Identification and Characterization of *Pseudomonas fuscovaginae*, the Causal Agent of Bacterial Sheath Brown Rot of Rice, from Madagascar and Other Countries

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ABSTRACT

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Four unidentified bacterial strains that caused sheath necrosis and sterility of rice spikelets in Madagascar were compared with nine known strains of *Pseudomonas fuscovaginae*. All 13 strains were pathogenic on rice seedlings and caused symptoms identical to those described for bacterial sheath brown rot. Based on 151 cultural, cytological, physiological, and biochemical characteristics, we demonstrated that the unidentified strains were *P. fuscovaginae*. Several characteristics distinguish this species from *P. marginalis*: production of levan, potato soft rot, nitrate reduction, production of 2-ketogluconate, the formation of pits on polypectate gels, and the use of 2-ketogluconate, polygalacturonic acid, and various carbohydrates. The four native strains and nine reference strains of *P. fuscovaginae* differed in agglutination and indirect immunofluorescence tests using two sera suggesting intraspecies differences. It was possible to distinguish four serological groups. Since various serological results were observed with different reference strains obtained from different collections and originated from the same initial isolation, divergency arising from subculturing must be considered. Four strains of *P. marginalis* and *P. fluorescens* tested were only distantly related to *P. fuscovaginae*, if at all.

Marked symptoms of leaf sheath rot, necrosis, and sterility of spikelets have been observed for the last few years in paddy fields of the Madagascar highlands. Their cause was either unknown or else attributed to a poor adaptation to climatic conditions (cold), or to infection by *Sarocladium oryzae* (Sawada) W. Gams and D. Hawks (ex *Acrocylindrium oryzae* Sawada). After a detailed study of the different symptoms, we suspected bacterial sheath brown rot, a disease first described in Japan (19). According to Miyajima et al (12), *Pseudomonas fuscovaginae* is a fluorescent pseudomonad that is positive for the arginine dihydrolase and oxidase reactions, but possesses characteristics that are distinct from other species within this group. This identification was confirmed by Duveiller et al (3). However, Zeigler and Alvarez (20) questioned the designation *P. fuscovaginae* and suggested that the causal agent of bacterial sheath brown rot of rice in Latin America resembled *Pseudomonas marginalis*.

The aim of this work was to identify and characterize the causal agent of the symptoms of sheath necrosis and sterility of rice spikelets in Madagascar and to compare these strains with *P. fuscovaginae* strains from Japan, Burundi, and Colombia. A prior study (4) described the pathogenicity of bacterial rice strains in Madagascar.

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MATERIALS AND METHODS

Plant material. The Madagascar plant material was obtained in March 1986, at Vinaninony, from a highland rice project conducted by the FOFIFA (Centre National de Recherche Appliquée pour le Développement Rural, Antananarivo, République Démocratique Malgache), and IRAT/CIRAD (Institut de Recherches Agronomiques Tropicales et des Cultures Vivrières, Département du Centre de Coopération Internationale en Recherche Agronomique pour le Développement [CIRAD]), and financed by the European Economic Community (contract TSD-084 F).

Isolation of bacteria. Pieces of leaf sheath from rice were macerated in sterile distilled water. Loopfuls of macerated tissue were plated on agar medium B (KB) of King et al (5). Rice grains were washed in running tap water for 1 hr

Table 1. References of the identified *Pseudomonas fuscovaginae*, *P. fluorescens*, and *P. marginalis* strains

Species	Strain number	Registered official strain number[a]	Host	Source	Obtained from or isolated by
P. fuscovaginae	2065	CFBP 2065 PDDCC 5940 NCPPB 3085	*Oryza sativa*	Japan	INRA Angers
P. fuscovaginae	HMB304	CFBP 2065 PDDCC 5940 NCPPB 3085	*O. sativa*	Japan	H. Maraite
P. fuscovaginae	BCE3	CFBP 2065 PDDCC 5940 NCPPB 3085	*O. sativa*	Japan	R. S. Zeigler
P. fuscovaginae	6801	CFBP 2065 PDDCC 5940 NCPPB 3085	*O. sativa*	Japan	K. Miyajima
P. fuscovaginae	7103	PDDCC 5939	*O. sativa*	Japan	K. Miyajima
P. fuscovaginae	BM1	PDDCC 5941	*O. sativa*	Japan	K. Miyajima
P. fuscovaginae	HMB264	...	*O. sativa*	Burundi	H. Maraite
P. fuscovaginae	BCE32	...	*O. sativa*	Colombia	R. S. Zeigler
P. fuscovaginae	532	...	*O. sativa*	Colombia	R. S. Zeigler
P. fluorescens biovar 1	2102	CFBP 2102 NCPPB 1964 ATCC 13 525	...	Great Britain	INRA Angers
P. marginalis pv. *marginalis*	2037	CFBP 2037 ATCC 10844	*Cichorium intybus*	United States	INRA Angers
P. marginalis pv. *pastinacea*	2038	CFBP 2038 ATCC 13889	*Pastinaca sativa*	United States	INRA Angers
P. marginalis pv. *alfalfae*	2039	CFBP 2039 NCPPB 2644	*Medicago sativa*	United States	INRA Angers

[a] CFBP = Collection Française de Bacteries Phytopathogenes, Station de Pathologie Végétale et de Phytobactériologie, INRA, Beaucouzé, 49000 Angers, France. PDDCC = Plant Diseases Division Culture Collection, Auckland, New Zealand. NCPPB = National Collection of Plant Pathogenic Bacteria, Plant Pathology Laboratory, Ministry of Agriculture, Harpenden, Hertfordshire, England, UK. ATCC = American Type Culture Collection, Rockville, Maryland, USA.

before placing on KB medium. The source of reference strains *P. fuscovaginae*, *P. marginalis*, and *P. fluorescens* appears in Table 1. The international type strain of *P. fuscovaginae* NCPPB 3085 (PDDCC 5940) was obtained from four different sources: the laboratory of K. Miyajima (12) and three other laboratories (Table 1). The bacteria were stored in sterile distilled water at −20 C.

Microbiological characteristics. The general characteristics of bacterial strains were determined by standard microbiological techniques (5,8,10,13,15–17). The API Galleries for carbohydrates were used according to the manufacturer's specifications and with the assimilation medium for gram-negative bacilli LRA 88803 (Api System, La Balme-les Grottes, 38390 Montalieu-Vercieu, France). The antibiogram for antibiotic resistance was determined using Bio-Mérieux disks (BioMérieux, Marcy-l'Etoile, 69260 Charbonnières-les-Bains, France) on Mueller-Hinton medium (1).

Serology. The antistrain GR2 serum was prepared by immunizing a New Zealand rabbit as follows. Bacteria from YPGA (yeast extract 7 g, bactopeptone 7 g, glucose 7 g, agar 15 g, distilled water 1 L, pH 7) cultures were washed three times with saline solution (NaCl 8 g, distilled water 1 L) by centrifugation at 27,000 *g* for 10 min. The bacterial suspension was then diluted to 10^9 cfu/ml based on turbidimetry in a fresh saline solution, and killed by heating (100 C, 1 hr). Bacterial suspensions without any adjuvant were injected on the first (0.5 ml), fourth (0.5 ml), seventh (0.5 ml), ninth (1.0 ml), 11th (1.0 ml), and 14th (2.0 ml) days of treatment into the marginal vein of the rabbit's ear. On the 14th day, 2.0 ml were also injected subcutaneously and a blood sample was taken on the 24th day. The characteristics of the anti-*P. fuscovaginae* serum HMB 266, supplied by H. Maraite, have been described (3).

The sera were stored at −20 C in glycerol (50%). Bacteria were grown at 26 ± 1 C on KB medium for the immunofluorescence test (6), and on nutrient agar (Difco) for the tube agglutination test. For tube agglutination, 0.5 ml of antiserum was mixed with 0.5 ml of bacterial suspension (10^9 cfu/ml). After 1 hr, the appearance of a flocculent precipitate was observed with and without 10× magnification. The titer of the anti-GR2 serum for strain GR2 was 1/20,480 with immunofluorescence and 1/800 with agglutination. The titer of the anti-*P. fuscovaginae* serum HMB266 for strain HMB264 was 1/5,120 with immunofluorescence and 1/800 with agglutination.

Test of pathogenicity. Bacterial cultures (24 hr old) on KB medium were harvested in sterile distilled water. A hypodermic syringe was used to inject 10^8 cfu/ml of aqueous cell suspensions into 3-wk-old rice seedlings of variety IRAT 13. The point of injection was between leaf sheaths located about 5 cm above soil and the amount injected filled up intersheath spaces. The same type of inoculation was performed on the winded flag leaf sheath of plants at the booting stage. Plants were grown in a greenhouse on horticultural compost composed of peat and pozzolana (volcanic sand). The photoperiod was natural (14 hr of daylight) and the temperature varied between 19 C at night and 31 C during the day.

RESULTS

Numerous fluorescent bacterial cultures were isolated from grains of rice variety China 1039 from Madagascar. Representative strains (GR1, GR2, GR3, and GR4) were pathogenic on rice seedlings. Two to three days after the infiltration of bacteria between the leaf sheaths, elongated water-soaked blotches were observed. These blotches quickly developed into brownish-black necrosis that eventually spread the full length of the sheaths. Inoculated rice seedlings often died after 7–14 days (Table 2). The single strain GR2 was inoculated on the flag leaf sheath and caused elongated brown blotches and large lesions with a light brown to gray dry center. Lesions were often surrounded by an indefinite brownish-black border. Pathogenic bacteria were readily reisolated from these inoculated plants. In fields in Madagascar, the same type of symptoms were observed at booting stage on flag leaf sheaths. Other symptoms related to the disease were a poor or total lack of panicle emergence, rot of grains, and sterility of spikelets.

All the reference strains of *P. fuscovaginae* were pathogenic on rice seedlings (Table 2) and could be reisolated from diseased plants. They induced necrosis on leaf sheaths identical to that already described for strains GR1, GR2, GR3, and GR4. However, strain 532 from Colombia was only very slightly pathogenic in our experimental conditions (three out of 15 plants had symptoms of sheath necrosis). The reference strains of *P. fuscovaginae* did not kill the rice seedlings, whereas GR1–GR4 did. The four strains of *P. marginalis* and *P. fluorescens* were not pathogenic on rice seedlings (Table 2).

Characterization of the pathogen. On KB medium, the strains GR1–GR4 formed colonies with entire margins that were convex and creamy white in color. They appeared after 24–48 hr of growth, and their average size was 2 mm after 5 days. The development of crystals was observed in the medium under bacterial cultures after 24–48 hr of incubation. The nature and significance of these crystals were unknown. The nine *P. fuscovaginae* reference strains (Table 1) had different morphologies on KB medium. While all colonies were creamy white, some were convex and circular with entire margins (BCE3, 6801, 7103, HMB264, BCE32, 532), and others were relatively flat and circular with erose margins (2065, HMB304, BM1). Colonies appeared after 24–48 hr of growth, and attained 2–5 mm after 5 days. Crystals similar to those produced by GR1–GR4 were also visible and their size, shape, and number

Table 2. Pathogenicity of *Pseudomonas fuscovaginae, P. fluorescens, P. marginalis* strains, and bacterial strains of Madagascar rice

Strain			Number of plants[a]		
Species	Source	Number	Without visible symptoms	With necrosis of foliar sheath	Dead
P. fuscovaginae	Japan	2065	7	8	0
P. fuscovaginae	Japan	HMB304	4	11	0
P. fuscovaginae	Japan	BCE3	2	13	0
P. fuscovaginae	Japan	6801	2	13	0
P. fuscovaginae	Japan	7103	2	13	0
P. fuscovaginae	Japan	BM1	6	9	0
P. fuscovaginae	Burundi	HMB264	1	14	0
P. fuscovaginae	Colombia	BCE32	2	13	0
P. fuscovaginae	Colombia	532	12	3	0
Unidentified	Madagascar	GR1	0	4	11
Unidentified	Madagascar	GR2	0	6	9
Unidentified	Madagascar	GR3	0	2	13
Unidentified	Madagascar	GR4	0	3	12
P. fluorescens	Great Britain	2102	15	0	0
P. marginalis pv. *marginalis*	United States	2037	15	0	0
P. marginalis pv. *pastinacea*	United States	2038	15	0	0
P. marginalis pv. *alfalfae*	United States	2039	15	0	0
Control (distilled water)	...	...	15	0	0

[a] Observed 20 days after inoculation. Each strain is inoculated on 15 3-wk-old plants of variety IRAT 13.

varied according to the strains. On nutrient agar medium, all of the bacterial strains studied formed smooth circular colonies with entire margins that were convex and creamy white in color. The average size of the colonies was between 3 and 5 mm after 5 days of incubation. Unlike what occurred on KB medium, no strain produced visible crystals in the nutrient agar medium. The cytological, physiological, and biochemical properties of the pathogenic strains of rice in Madagascar and the identified *P. fuscovaginae* strains appear in Tables 3, 4, and 5.

Serological properties. Apart from BCE32 and 532, all of the reference strains of *P. fuscovaginae* and the strains from Madagascar reacted with the anti-*P. fuscovaginae* HMB266 serum using the tube agglutination technique (Table 6). Only the Madagascar strains and reference strains BCE3, 6801, and HMB264 reacted positively with anti-GR2 serum. The four *P. fluorescens* and *P. marginalis* strains reacted negatively with both sera. When the indirect immunofluorescence technique was used, all of the bacterial strains reacted with the anti-GR2 serum diluted to 1/200. On the other hand, only the Madagascar strains and reference strains BCE3, 6801, and HMB264 had a clear positive reaction with the 1/2,000 dilution of anti-GR2 serum. This result was also observed with the anti-*P. fuscovaginae* HMB266 serum diluted to 1/640 (Table 6).

DISCUSSION

The plant pathogen *P. fuscovaginae* is a fluorescent pseudomonad that does not produce levan on a sucrose-rich medium and does not cause potato soft rot, but is oxidase and arginine dihydrolase positive; its hypersensitive reaction on tobacco is

Table 3. Characteristics of *Pseudomonas fuscovaginae* strains and pathogenic strains of Madagascar rice

Test	*P. fusco-vaginae*[a]	Strains GR1, GR2, GR3, GR4 (Madagascar)
Gram KOH (5)	–[b]	–[b]
Motility in peptoned water	+	+
Flagellation (16)	1–4 polar	1–4 polar
Oxidative on glucose (5)	+	+
Inert on glucose (5)	–	–
Catalase (17)	+	+
Nitrate reduction (8)	–	–
Fluorescent pigment (5)	+	+
Levan production (5)	–	–
Kovacs's oxidase (5)	+	+
Potato soft rot (17)	–	–
Arginine dihydrolase (5)	+	+
Tobacco hypersensitivity (5)	d,v	d,v
Pectinolytic activity (15)	–	–
Pit formation on polypectate gel (17):		
pH 4.9–5.1	–	–
pH 6.9–7.1	–	–
pH 8.3–8.5	–	–
Starch hydrolysis (5)	d	+w
Esculin hydrolysis (10,17)	–	–
Cellulolytic activity (13)	–	–
Acid production from (medium of Ayers et al [5]):		
Sucrose	–	–
Trehalose	+	+
Inositol	–	–
Sorbitol	–	–
Mannitol	+	+
Utilization of (medium of Ayers et al [5]):		
Acetate	+	+
Citrate	+	+
Malonate	+	+
Succinate	+	+
Milk peptonization (5)	d	+w
Milk acidification (5)	–	–
Gelatin hydrolysis (5)	d	+
Indole production (10)	–	–
Urease production (10)	–	–
Methyl red test (5)	–	–
Voges-Proskauer test (5)	–	–
Tween 80 hydrolysis (5)	d	+
Growth on nutrient agar + NaCl 5%	d	+
2-Ketogluconate production (5)	–	–
H_2S production (5)	–	–

[a] Nine strains from Table 1.
[b] – = Negative reaction, + = positive reaction, +w = weakly positive reaction, d = variable reaction between strains, v = variable reaction for same strain.

Table 4. Carbohydrate utilization (API 50 CH galleries)

Tube (no.)	Carbohydrate	*Pseudomonas fuscovaginae*[a]	Strains GR1, GR2, GR3, GR4 (Madagascar)
0	Control	–[b]	–[b]
1	Glycerol	+	+
2	Erythritol	–	–
3	D-Arabinose	–	–
4	L-Arabinose	+	+
5	Ribose	+	+
6	D-Xylose	d	+
7	L-Xylose	–	–
8	Adonitol	–	–
9	β-Methyl-xyloside	–	–
10	Galactose	+	+
11	Glucose	+	+
12	Fructose	+	+
13	Mannose	+	+
14	Sorbose	–	–
15	Rhamnose	–	–
16	Dulcitol	–	–
17	Inositol	–	–
18	Mannitol	+	+
19	Sorbitol	–	–
20	α-Methyl-mannoside	–	–
21	α-Methyl-glucoside	–	–
22	*N*-Acetyl-glucosamine	d	–
23	Amygdalin	–	–
24	Arbutin	–	–
25	Esculin	–	–
26	Salicin	–	–
27	Cellobiose	–	–
28	Maltose	–	–
29	Lactose	–	–
30	Melibiose	–	–
31	Sucrose	–	–
32	Trehalose	+	+
33	Inulin	–	–
34	Melezitose	–	–
35	D-Raffinose	–	–
36	Starch	–	–
37	Glycogen	–	–
38	Xylitol	–	–
39	β-Gentiobiose	–	–
40	D-Turanose	–	–
41	D-Lyxose	d–	–
42	D-Tagatose	–	–
43	D-Fucose	–	–
44	L-Fucose	–	–
45	D-Arabitol	+	+
46	L-Arabitol	–	–
47	Gluconate	+	+
48	2-Ketogluconate	d–	–
49	5-Ketogluconate	–	–

[a] Nine strains from Table 1.
[b] – = Negative reaction (nonbacterial growth), + = positive reaction (bacterial growth), d = variable reaction between strains, d– = negative reaction except for one strain, d+ = positive reaction except for one strain. Results recorded after 7 days of incubation.

variable (Table 7). These characteristics place *P. fuscovaginae* in group V of the fluorescent pseudomonads, according to the classification of Lelliott et al (9). In contrast, *P. marginalis*, a member of group IV, produces levan, soft rots potato, reduces nitrate, produces 2-ketogluconate, forms pits on polypectate gels, and uses 2-ketogluconate, polygalacturonic acid, and various carbohydrates (Table 7). Our results with bacterial strains from Madagascar, Japan, Burundi, and Colombia confirm those of Duveiller et al (3) and Miyajima et al (11,12).

In the analysis of 151 different bacteriological characteristics (cultural, physiological, cytological, and biochemical properties; use of carbohydrates and organic acids; antibiotic sensitivities) the four pathogenic bacterial strains from Madagascar rice had properties similar to those of the nine known *P. fuscovaginae* strains. All strains were pathogenic in inoculated rice seedlings. In all cases, they induced the development of leaf sheath necrosis. The Madagascar strains were the most pathogenic, causing the death of rice seedlings. In contrast, four strains of *P. marginalis* and *P. fluorescens* were not pathogenic on rice seedlings.

Variability of the serological properties within *P. fuscovaginae* exists, and different groupings can be made according to the antiserum and technique used. A general analysis of the serological properties, which takes into account the two antisera and the two techniques employed (immunofluorescence and agglutination), makes it possible to distinguish four groups: 1) the pathogenic strains of Madagascar rice (GR1, GR2, GR3, GR4), HMB 264 from Burundi, and strains BCE3 and 6801 from Japan; 2) strains 2065, HMB304, and BM1 from Japan; 3) strain 7103 from Japan; and 4) strains BCE32 and 532 from Colombia, which have a positive serological reaction only in immunofluorescence with the anti-GR2 serum diluted to 1/200. It should be noted that this dilution is

Table 5. Reaction of *Pseudomonas fuscovaginae* strains and pathogenic bacterial strains of Madagascar rice to 22 antibiotics

Antibiotic			Reaction	
Denomination	Code	Charge in μg or units	*P. fuscovaginae*[a]	Strains GR1, GR2, GR3, GR4 (Madagascar)
Ampicillin	AM	10	d[b]	R[b]
Bacitracin	B	10U	R	R
Cefalotin	CF	30	R	R
Chloramphenicol	C	30	S	S
Chlortetracycline	A	30	S	S
Colistin	CL	10	S	S
Erythromycin	E	15	d	R
Flumequine	AR	30	S	S
Gentamycin	GM	10	S	S
Kanamycin	K	30	S	S
Lincomycin	L	2	R	R
Mezlocillin	MZ	75	S	S
Nalidixic acid	NA	30	S	S
Oleandomycin	OL	15	d	R
Oxacillin	OX	1	R	R
Oxytetracycline	T	30	S	S
Penicillin G	P	10U	R	R
Rifampicin	RA	30	S	S
Rifamycin	RF	30	S	S
Streptomycin	S	10	S	S
Tetracycline	TE	30	S	S
Trimethoprim-sulfamides	SxT	T-1,25+ SX-23,75	S	S

[a] Nine strains from Table 1.
[b] S = sensitivity (inhibition zone around the antibiotic disk), R = resistance (no inhibition zone around the antibiotic disk), d = variable reaction between strains.

Table 6. Serological properties of *Pseudomonas fuscovaginae*, *P. fluorescens*, and *P. marginalis* strains, and pathogenic bacterial strains of Madagascar rice

Strain			Reaction[a]				
			Tube agglutination		Immunofluorescence		
			Dilution of antiserum		Dilution of antiserum		
			Strain GR2	*P. fuscovaginae* HMB266	Strain GR2		*P. fuscovaginae* HMB266
Species	Source	Number	1/200	1/200	1/200	1/2,000	1/640
P. fuscovaginae	Japan	2065	−	+	+	+w	+w
P. fuscovaginae	Japan	HMB304	−	+	+	+w	+w
P. fuscovaginae	Japan	BCE3	+	+	+	+	+
P. fuscovaginae	Japan	6801	+	+	+	+	+
P. fuscovaginae	Japan	7103	−	+	+	−	−
P. fuscovaginae	Japan	BM1	−	+	+	+w	+w
P. fuscovaginae	Burundi	HMB264	+	+	+	+	+
P. fuscovaginae	Colombia	BCE32	−	−	+	−	−
P. fuscovaginae	Colombia	532	−	−	+	−	−
P. fuscovaginae	Madagascar	GR1	+	+	+	+	+
P. fuscovaginae	Madagascar	GR2	+	+	+	+	+
P. fuscovaginae	Madagascar	GR3	+	+	+	+	+
P. fuscovaginae	Madagascar	GR4	+	+	+	+	+
P. fluorescens	Great Britain	2102	−	−	+	−	−
P. marginalis pv. *marginalis*	United States	2037	−	−	+	+w	−
P. marginalis pv. *pastinacea*	United States	2038	−	−	+	+w	−
P. marginalis pv. *alfalfae*	United States	2039	−	−	+	+w	−

[a] Agglutination: + = flocculent precipitate, − = no flocculent precipitate. Immunofluorescence: + = bacterial cells visible, +w = bacterial cells weakly fluorescent, − = bacterial cells not visible.

Table 7. Comparison of the characteristics of *Pseudomonas fuscovaginae* and *P. marginalis* published by different researchers

Characters	Sources of *P. fuscovaginae*[a]				
	Madagascar, Burundi, Japan, Colombia[b]	Japan[c]	Burundi[d]	Colombia[e]	*P. marginalis*[f]
Fluorescent pigment	+	+	+	+	+
Levan production	−	−	−	+	+
Kovacs's oxidase	+	+	+	+	+
Potato soft rot	−	−	−	+	+
Arginine dihydrolase	+	+	+	+	+
Tobacco hypersensitivity	d,v	+	d	+	−
Starch hydrolysis	d	+	d	−	−
Nitrate reduction	−	−	−	d	+
Denitrification	ND	−	−	ND	+
2-Ketogluconate production	−	−	−	ND	+
Pit formation on polypectate gel	−	−	−	−	+
Growth at 41 C	ND	ND	ND	−	−
Growth at 4 C	ND	ND	ND	−	+
β-Glucosidase	−	−	−	ND	+
Acid production from:					
Sucrose	−	−	ND	+	+
Sorbitol	−	−	−	+	ND
Inositol	−	−	−	+	ND
Raffinose	ND	−	ND	+	ND
Utilization (for growth):					
Sucrose	−	ND	−	ND	+
Sorbitol	−	−	−	ND	+
Inositol	−	−	−	ND	+
2-Ketogluconate	−	−	−	−	+
Polygalacturonic acid	ND	−	−	−	+

[a] + = More than 90% strains positive, − = more than 90% strains negative, d = results variable between strains, v = results variable for same strain, ND = not determined.
[b] Published in this study.
[c] Miyajima (11) and Miyajima et al (12).
[d] Duveiller et al (3).
[e] Zeigler and Alvarez (20).
[f] Hildebrand and Schroth (7), Lelliott et al (9), Palleroni (14), and Stanier et al (18).

relatively low considering the titer of the serum (1/20,480). It is, therefore, possible that there were cross-serological reactions with other bacterial species, such as *P. fluorescens* or *P. marginalis*, under these conditions.

It also appears that strains 2065, HMB304, BCE3, and 6801 were not in the same serological group. This result is surprising, as these four strains are subcultures of the original type strain of *P. fuscovaginae* (NCPPB 3085, PDDCC 5940). This phenomenon was also observed for colony appearance on KB medium: BCE3 and 6801 formed convex colonies that were circular with entire margins, whereas 2065 and HMB304 formed relatively flat colonies that were circular with erose margins. Thus, it is possible that certain cultural and serological properties of *P. fuscovaginae* may change during subculturing.

The consideration of all of the results described and discussed above enable us to affirm that *P. fuscovaginae* is present in Madagascar and induces a disease known as bacterial sheath brown rot of rice. The causal bacterium has characteristics that distinguish it from other species within the fluorescent pseudomonads (12), but determination of differences between *P. fuscovaginae* and related bacteria is time-consuming. Use of a rapid serological technique for the diagnosis of this disease would be a great advantage. In studies already published on the serological characterization of *P. fuscovaginae*, antisera produced against this bacterium were relatively specific in regard to other bacterial species (3,11,21). It is possible that cross-reactivity problems could be resolved by use of monoclonal antibodies (2). However, based on tests reported here, serological variability within the *P. fuscovaginae* species must also be considered if accurate serological techniques are to be developed for the diagnosis of the disease or detection of the bacterium.

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