms were generally the same, the severity of the produced lesions greatly varied among the strains. Variability in virulence among the 204 strains appeared later to correspond more or less to the different groups in the Biolog clustering (1). Table 3 shows the mean percentage of plants affected by the strains grouped according to the Biolog clusters (1) together with the lowest and highest percentage of diseased plants. The *A. a.* subsp. *avenae* (cluster E) and *B. glumae* (cluster D1) strains were able to induce symptoms on more than 95% of the total number of seedlings tested. Their pathogenicity was obvious and clear-cut. With the modified method of Shakya and Chung (16), the characteristic brown stripe associated with *A. a.* subsp. *avenae* could not unequivocally be identified among the browning patterns. Symptoms were the same on seedlings treated with 230 ppm urea solution or with distilled water (results not shown). The mean infection rate of the fluorescent pathogens of clusters A and B was low, as they infected less than 50% of the total number of seedlings tested (Table 3). The *P. fuscovaginae* group (cluster B1) showed a higher virulence, but five strains produced symptoms on over 95% of the test plants, whereas six strains were pathogenic on less than 40% of the plants. Some of the fluorescent strains may have lost their pathogenicity, as they were able to induce symptoms in the first two trials only (Table 2). This was especially the case for strains belonging to the A clusters.

Only a few of the 204 pathogenic strains induced symptoms on TN1 plants inoculation at the booting stage. Moreover, most were only slightly pathogenic in our experimental conditions, as slightly less than half of the inoculated plants developed symptoms of sheath necrosis. All suspected *B. glumae* strains caused blotching and brown necrosis on the flag leaf sheath. Only 45% of the suspected *P. fuscovaginae* strains and almost 35% of the strains from cluster B2 produced leaf sheath symptoms. Some of these strains caused severe discoloration and rotting of the flag leaf sheath. The other strains, including suspected *A. a.* subsp. *avenae* strains, caused only slight discoloration of the sheath. The symptoms ranged from a restricted dark brown zone around the inoculation site, to small brown spots that occasionally coalesced to form large indistinct blotches on the flag leaf sheath.

**Table 1.** Origin and isolation frequency of pathogenic strains associated with sheath rot complex and grain discoloration of rice in the Philippines

| Province | Pathogens (total)[a] | Samples (total)[b] | Biolog cluster[c] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $A_2$ | $A_3$ | $A_4$ | $A_5$ | $A_6$ | $B_1$ | $B_2$[d] | D1 | E |
| Albay | 1(358) | 1(23) | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Cagayan | 5(91) | 1(4) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| Camarines Sur | 3(128) | 2(11) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 |
| South Cotabato | 1(400) | 3(16) | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Davao | 9(373) | 7(34) | 0 | 0 | 5 | 2 | 1 | 1 | 0 | 0 | 0 |
| Ifugao | 69(983) | 18(43) | 2 | 0 | 4 | 0 | 0 | 4 | 59 | 0 | 0 |
| | 32(289)* | | 0 | 3 | 19 | 1 | 7 | 2 | 0 | 0 | 0 |
| Iloilo | 39(389) | 15(26) | 0 | 0 | 4 | 0 | 1 | 0 | 34 | 0 | 0 |
| Isabela | 2(7) | 1(1) | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| Laguna | 17(780) | 9(64) | 0 | 0 | 3 | 0 | 0 | 2 | 3 | 0 | 9 |
| | 8(710)* | | 1 | 0 | 3 | 1 | 2 | 0 | 1 | 0 | 0 |
| Nueva Vizcaya | 9(111) | 3(5) | 0 | 0 | 1 | 0 | 0 | 0 | 8 | 0 | 0 |
| Palawan | 5(256) | 3(26) | 0 | 0 | 2 | 0 | 1 | 2 | 0 | 0 | 0 |
| Sultan Kudarat | 1(283) | 1(10) | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |

[a] Number of pathogenic strains and (total strains isolated). Asterisks refer to the strains from seed of IR54 (Los Baños, Laguna) and IR8866 (Banaue, Ifugao).

[b] Number of samples from which the pathogenic strains were isolated and (total samples).

[c] Number of strains grouped according to the numerical analysis of Biolog data. Clusters $A_2$, $A_3$, $A_4$, and $A_6$ remained unidentified because they did not contain reference strains. Cluster A5 was identified as *Pseudomonas aeruginosa* and cluster B1 as *P. fuscovaginae*. Cluster B2 was related to *P. aureofaciens*, *P. corrugata*, *P. fluorescens*, and *P. marginalis*. Cluster D1 was identified as *Burkholderia glumae* and cluster E as *Acidovorax avenae* subsp. *avenae*.

[d] Three strains originally in cluster $B_2$ were lost and were not included in this table.

**Table 2.** Tissue of origin of the pathogenic strains and their reactions over five successive pathogenicity tests on 21-day-old TN1 seedlings and on TN1 plants at the booting stage in the IRRI greenhouse

| Biolog cluster[a] | IRRI number | Tissue origin[b] | S1[c] | S2 | S3 | S4 | S5 | P (total)[d] | BT[e] |
|---|---|---|---|---|---|---|---|---|---|
| $A_2$ | 4937 | sheath$^2$ | 2 | 4 | 0 | 0 | 0 | 6(29) | 4 |
| | 5405 | sheath$^0$ | 2 | 4 | 0 | 0 | 0 | 6(29) | 0 |
| | 5440 | seed$^0$ | 3 | 4 | 0 | 0 | 0 | 7(33) | 4 |
| $A_3$ | 6239 | seed$^2$ | 2 | 4 | 6 | 0 | 0 | 12(33) | 0 |
| | 6244 | seed$^2$ | 2 | 4 | 0 | 0 | 0 | 6(33) | 0 |
| | 6269 | seed$^2$ | 2 | 4 | 0 | 0 | 0 | 6(33) | 0 |
| $A_4$ | 2128 | seed$^3$ | 3 | 7 | 16 | 0 | 0 | 26(33) | 4 |
| | 3474 | seed$^2$ | 3 | 8 | 8 | 0 | 0 | 19(33) | 0 |
| | 3680 | seed$^3$ | 2 | 8 | 6 | 0 | 0 | 16(33) | 0 |
| | 3953 | seed$^3$ | 3 | 8 | 8 | 0 | 0 | 19(32) | 0 |
| | 4008 | seed$^3$ | 2 | 8 | 10 | 0 | 0 | 20(33) | 0 |
| | 4142 | seed$^2$ | 2 | 8 | 5 | 0 | 0 | 15(32) | 0 |
| | 4184 | seed$^2$ | 3 | 2 | 12 | 0 | 0 | 17(33) | 0 |
| | 4276 | seed$^2$ | 3 | 8 | 6 | 0 | 0 | 17(33) | 0 |
| | 4704 | sheath$^2$ | 2 | 8 | 5 | 3 | 3 | 21(25) | 0 |
| | 4707 | sheath$^2$ | 3 | 8 | 7 | 0 | 0 | 18(33) | 0 |
| | 4735 | sheath$^2$ | 3 | 8 | 3 | 0 | 0 | 14(33) | 0 |
| | 4736 | sheath$^2$ | 3 | 8 | 15 | 3 | 3 | 32(33) | 0 |
| | 5268 | sheath$^2$ | 2 | 4 | 0 | 0 | 0 | 6(29) | 0 |
| | 6464 | sheath$^2$ | 3 | 4 | 9 | 0 | 0 | 16(29) | 0 |
| | 6591 | sheath$^2$ | 3 | 4 | 5 | 0 | 0 | 12(29) | 0 |
| | 6593 | sheath$^2$ | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 6594 | sheath$^2$ | 2 | 4 | 3 | 0 | 0 | 9(28) | 0 |
| | 6595 | sheath$^2$ | 3 | 4 | 16 | 0 | 0 | 23(29) | 0 |
| | 6625 | sheath$^2$ | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 6687 | seed$^2$ | 2 | 4 | 16 | 0 | 0 | 22(29) | 0 |
| | 6717 | seed$^1$ | 3 | 4 | 4 | 0 | 0 | 11(29) | 0 |
| | 6720 | seed$^3$ | 3 | 4 | 16 | 0 | 0 | 23(29) | 4 |
| | 6726 | sheath$^1$ | 3 | 4 | 16 | 0 | 0 | 23(29) | 0 |
| | 6730 | sheath$^1$ | 3 | 0 | 13 | 0 | 0 | 16(29) | 0 |
| | 7073 | seed$^0$ | 3 | 4 | 0 | 0 | 0 | 7(33) | 0 |
| | 7160 | seed$^0$ | 2 | 4 | 16 | 0 | 0 | 22(33) | 0 |
| | 7161 | seed$^0$ | 3 | 4 | 16 | 0 | 0 | 23(33) | 0 |
| | 7164 | seed$^0$ | 2 | 4 | 7 | 0 | 0 | 13(33) | 0 |
| | 7173 | seed$^0$ | 3 | 4 | 15 | 0 | 0 | 22(33) | 0 |
| | 7174 | seed$^0$ | 3 | 4 | 16 | 0 | 0 | 23(33) | 0 |
| | 7245 | seed$^0$ | 2 | 4 | 16 | 0 | 0 | 22(33) | 0 |
| | 7252 | seed$^0$ | 3 | 4 | 6 | 0 | 0 | 13(33) | 0 |
| | 7277 | seed$^0$ | 3 | 4 | 0 | 0 | 0 | 7(33) | 0 |
| | 7285 | seed$^0$ | 3 | 4 | 16 | 0 | 0 | 23(33) | 0 |
| | 7308 | seed$^0$ | 3 | 4 | 16 | 0 | 0 | 23(33) | 0 |
| | 7392 | seed$^0$ | 3 | 4 | 0 | 0 | 0 | 7(33) | 0 |
| | 7406 | seed$^0$ | 3 | 4 | 0 | 0 | 0 | 7(33) | 0 |
| | 7407 | seed$^0$ | 3 | 4 | 16 | 0 | 0 | 23(33) | 0 |
| | 7470 | seed$^0$ | 3 | 4 | 16 | 0 | 0 | 23(33) | 0 |
| | 7471 | seed$^0$ | 3 | 4 | 0 | 0 | 0 | 7(33) | 0 |
| | 7475 | seed$^0$ | 3 | 4 | 16 | 0 | 0 | 23(33) | 0 |
| | 7478 | seed$^0$ | 3 | 4 | 16 | 0 | 0 | 23(33) | 0 |
| | 7479 | seed$^0$ | 3 | 4 | 16 | 0 | 0 | 23(33) | 0 |
| $A_5$ | 5459 | seed$^0$ | 3 | 4 | 0 | 0 | 0 | 7(33) | 0 |
| | 7342 | seed$^2$ | 3 | 3 | 16 | 0 | 0 | 22(29) | 0 |
| | 7343 | seed$^2$ | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 7358 | seed$^0$ | 3 | 4 | 0 | 0 | 0 | 7(33) | 0 |
| $A_6$ | 3678 | seed$^2$ | 2 | 6 | 6 | 0 | 0 | 14(33) | 4 |
| | 4185 | seed$^2$ | 3 | 8 | 7 | 0 | 0 | 18(32) | 1 |
| | 5164 | seed$^0$ | 3 | 4 | 0 | 0 | 0 | 7(33) | 4 |
| | 6245 | seed$^2$ | 2 | 4 | 0 | 0 | 0 | 6(33) | 0 |
| | 6251 | seed$^2$ | 2 | 4 | 4 | 0 | 0 | 10(33) | 0 |
| | 6257 | seed$^2$ | 2 | 4 | 0 | 0 | 0 | 6(32) | 0 |

*(continued on next page)*

[a] From the numerical analysis of Biolog data. No reference strains were grouped in clusters $A_2$, $A_3$, $A_4$, and $A_6$. Cluster $A_5$ was identified as *Pseudomonas aeruginosa* and $B_1$ as *P. fuscovaginae.* Cluster $B_2$ was related to *P. aureofaciens, P. corrugata, P. fluorescens,* and *P. marginalis.* Cluster $D_1$ was identified as *Burkholderia glumae* and cluster E as *Acidovorax avenae* subsp. *avenae.*

[b] With symptom description: 0 = symptomless; 1 = slight infection (few small lesions); 2 = coalesced lesions to browning all over; 3 = no description available.

[c] Number of plants with leaf sheath necrosis, observed 1 week after inoculation for: S1 = first inoculation on three seedlings; S2 = second inoculation on four or eight seedlings; S3 = third inoculation on 16 seedlings; S4 = fourth inoculation on three seedlings; S5 = fifth inoculation on three seedlings.

[d] Total positive reactions (total of tested plants) over the five seedling inoculation tests.

[e] Number of plants showing leaf sheath necrosis on a total of seven plants, observed 2 weeks after inoculation at the booting stage.

[f] Three strains pertaining to cluster $B_2$ are not available anymore, hence are not included.

**Table 2.** (*continued from preceding page*)

| Biolog cluster[a] | IRRI number | Tissue origin[b] | S1[c] | S2 | S3 | S4 | S5 | P (total)[d] | BT[e] |
|---|---|---|---|---|---|---|---|---|---|
| $A_{6\ (cont)}$ | 6535 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 6702 | seed[1] | 2 | 4 | 0 | 0 | 0 | 6(28) | 0 |
| | 6827 | sheath[1] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 7104 | seed[0] | 2 | 4 | 1 | 0 | 0 | 7(33) | 0 |
| | 7270 | seed[0] | 3 | 4 | 16 | 0 | 0 | 23(33) | 0 |
| | 7346 | seed[0] | 3 | 4 | 0 | 0 | 0 | 7(33) | 0 |
| | 7391 | seed[0] | 3 | 4 | 0 | 0 | 0 | 7(33) | 0 |
| $B_1$ | 4521 | sheath[3] | 3 | 8 | 8 | 0 | 0 | 19(33) | 0 |
| | 4605 | sheath[3] | 3 | 8 | 16 | 3 | 2 | 32(33) | 0 |
| | 5793 | seed[2] | 2 | 4 | 5 | 0 | 0 | 11(28) | 3 |
| | 5801 | seed[2] | 3 | 4 | 16 | 3 | 3 | 29(29) | 6 |
| | 5803 | seed[2] | 3 | 4 | 16 | 3 | 3 | 29(29) | 0 |
| | 6031 | seed[2] | 3 | 4 | 3 | 0 | 0 | 10(29) | 0 |
| | 6202 | seed[2] | 3 | 4 | 0 | 0 | 0 | 7(33) | 1 |
| | 6235 | seed[2] | 3 | 4 | 3 | 0 | 0 | 10(33) | 0 |
| | 6609 | sheath[2] | 3 | 4 | 16 | 3 | 3 | 29(29) | 3 |
| | 7007 | sheath[2] | 3 | 4 | 16 | 0 | 0 | 23(29) | 0 |
| | 7008 | sheath[2] | 3 | 4 | 16 | 3 | 3 | 29(29) | 2 |
| $B_2$[f] | 4784 | sheath[2] | 3 | 4 | 15 | 0 | 0 | 22(28) | 4 |
| | 4790 | sheath[1] | 3 | 4 | 15 | 0 | 0 | 22(29) | 4 |
| | 4792 | sheath[1] | 2 | 4 | 14 | 0 | 0 | 20(27) | 4 |
| | 4794 | sheath[1] | 3 | 4 | 0 | 0 | 0 | 7(29) | 2 |
| | 4797 | sheath[1] | 3 | 4 | 13 | 3 | 3 | 26(27) | 4 |
| | 4799 | sheath[1] | 3 | 4 | 15 | 0 | 0 | 22(29) | 2 |
| | 4807 | sheath[2] | 3 | 4 | 15 | 0 | 0 | 22(28) | 4 |
| | 4808 | sheath[1] | 2 | 4 | 14 | 0 | 0 | 20(27) | 3 |
| | 4809 | sheath[2] | 3 | 4 | 13 | 0 | 0 | 20(27) | 4 |
| | 4830 | sheath[2] | 3 | 4 | 15 | 2 | 3 | 27(28) | 2 |
| | 4831 | sheath[2] | 3 | 4 | 11 | 0 | 0 | 18(28) | 4 |
| | 4832 | sheath[1] | 3 | 4 | 1 | 0 | 0 | 8(28) | 4 |
| | 4833 | sheath[2] | 2 | 4 | 0 | 0 | 0 | 6(29) | 4 |
| | 4834 | sheath[1] | 2 | 4 | 0 | 0 | 0 | 6(29) | 4 |
| | 4836 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 2 |
| | 4840 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 4 |
| | 4841 | sheath[2] | 3 | 4 | 1 | 0 | 0 | 8(29) | 4 |
| | 4842 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 4 |
| | 4845 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 4 |
| | 4846 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 4 |
| | 4847 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 4 |
| | 4849 | sheath[2] | 3 | 4 | 1 | 0 | 0 | 8(29) | 4 |
| | 4855 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 4 |
| | 4863 | sheath[2] | 2 | 4 | 0 | 0 | 0 | 6(29) | 3 |
| | 4866 | sheath[2] | 2 | 4 | 2 | 0 | 0 | 8(29) | 4 |
| | 4868 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 4 |
| | 4882 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 4 |
| | 4902 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 4 |
| | 4909 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 4 |
| | 4915 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 1 |
| | 4924 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 4 |
| | 4928 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 4 |
| | 4967 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 3 |
| | 5067 | sheath[1] | 3 | 4 | 0 | 0 | 0 | 7(29) | 2 |
| | 5068 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 1 |
| | 5191 | sheath[0] | 2 | 4 | 1 | 0 | 0 | 7(27) | 4 |
| | 5200 | sheath[0] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5229 | sheath[0] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5232 | sheath[0] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5237 | sheath[2] | 3 | 4 | 1 | 0 | 0 | 8(29) | 0 |
| | 5244 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5245 | sheath[1] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5247 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5256 | sheath[0] | 3 | 4 | 0 | 0 | 0 | 7(29) | 4 |
| | 5263 | sheath[2] | 3 | 4 | 2 | 0 | 0 | 9(29) | 0 |
| | 5270 | sheath[2] | 3 | 4 | 16 | 0 | 0 | 23(29) | 0 |
| | 5273 | sheath[0] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5274 | sheath[0] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5276 | sheath[0] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5280 | sheath[0] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5301 | sheath[0] | 2 | 4 | 0 | 0 | 0 | 6(29) | 0 |
| | 5310 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5313 | sheath[0] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5314 | sheath[0] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |

(*continued on next page*)

**Table 2.** (*continued from preceding page*)

| Biolog cluster[a] | IRRI number | Tissue origin[b] | S1[c] | S2 | S3 | S4 | S5 | P (total)[d] | BT[e] |
|---|---|---|---|---|---|---|---|---|---|
| $B_2$ (cont) | 5317 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5318 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5321 | sheath[0] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5328 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5329 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5337 | sheath[2] | 3 | 4 | 16 | 2 | 3 | 28(29) | 0 |
| | 5371 | sheath[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5372 | sheath[0] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 5373 | sheath[2] | 2 | 3 | 0 | 0 | 0 | 5(28) | 0 |
| | 5395 | sheath[0] | 3 | 4 | 1 | 0 | 0 | 8(24) | 0 |
| | 5397 | sheath[0] | 2 | 4 | 1 | 0 | 0 | 7(26) | 0 |
| | 5418 | sheath[2] | 3 | 4 | 1 | 0 | 0 | 8(28) | 0 |
| | 5463 | seed[0] | 3 | 4 | 0 | 0 | 0 | 7(33) | 0 |
| | 5614 | seed[2] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 6182 | sheath[1] | 3 | 4 | 0 | 3 | 3 | 13(29) | 0 |
| | 6183 | sheath[1] | 2 | 4 | 13 | 3 | 3 | 25(29) | 0 |
| | 6190 | sheath[0] | 2 | 4 | 0 | 0 | 0 | 6(29) | 0 |
| | 6192 | sheath[1] | 2 | 4 | 13 | 2 | 3 | 24(28) | 0 |
| | 6193 | sheath[1] | 2 | 4 | 16 | 3 | 0 | 25(29) | 0 |
| | 6194 | sheath[0] | 2 | 4 | 16 | 0 | 0 | 22(29) | 0 |
| | 6217 | sheath[0] | 2 | 4 | 16 | 0 | 0 | 22(29) | 0 |
| | 6221 | sheath[1] | 3 | 4 | 2 | 0 | 0 | 9(29) | 0 |
| | 6285 | sheath[2] | 3 | 4 | 10 | 0 | 0 | 17(29) | 0 |
| | 6287 | sheath[0] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 6288 | sheath[2] | 3 | 4 | 16 | 0 | 0 | 23(29) | 0 |
| | 6291 | sheath[1] | 3 | 4 | 16 | 0 | 0 | 23(29) | 0 |
| | 6309 | sheath[1] | 3 | 4 | 16 | 0 | 0 | 23(29) | 0 |
| | 6316 | sheath[1] | 3 | 4 | 16 | 0 | 0 | 23(29) | 0 |
| | 6318 | sheath[1] | 2 | 4 | 16 | 0 | 0 | 22(29) | 0 |
| | 6333 | sheath[1] | 2 | 4 | 0 | 0 | 0 | 6(29) | 0 |
| | 6334 | sheath[1] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 6347 | sheath[1] | 2 | 4 | 0 | 0 | 0 | 6(28) | 0 |
| | 6348 | sheath[1] | 3 | 4 | 15 | 0 | 0 | 22(29) | 0 |
| | 6356 | sheath[1] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 6357 | sheath[1] | 3 | 4 | 16 | 0 | 0 | 23(29) | 0 |
| | 6358 | sheath[1] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 6362 | sheath[1] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 6368 | sheath[1] | 2 | 4 | 16 | 0 | 0 | 22(29) | 0 |
| | 6369 | sheath[1] | 2 | 4 | 16 | 0 | 0 | 22(29) | 0 |
| | 6370 | sheath[1] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 6371 | sheath[1] | 3 | 4 | 16 | 0 | 0 | 23(29) | 0 |
| | 6372 | sheath[1] | 3 | 4 | 0 | 0 | 0 | 7(29) | 0 |
| | 6375 | sheath[1] | 3 | 4 | 6 | 0 | 0 | 13(29) | 0 |
| | 6376 | sheath[1] | 2 | 4 | 16 | 2 | 3 | 27(29) | 0 |
| | 6400 | sheath[1] | 2 | 4 | 0 | 0 | 0 | 6(29) | 0 |
| | 6402 | sheath[1] | 3 | 4 | 16 | 0 | 0 | 23(29) | 0 |
| | 6408 | sheath[1] | 3 | 4 | 16 | 0 | 0 | 23(29) | 0 |
| | 6415 | sheath[2] | 2 | 4 | 16 | 0 | 0 | 22(29) | 0 |
| | 7246 | sheath[1] | 2 | 3 | 16 | 0 | 0 | 21(28) | 0 |
| | 7253 | sheath[1] | 2 | 4 | 16 | 3 | 2 | 27(29) | 0 |
| | 7405 | sheath[1] | 2 | 4 | 16 | 0 | 0 | 22(29) | 0 |
| $D_1$ | 1857 | sheath[2] | 3 | 7 | 14 | 3 | 3 | 30(33) | 4 |
| | 1858 | sheath[2] | 3 | 7 | 16 | 3 | 3 | 32(33) | 7 |
| | 2056 | seed[0] | 2 | 8 | 16 | 3 | 3 | 32(33) | 7 |
| | 2057 | seed[0] | 3 | 8 | 16 | 3 | 3 | 33(33) | 3 |
| | 2076 | seed[0] | 3 | 8 | 16 | 3 | 3 | 33(33) | 7 |
| E | 1837 | seedling[1] | 3 | 6 | 16 | 3 | 3 | 31(33) | 0 |
| | 1840 | seedling[1] | 2 | 1 | 16 | 3 | 3 | 25(33) | 5 |
| | 1845 | seedling[1] | 3 | 6 | 16 | 3 | 3 | 31(33) | 0 |
| | 1851 | seedling[1] | 3 | 7 | 16 | 3 | 3 | 32(33) | 1 |
| | 1891 | seedling[1] | 2 | 7 | 16 | 3 | 3 | 31(33) | 2 |
| | 7010 | seedling[1] | 3 | 4 | 16 | 3 | 3 | 29(29) | 2 |
| | 7012 | seedling[1] | 3 | 4 | 16 | 3 | 3 | 29(29) | 0 |
| | 7014 | seedling[1] | 2 | 4 | 16 | 3 | 3 | 28(28) | 0 |
| | 7015 | seedling[1] | 2 | 4 | 16 | 3 | 3 | 28(28) | 0 |
| | 7017 | seedling[1] | 3 | 4 | 16 | 3 | 3 | 29(29) | 0 |
| | 7018 | seedling[1] | 2 | 4 | 16 | 3 | 3 | 28(28) | 1 |
| | 7019 | seedling[1] | 3 | 4 | 16 | 3 | 3 | 29(29) | 1 |
| | 7021 | seedling[1] | 3 | 4 | 16 | 3 | 3 | 29(29) | 0 |
| | 7023 | seedling[1] | 3 | 4 | 16 | 3 | 3 | 29(29) | 1 |

Suspected *B. glumae*, *A. a.* subsp. *avenae*, and *P. fuscovaginae* strains were further characterized with the seed-soaking method of inoculation (Table 4). Both cultivars TN1 and IR24 gave the same results. None of the *P. fuscovaginae* strains induced any symptom on the seedlings by this inoculation method. *A. a.* subsp. *avenae* strains induced browning of the sheaths, with the youngest leaf becoming dry or having a brown stripe on the midrib (Fig. 2B). *B. glumae* strains reduced the germination rate by causing soft rotting of the soaked seeds. Resulting seedlings also were smaller and mostly displayed brown rotting sheaths. Twisting, curling, and whitening of the leaves were also observed 1 to 2 weeks after inoculation. However, the virulence of these *B. glumae* strains was considerably different; strains 2056, 2057, and 2076 (isolated from apparently symptomless seeds from a field in the province of Camarines) were consistently virulent, while 1857 and 1858 (isolated from discolored sheath material in the province of Isabela) displayed a less severe type of lesions.

IR24 plants injected with suspected *B. glumae* and *P. fuscovaginae* strains at maximum tillering stage developed severe dark browning of the sheath. On IR64 plants in the booting stage, very distinct brown discolorations and dry rotting of the flag leaf sheath were observed (Fig. 3A). The symptoms appeared as early as 2 to 3 days after inoculation. Also, spikelet sterility appeared (Fig. 3B). At maturity, the grains showed brown discoloration ranging from small spots on the hull to discoloration of the whole grain for both *P. fuscovaginae* and *B. glumae* strains inoculated (Table 5). Some grains, however, displayed the characteristic brown streak on the hulls typical for *B. glumae* (Fig. 2A).

## DISCUSSION

It is clear that the sheath rot complex and grain discoloration syndrome of rice involves a complex group of *Pseudomonads* spp. in addition to *S. oryzae*, other fungal pathogens, insects, and abiotic factors (7,13,21,23,24). Sarocladium sheath rot causes sheath discoloration to rotting (dry rot) of the sheath tissues and abortion of the panicle exertion (13). *S. oryzae* may be isolated from lesions of sheath browning, but sheath browning does not always produce *S. oryzae* (T. W. Mew, *unpublished*). In recent years, several pathogenic pseudomonads have been reported to cause a similar syndrome of sheath rot complex and grain discoloration (3,10,15,17,23,24). The distinct features of bacterial sheath brown rot of *P. fuscovaginae* reported by Tanii et al. (17) were not observed throughout our field survey in the Philippines. However, the bacterial pathogen has been isolated from seed obtained from this country (3).

Our survey throughout the major rice growing districts in the Philippines was conducted from 1988 to 1990. Of the total collected bacterial strains from rice plants with the sheath rot complex and grain discoloration, only 3.6% was pathogenic. Detailed studies to characterize the large remaining portion of isolated nonpatho-

**Table 3.** Percentage of plants showing symptoms after inoculation

| Biolog cluster | Mean % ± SD[a] | Range |
|---|---|---|
| A2 | 21.0% | 21% |
| A3 | 24.2 ± 10.5% | 18–36% |
| A4 | 54.6 ± 20.9% | 21–97% |
| A5 | 35.6 ± 26.9% | 21–76% |
| A6 | 30.0 ± 16.2% | 18–70% |
| B1 | 69.0 ± 32.7% | 21–100% |
| B2 | 42.4 ± 26.5% | 21–100% |
| D1 | 97.0 ± 3.7% | 91–100% |
| E | 96.8 ± 6.6% | 76–100% |

[a] The average is for all strains per Biolog cluster.

**Table 4.** Symptoms induced on IR24 seedlings by selected strains 14 days after seed-soak inoculation of 100 seeds per strain

| IRRI no. | Biolog identity at sowing | Soft rotting of seeds at sowing | Brown striping of sheath and leaf midrib | Sheath brown, leaves curled, whitened |
|---|---|---|---|---|
| 1840 | *Acidovorax avenae* | – | + | – |
| 1851 | *A. avenae* | – | + | – |
| 7015 | *A. avenae* | – | + | – |
| 1857 | *Burkholderia glumae* | + | – | + |
| 1858 | *B. glumae* | + | – | + |
| 2056 | *B. glumae* | + | – | + |
| 2057 | *B. glumae* | + | – | + |
| 2076 | *B. glumae* | + | – | + |
| 6031 | *Pseudomonas fuscovaginae* | – | – | – |
| 6235 | *P. fuscovaginae* | – | – | – |
| 7008 | *P. fuscovaginae* | – | – | – |

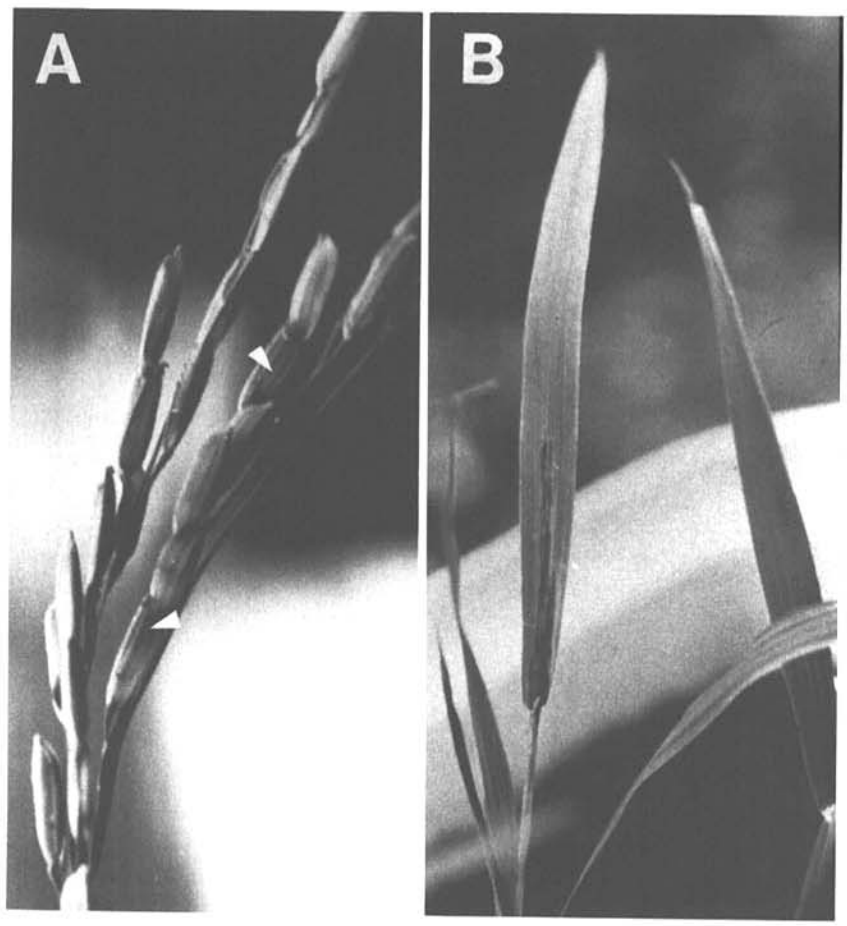


**Fig. 2.** Disease symptoms induced by selected nonfluorescent strains: **(A)** brown striping (arrows) on the grains of IR64 observed at maturity after injection of the flag leaf sheath at booting stage with strain 2056 (suspected *Burkholderia glumae*); **(B)** water-soaked brown lesion along the midrib of TN1 seedlings observed 2 weeks after seed-soak inoculation with strain 1840 (suspected *Acidovorax avenae*).

genic bacteria, some showing antagonistic properties against rice fungal pathogens, are in progress. Although there might have been a bias toward isolation of fluorescent strains on King's medium B, the group of collected pathogenic bacteria consisted of 19 nonfluorescent and a majority of 185 fluorescent strains. All were pathogenic on rice seedlings by the inoculation method of injection, and they induced the development of leaf sheath necrosis or browning (Table 2). The pattern of leaf sheath necrosis differed among the strains, but the syndrome was very similar, consequently preventing diagnosis based solely on symptomatology, as was also reported in earlier studies (23,24). The nonfluorescent strains were generally very consistent in producing leaf sheath necrosis or browning in the different pathogenicity tests. *A. a.* subsp. *avenae* was only isolated from diseased seedlings. *A. a.* subsp. *avenae* is seedborne and has been detected in seed lots from many countries (16). Its role in the sheath rot complex is not clear, as opposed to an earlier report (25). *B. glumae,* which also infects seedlings (19), was obtained from discolored leaf sheaths and apparently healthy seeds. The strains grouped into *B. glumae* produced very consistent sheath browning (Tables 4 and 5). The severity, however, varied among strains at the seedling, maximum tillering, and booting stages.

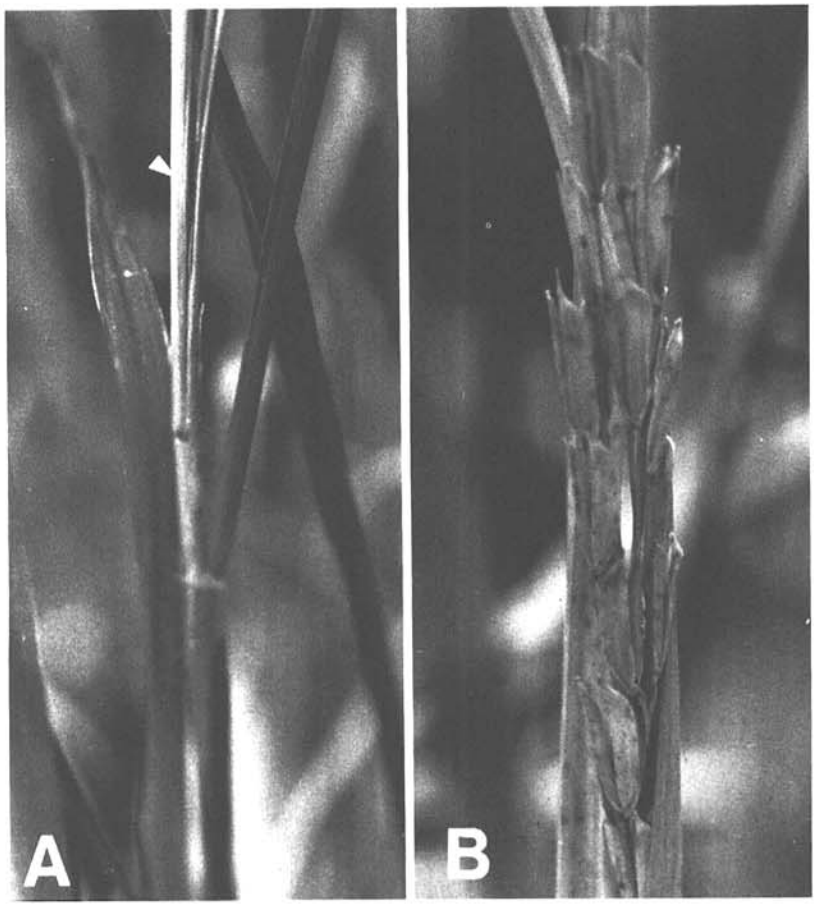


**Fig. 3.** Disease symptoms induced by suspected *Pseudomonas fuscovaginae* strains on IR64 rice plants at the booting stage: **(A)** necrotic leaf 8 days after inoculation of the flag leaf sheath with strain 7008; **(B)** spikelet sterility observed 14 days after inoculation with strain 6031.

**Table 5.** Symptoms produced by suspected *Burkholderia glumae* and *Pseudomonas fuscovaginae* strains inoculated into rice plants at maximum tillering stage/booting stage

| IRRI no. | Biolog identity | Discolored grain (%)[a] | Sheath browning | |
|---|---|---|---|---|
| | | | MT[b] | BT[c] |
| 1857 | *Burkholderia glumae* | 50.1 | + | + |
| 1858 | *B. glumae* | 28.8 | + | + |
| 2056 | *B. glumae* | 47.0 | + | + |
| 2057 | *B. glumae* | 18.1 | + | + |
| 2076 | *B. glumae* | 19.5 | + | + |
| 6202 | *Pseudomonas fuscovaginae* | 40.8 | + | + |
| 4521 | *P. fuscovaginae* | 64.9 | + | + |
| 4605 | *P. fuscovaginae* | 51.5 | + | + |
| 5793 | *P. fuscovaginae* | 24.1 | + | + |
| 5801 | *P. fuscovaginae* | 17.8 | + | + |
| 5803 | *P. fuscovaginae* | 39.7 | + | + |
| 6031 | *P. fuscovaginae* | 36.3 | + | + |
| 6235 | *P. fuscovaginae* | 41.9 | + | + |
| 6609 | *P. fuscovaginae* | 19.7 | + | + |
| 7007 | *P. fuscovaginae* | 37.0 | + | + |
| 7008 | *P. fuscovaginae* | 19.6 | + | + |

[a] On a total of 200 grains.
[b] Observed 7 to 14 days after inoculation. Each strain was inoculated onto 20 plants of cultivar IR24 at the maximum tillering (MT) stage.
[c] Observed 7 to 14 days after inoculation up to grain maturity. Each strain was inoculated onto four plants of cultivar IR64 at the booting (BT) stage.

The fluorescent strains caused indistinguishable symptoms by the methods used for the pathogenicity tests. The extent of sheath necrosis or browning, by the injection method either at booting or at seedling stage, varied among the strains. There is a marginal relationship between the degree of leaf sheath necrosis or browning and the obtained classification into Biolog clusters. The individual strains within a Biolog cluster may produce a diversity in leaf sheath browning patterns and rotting. The capability of each cluster to cause sheath discoloration is reflected in the mean percentage of affected plants (Table 3).

Generally, most of the strains from seeds were grouped in the A clusters (Table 2). Strains derived from leaf sheath samples were mainly grouped in cluster B2. Cluster B1 contained a small number of strains derived both from seeds and sheaths. Cluster B2, with 108 strains, represented more than half of the obtained pathogenic forms and was closely related to cluster B1 (1). Cluster B2 strains produced a wide range of sheath browning and rotting and, based on the types of lesions produced, were indistinguishable from cluster B1. However, the mean percentage of plants affected by cluster B2 strains was lower than that for cluster B1 (Table 3). Furthermore, cluster B2 was phenotypically distinct from cluster B1 (1).

Among the pathogenic forms isolated from leaf sheaths, seeds, and seedlings, only a small portion clustered with *P. fuscovaginae* (11 strains), *B. glumae* (five strains), and *A. a.* subsp. *avenae* (14 strains). In fact, the majority (85%) of the pathogenic forms was grouped by the Biolog identification system into either unidentified clusters or clusters containing *Pseudomonas* spp. usually regarded as saprophytes (1). In the literature, these

*Pseudomonas* spp. are not considered pathogens (3,10,14,24); whether they are opportunists needs further verification. Their role in the development of the disease syndrome has not been recognized so far.

In our survey, 52 pathogenic strains were obtained from apparently symptomless leaf sheaths or grains (28 strains from the two seed batches and 24 strains from seven of the 26 samples containing symptomless material in the total of 304 samples). This appeared to confirm the finding of Miyajima and Akita (11) that rice plants harbor many pathogenic bacteria that reside for a long time on or in the leaf sheath and cause disease at the booting stage. Pathogenic bacteria were also isolated from both discolored and healthy seed in a single seed batch. It is difficult to ascertain if there had been contamination during seed harvesting and processing, or if these strains were naturally associated with the seed. In the present survey, no pathogenic strains were isolated from more than 70% of leaf sheaths with browning or rotting and discolored seeds. It is premature to assume that pathogenic bacteria are involved in all cases of the sheath rot complex and grain discoloration syndrome. Earlier reports recognized the difficulty of recovering pathogenic pseudomonads from rice tissues (10,25). The large number of samples that did not produce any pathogenic strain could also be related to the efficiency of the isolation method used in the current study. A recent endeavor to improve the method of isolation of these bacteria from rice seed produced a higher recovery (G. L. Xie, *personal communication*).

Although there were strains resembling *P. fuscovaginae*, they were few in number but originated from a wide array of locations in the Philippines representing both tropical lowland (Laguna, Palawan, Davao) and tropical highland areas (Banaue, Ifugao at an elevation of more than 950 m above sea level). Although clustered with *P. fuscovaginae* by the Biolog system, none of the strains was identical to the type culture of *P. fuscovaginae* described by Miyajima et al. (1,12). In addition, pathogenicity tests did not produce the typical symptoms of bacterial sheath brown rot caused by *P. fuscovaginae* (17). Strains clustered with *A. a.* subsp. *avenae* produced the typical brown stripe lesions on leaves of rice seedlings. Strains clustered with *B. glumae*, however, did not produce the typical seedling rot, nor the grain rot or glume blight caused by *B. glumae* (19). Strains of *A. a.* subsp. *avenae* and *B. glumae* all originated from tropical lowland areas. Further specification of *P. fuscovaginae* and *B. glumae* in relation to the sheath rot complex and grain discoloration is in progress. The influence of environmental factors and rice group, i.e., the indica (under tropical climate) and the japonica (under temperate climate), on the pathogenicity of these bacteria is an important area to be addressed in further studies. Because a large number of pseudomonads considered nonpathogenic in the literature were isolated from tissues with the sheath rot complex and grain discoloration, their roles in the syndrome, especially in relation to the pathogenic forms, need further research effort.


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