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Pseudomonas avenae Causing Bacterial Brown Stripe Disease of Rice in Korea

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*Pseudomonas avenae*에 의한 벼·세균성 줄무늬병

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ABSTRACT

A bacterial brown stripe disease new to Korea was noted for the first time on rice seedlings grown in the nursery pots and in a field nursery. Artificial hypodermic injection and pricking inoculation with isolates obtained from lesions of naturally infected plants produced symptoms similar to those occurring under natural condition. Among eleven species of ten genera of gramineous plants *Echinochloa crusgalli*, *Digitaria sanguinalis* and *Setaria viridis* were the new hosts for the pathogen. On the basis of bacteriological and biochemical tests of isolates from infected rice seedlings, the causal bacterium is considered to be *Pseudomonas avenae* Manns.

Key words: Pseudomonas avenae, rice.

要 約

韓國에서 처음으로 벼못자리와 못트에 자란 모에서 세균성갈색줄무늬병을 記錄하였다. 圃場에서 자란 罹病植物로부터 分離한 菌株를 注射 또는 針接種한 結果, 自然狀態의 病徵과 비슷하였다. 供試한 10科 11種의 禾本科植物 중에서 *Echinochloa crusgalli*, *Digitaria sanguinalis*, *Setaria viridis*는 本 病原細菌의 새로운 寄主로 밝혀졌다. 생화학적 특성 및 병원성등을 토대로 本 細菌을 *Pseudomonas avenae* Manns로 同定하였다.

INTRODUCTION

A bacterial brown stripe disease of rice new to Korea was found for the first time in nursery pots at the College of Agriculture, Seoul National University, Suweon, Korea in July 1979. Later the disease was also found in the nursery beds in the experimental farm of the same college. The symptoms were characterized by distinct brown stripe lesions on leaf sheath and leaf blade(Fig. 1). These systoms were similar to those previously described for rice by some workers(1,3,11). Heavily infected coleoptiles and first leaves were commonly noted on the

diseased seedlings. However, these symptoms had not been mentioned previously. As no such bacterial brown disease has been known to occur in Korea except as reported previously by the authors(16), attemps were made to identify the causal organism on the basis of morphological and biochemical characteristics and the host range.

MATERIALS AND METHODS

Bacterial isolates: The isolates used in this study were obtained from lesions of naturally infected seedlings. Isolates PM1, and PM2 were from the sheath and PM 4 was from the leaf of the cultivar Milyang 23 grown in the pot.

Pathogenicity test: Three-week old rice seedlings were inoculated with a bacterial suspension (10^7 cells/ml) from cultures grown for 48 hours on yeast extract-dextrose-calcium carbonate (YDC) medium. The methods of inoculation employed were hypodermic injection, needle pricking and spraying. For the host range test eleven species with ten different genera of the family Gramineae were inoculated with all four isolates by hypodermic injection. All test plants were kept under a humidity tent for two days at temperatures ranging from 20 to 33°C.

Morphology and culture characteristics: Gram stain for light microscopy and two percent potassium phosphotungstate for electron microscopy were used to observe the morphological characteristics of the isolates. The media used for the culturing bacteria were nutrient agar, King'B and YDC.

Biochemical and biological tests: All test methods used in this study were those given in the Mannual of Mircobiological Method (9) unless otherwise stated. The production of fluorescence was followed to King et al.(5). The oxidase test was according to Kovacs (7) but N, N-dimethyl p-phenylene diaminedihydrochloride, arginine dihydrolase and lipase production were carried out according to Misaghi and Grogan (10). Levan production, lypolysis of margarine and the catalase reaction were determined according to Lelliott et al.(8). The esculin test and tolerance of NaCl (5%) in peptone test was according to Rhodes (13) and Phenylalanine was carried out as given by Shaw (17). The tobacco hypersensitive test was conducted as described by Klementz et al. (6).

The effect of temperature on the growth of isolates at 25-29°C, 36°C, 42°C and 51°C in 5 ml nutrient broth and YDC slants was examined after 2 days of incubation.

RESULTS

Pathogenicity tests: Rice seedlings inoculated by injection as well as the needle pricking method developed water-soaked stripe after 48 hrs. The water soaked stripes turned into dark brown colour within 4 to 5 days. These symptoms were similar to those observed under field conditions(Fig. 1). The lesions were more elongated running along the midrib by the injection method than by the needle pricking method. However, the symptoms did not develop by spray inoculation method. The host range test results showed that *Hordeum vulgare*, *Avenae sativum*, *Secale cereales*, *Setaria viridis* and *Echinochloa crusgalli* were highly suscepible to all isolates of the bacterial brown stripe organism producing more than 10 cm long stripe lesisons. *Triticum aestivum* and *Setaria italica* developed lesions a few cm beyond the point of inoculation. While

Fig. 1. Bacterial brown stripe symptoms on leaf sheath, leaf blade and drying of heavily infected leaves (left) on naturally infected rice seedlings.

Table 1. Reactions of gramineous plants to 4 isolates of the bacterial brown stripe bactorium when inoculated by hypodermic injection

Test Plant	Isolates			
	PM1	PM2	PM3	PM4[a]
Triticum alstivum L.	MS	MS	MS	MS
Hordeum vulgare L.	HS	HS	HS	HS
Zea mays L.	MR	MR	MR	MR
Avenae sativs L.	HS	HS	HS	HS
Secale cereales L.	HS	HS	HS	HS
Sorghum nervosum Bess. et Schult	MR	MR	MR	MR
Panicum miliaceum L.	S	S	S	S
Setaria italica Beauce	MS	MS	MS	MS
Setaria viridis Beauce	HS	HS	HS	HS
Echinochloa crusgalli Becuce	HS	HS	HS	HS
Digitaria sanguinalis Scopoli	S	S	S	S

a MR : lesions limited around the point of inoculation,
MS : lesions 1 to 2 cm long,
S : lesions extended a few cm on leaf blade,
HS : lesions elongated more than 10 cm extending along leaf blade.

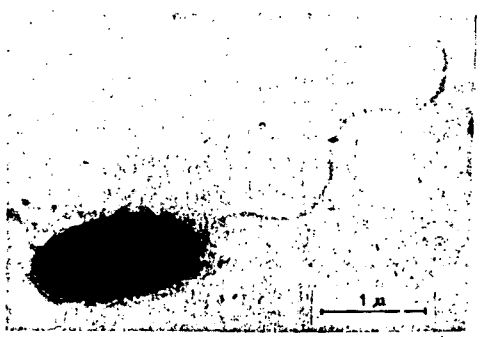

Fig. 2. Electron micrography of the bacterium with a pollar flagellum causing bacterial brown stripe disease of rice seedling.

the lesions were limited to the point of inoculation in the case of *Zea mays* and *Sorghum nervosum*, only 0.5 to 2 cm lesions on the leaf sheath were produced after inoculation of *Digitaria sanguinalis* and *Panicum miliaceum* with the test organism (Table 1).

Morphology and Gram reaction: The bacterial cells of all isolates were Gram negative, non-acid fast, nonsporing rod with round ends. They occurred mostly singly or pairs, measuring 0.5-1.0 by 1.5-3.0μ in size. The organism was motile by means of single or very rarely two polar flagella (Fig. 2).

Culture characteristics: On nutrient agar, yeast extract-dextrose-calcium carbonate (YDC) and King's B medium, the colonies were visible within 24 to 48 hrs. The colonies on King's B and nutrient agar were white, round, raised, smooth, translucent, entire margin and sticky. On these media the colonies were surrounded by a colorless halo; later the halo collpased and encircled whole colonies along the line of streak. This was more prominent where the colonies were densely arranged. The colonies became dirty white on older plates. Growth was slow on nutrient agar and colonies were small, while they measured 1 to 4.5 mm in diameter on YDC and King'B media after the same incubation period.

Physiological and biochemical characteristics: All isolates were non-fluorescent and induced a strong hypersensitive reaction in tobacco. These isolates were positive in oxidase, nitrate reduction, lipase, strach hydrolysis, NH_3 production, catalase and aesculine hydrolysis but were negative in arginine dihydrolase, indol production, VP and MR tests, levan production and phenyl-alanine deaminase. Litmus milk was completly digested and slightly pink colour was obsevered. Acid but not gas was produced from mannitol and galactose but delayed in xylose. No acid and gas were produced from glucose, sucrose, lactose, maltose, inulin,

Table 2. Comparisions of diagnostic characters of present isolates with previously reported descriptions of *Pseudomonas alboprecipitans*

Characteristics	present isolates[a]				P. alboprecipitants[b]			
	PM1	PM2	PM3	PM4	11	12	14	19
Fluorescent pigment	—	—	—	—[c]	—	—		—
Arginine dihydrolase	—	—	—	—	—	—		—
Kovacs' oxidase	+	+	+	+	+	+		+
Nitrate reduction	+	+	+	+	+	+	+	+
Lipase	+	+	+	+	+	+	+	+
Gelatin liquefaction	—	—	—	—	—	—	—	v
H_2S production	(+)	(+)	(+)	(+)	—	(+)	—	v
Indol production	—	—	—	—	—	—	—	—
Strach hydrolysis	+	+	+	+	v	w	+	+
NH_3 production	+	+	+	+	+	+	+	
Catalase	+	+	+	+				
Milk	D	D	D	D	Al	Al		
MR & VP	—	—	—	—				
Growth in 5% NaCl	—	—	—	—				
Phenylalanine deaminase	—	—	—	—	—			
Aesculine hydrolase	+	+	+	+	—			
Temparature growth:								
25±2 C	+	+	+	+				
36 C	opt.	opt.	opt.	opt.	opt.	opt.	opt.	opt.
42 C	+	+	+	+	+	+	+	+
Acid production but no gas from:								
Glucose	—	—	—	—	+	+	—	+
Sucrose	—	—	—	—	—	—	—	—
Lactose	—	—	—	—	—	—	—	—
Mannitol	+	+	+	+	+	+	+	+
Glycerine	w	w	w	w	+	—	—	+
Galactose	+	+	+	+	+	+	—	+
Maltose	—	—	—	—	—			
Xylose	(+)	(+)	(+)	(+)	v			
Inulin	—	—	—	—				
Dextrin	—	—	—	—				
Salicin	—	—	—	—				
Tobacco hypersensitivity	+	+	+	+				

a Authors' isolates

b 11: Miyazima 1974, 12: Nishiyama et al. 1979, 14: Rosen 1922, 19: Tominaga 1971

c —: negative, v: varied, +: positive, D: digested, (): delayed, Al: alkine, w: weak, opt.: optium temperature.

dextrin and salicin. The maximum growth temperature of the isolates was above 42°C. The comparison of the charateristics of the present isolates with *P. alboprecipitans* (11,12,14,19) was given in Table 2.

DISCUSSION

The symptoms of the present bacterial disease of rice seedling that had been noted in nursery pots and nursery beds in the experimental farm in Korea, were similar with previously described bacterial brown stripe disease of rice which is known to be caused by *P. setariae* (3), *P. panici* (*P. panicimillicei* and *P. setariae* as synonyms) (1) and *P. alboprecipitans* (11,19). *P. alboprecipotans* was reported to cause stripe disease not only on rice but also on several gramineous plants (4, 12,14,19). The present isolates from rice seedlings were identical in their biochemical characteristics

with *P. alboprecipitans* reported by several authors (11,12,19) except in acid production from glucose, galactose (14) and aesculine hydrolysis (11). The diagonostic characters of *P. setariae* (3) such as nitrate reduction, maximum growth temperature above 42°C and no acid from sucrose were *Pseudomonas* and considered to be synonymous with *P. alboprecipitans* Rosen 1922. However, some workers did not consider *P. panici* as synonyms of *P. setariae* and *P. alboprecipitans* (3,19). *P. glumae* (20) also caused browning, stunting, and killing of the seedlings which were similar to the present stripe disease. But viscid colony, more than one flagella, negative H_2S production and positive oxidase tests were the characteristics of *P. glumae* (19,20) distinguished from the present isolates.

In the host range test the isolates infected oat, wheat, barley, corn, rye, sorghum, Italian millet and prosomillet. These plants are known to be susceptible to *P. alboprecipitans* and *P. setariae* (1, 3,11,12,19). In addition present isolates also infected grasses such as *Setaria viridis, Echinochloa crusgalli* and *Digitaria sanguinalis*. So far, these plants were not known as hosts of the casusal bacterium of brown stripe disease.

Furthermore, the cultural chacteristics of the present isolates such as butyrous, translucent and circular with colourless halo are similar with *P. alboprecipitans* of Rosen (14). *P. alboprecipitans* and *P. avenae* are considered as synonyms (15). *P. avenae* has been recommended to be adopted as the current nomenspecies (2). According to the new approved lists of phytopathogenic bacteria, therefore, the present bacterium causing the brown stripe disease of rice seedlings in Korea is indentified as *P. avenae* Manns.

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REFERENCES

1. GOTO, M. (1964). Nomenclature of the bacteria causing bacterial leaf streak and bacterial stripe of rice. *Bulletin of the Faculty of Agriculture, Schizuoka University* 14:3-10.
2. GOTO, M. (1980). A new approved lists of phytopathogenic bacteria. *Shokubutsu Boyeki* 34(1): 27-34. (In Japanese).
3. GOTO, K. & OHATA, K. I. (1961). Bacterial stripe of rice. *Special Publication, College of Agriculture, Taiwan University* 10:49-57.
4. JOHNSON, A. G., ROBERT, A. L. & CASH, L. (1949). Bacterial leaf blight and stalk rot of corn. *J. Agric. Res.* 78:719-733.
5. KING, E. O., WARD, N. K. & RANEY, D. E. (1954). Two simple media for demonstration of Phocynin and fluorescein. *J. Labor. Clin. Med.* 44:301-307.
6. KLEMENT, Z., FAKKS, G. L. & LOVEKOVITCH, L. (1964). Hypersensitive reaction induced by phytopathogenic bacteria in the tobacco leaf. *Phytopathology* 54:474-477.
7. KOVACS, N. (1956). Identification of *Pseudomonas pyocynea* by oxidase reaction. *Nature* 179:705.
8. LELLIOT, R. A., BILLING, E. & HAYWARD, A. C. (1966). A determinative scheme for fluorescent plant pathogenic *Pseudomonas*. *J. Appl. Bacteriol.* 29:470-489.
9. Manual of Microbiological Methods. (1957). Com. Bacterio. Tech. Ed. by Society of American Bacteriologists. McGrawHill, New York.
10. MISAGHI, I. & GROGAN, G. H. (1969). Nutritional and biochemical comparisions of plant pathogenic and saprophytic fluorescent *Pseudomonas*. *Phytopathology* 59: 1436-1450.
11. MIYAJIMA, K. (1974). Occurrence of bacterial brown stripe of rice. *Ann. Phytopath. Soc. Japan*. 40:119. (Abstract, In Japanese)
12. NISHIYAMA, K., NISHIHARA, N. & EZUKA, A. (1979). Bacterial brown stripe of ragicaused by *Pseudomonas alboprecipitans*. Ann. *Phy-*

topath. Soc. Japan 45:25-31 (In Japanese).

13. RHODES, M. E. (1959). The characteristics of *Pseudomonas fluorescens. J. Gen. Microbiol.* 21:221-263.
14. ROSEN, H. R. (1923). A bacterial disease of foxtail (*Cheatochloa lutascens*). *Rev. Appl. Mycol.* 2:447-8.
15. SCHAAD, N., KADO, C. & SUMMER, D. R. (1975). Synonyms of *Pseudomonas avenae* 1909 and *Pseudomonas alboprecipitans* Rosen 1922. *Int. J. Syst. Bacteriol.* 25:133-137.
16. SHAKYA, D. D. & CHUNG, H. S. (1980). Bacterial brown stripe on rice seedlings in Korea. Paper presented at *Annual Meeting of Korean Society of Plant Protection* p. 9 (Abstract).
17. SHAW, C. & CLARKE, P. H. (1955) Biochemical classification of Proteus and Providence cultures. *J. Gen. Microbiol.* 13:155-161.
18. SNEATH, P.H.A.(1956). Cultural and biochemical characteristics of the genus *Chromobacterium. J. Gen. Microbiol* 15:70-98.
19. TOMINAGA, T. (1971). Studies on the disease of forage crops in Japan. *Bull. Nat. Inst. Agric. Sci. Japan* 25(3): 205-505. (In Japanese, with English summary).
20. UEMATSU, T., YOSHIMURA, D., NISHIYAMA, K., IBARAGI, I. & FUJII, H. (1976). Pathogenic bacterium causing seedling rot of rice. *Ann. Phytopath. Soc. Japan* 42:464-471.