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Symptoms and Ecology of Bacterial Brown Stripe of Rice

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Abstract

Bacterial brown stripe of rice, caused by Pseudomonas avenae Manns is a disease that affects rice seedlings. The symptoms of the disease on rice plants in the seedling stage were divided into 4 types: i.e. inhibition of germination, brown stripes on a leaf, curving of a leaf sheath and abnormal elongation of a mesocotyl. After the infected seedlings were transplanted to paddy fields, the symptoms were masked. Natural occurrence of the disease was not seen after the tillering stage, except for the case where rice plants were submerged in the water by flood. At the heading stage, the causal bacteria invaded the seeds during several days around the flowering date without any symptoms. A small quantity of the bacteria increased rapidly during the germination period and became the source of infection in nursery boxes in the following year. An effective method for controlling the disease is to maintain an adequate level of temperature and humidity each in growing rice seedlings and to use pathogen-free seeds with a germination test before planting. In case where the seeds are contaminated with the pathogen, it is important to control the disease by a bactericide of kasugamycin.

Discipline: Plant disease
Additional key words: disease index, ecology, Kasugamycin, Pseudomonas avenae

Introduction

In growing rice plants in Japan, a method of transplanting by machine has come into wide use and contributed to saving hand labor tremendously. This method requires a large quantity of seedlings, which should be quite uniform in their growth. To ensure availability of such seedlings, rice seeds are densely planted in a small size of nursery box and grown under fairly warm temperature and high humidity. These conditions provide a favorable environment for the incidence of various types of bacterial diseases such as bacterial brown stripe (*Pseudomonas avenae*)[3,8,14], bacterial grain rot (*P. glumae*)[15] and bacterial seedling blight (*P. plantarii*)[1]. In case where such a incidence of the diseases takes place, the rice growers suffer from shortage of seedlings and poor quality of seedlings.

Among these diseases, the bacterial brown stripe has occurred most frequently in the Hokuriku area since 1976, when this disease was first recognized in a nursery box in Niigata Prefecture. This report presents characteristics of the pathogen, symptoms and ecology of this disease.

Isolation and identification of the pathogenic bacteria

Infected seedlings were collected in the Hokuriku area during the period 1982 to 1985 and bacterial isolates showing milky white or white colony color were isolated by the dilution plate technique with a nutrient agar medium. The pathogenicities of these isolates to rice seedlings were tested by the method of seed-soaking in bacterial suspensions. A species of the bacterial isolates was identified on the basis of Bergey's Mannual, 8th edition and other direction[2,13].

The bacteria were Gram-negative straight rod

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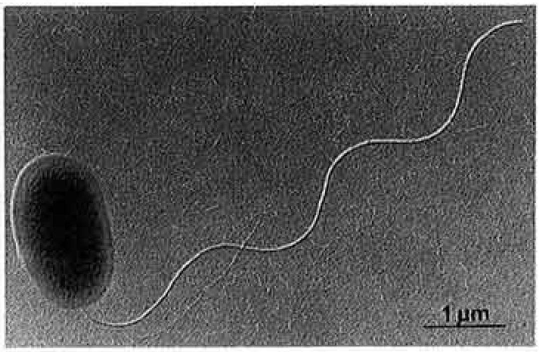

Plate 1. Electron micrograph of *P. avenae*

(0.9 × 2.1 µm), motile with one polar flagella (Plate 1) and aerobic. They accumulated poly-ß-hydroxybutyrate in their bodies. They did not require an organic growth factor and did not grow on the D-1 agar medium[7]. The colonies of all isolates on YDC agar medium[16] were translucent, glistening, convex, and round with entire edges and a smooth surface. The isolates utilized glucose oxidatively without production of acid. No fluorescent pigments were produced. They grew in peptone water at 41°C except for several isolates. Oxydase activity, reduction of nitrate and hydrolysis of starch were positive; arginine dihydrolase activity, production of levan, and liquefaction of gelatin were negative. Glucose, mannitol, sorbitol, ß-alanine and D-tartrate were used as a sole source of carbon; cellobiose, trehalose, L-rhamnose, and sucrose were not used (Table 1). These bacteriological properties of the isolates coincided with those of two isolates of *Pseudomonas avenae* (NIAES 1024, 1141) and did not differ from those recorded in Bergey's Manual[2]. These isolates were therefore identified as *Pseudomonas avenae* Manns[12].

Table 1. Bacteriological characteristics of the bacteria isolated from infected seedlings in nursery boxes

Characteristic	Bacterial isolates (79)[a]	*P. avenae* (2)	*P. glumae* (4)	*P. plantarii* (7)	*P. marginalis* (2)
Number of flagera	1	1	1–5	3–7	2–5
Relation to oxygen	S.A.[b]	S.A.	S.A.	S.A.	S.A.
Gram's reaction	–[c]	–	–	–	–
O-F test	O/–	O/–	O/+	O/+	O/+
Organic compounds requirement	–	–	–	–	–
Accumulation of PHB[d]	+[c]	+	+	+	–
Growth at 41 C	+	+	+	–	–
Fluorescent pigment	–	–	–	–	+
Revan production from sucrose	–	–	–	–	+
Arginine dihydrolase	–	–	–	–	+
Oxidase reaction	+	+	–	+	+
Lecithinase	–	–	+	+	+
Nitrate reduction	+	+	+	+	+
Gelatin liquefaction	–	–	–	+	+
Starch hydrolysis	+	+	d[c]	–	–
Utilization of:					
D-Arabinose	–	–	+	–	–
ß-Alanine	+	+	+	W[c]	+
Cellobiose	–	–	+	+	–
Glucose	+	+	+	+	+
Mannitol	+	+	+	+	+
L-Rhamnose	–	–	–	+	–
Sorbitol	+	+	+	+	+
Sucrose	–	–	–	–	–
D-Tartrate	+	+	–	+	
Trehalose	–	–	+	–	d
Potato soft rot	–	–	–	–	+
Tobacco hypersensitivity	+	+	+	+	–

a): Number of isolates. b): S.A. = Strict aerobes.
c): + ; Positive, – ; Negative, d; Varied reactions in different strains, W; Weak reaction.
d): PHB = Poly-ß-hydroxybutyrate.

Symptoms and ecology

1) Seedling stage

The symptoms of the disease on rice plants in the seedling stage were divided into 4 types; i.e. inhibition of germination, brown stripe on the leaf, curving of the leaf sheath and abnormal elongation of the mesocotyl[8].

Inhibition of germination was seen in the seeds which began to germinate. The coleoptile, approximately 1 cm grown, turned pale yellow-brown color accompanied by a water-soaked region and stopped growing. Subsequently, these seeds died without germination (Plate 2-1).

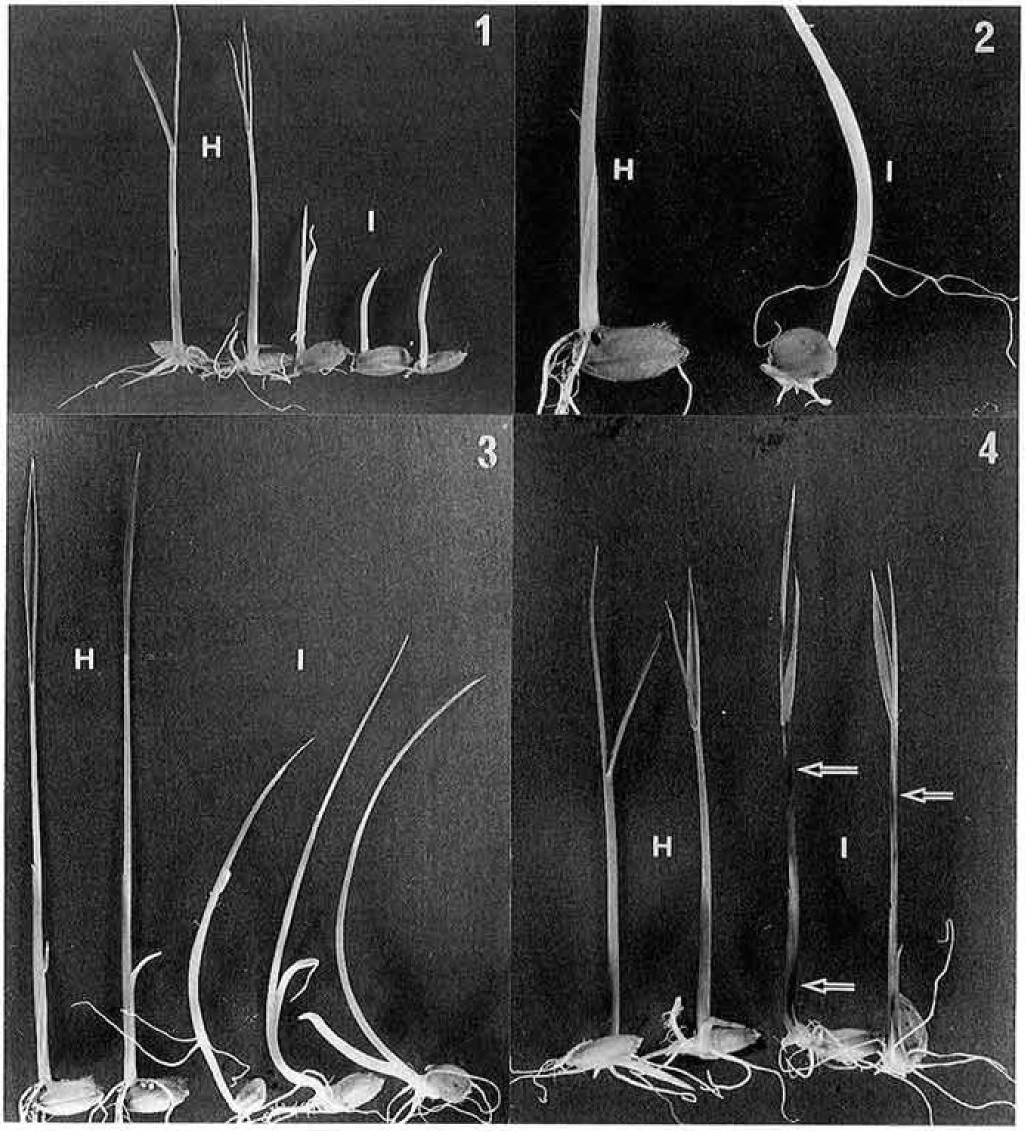

Plate 2. Symptoms of the diseased seedlings infected by *P. avenae*
1: Inhibited germination, 2: Abnormal elongation of mesocotyl,
3: Curving of the leaf sheath, 4: Brown stripe on the leaf (arrows).
H: Healthy seedlings, I: Infected seedlings.

Infected seedlings, though not dead at the germination stage, grew less in size than healthy ones did. One side of the leaf sheath grew better than the other, resulting in the leaf sheath curved as a bow (Plate 2-3). The degree of curving varied among the seedlings. Growth of the seminal roots of the curved seedlings was usually retarded remarkably. Mesocotyl was abnormally elongated and the crown root grew at the node of coleoptile (Plate 2-2). The majority of the seedlings showing these symptoms died before the 3rd or 4th leaf stage.

Brown stripe occurred on the coleoptile at first in the form of water-soaked dark-brown regions with a less than 1 mm width each. The regions extended to sheaths and blades of the 1st and 2nd leaves (Plate 2-4). This symptom emerged on the curved and mesocotyl-elongated seedlings at the same time. Most seedlings with this symptom died until the 2nd or 3rd leaf stage. After the 4th leaf stage, this symptom appeared only on the lower leaves and had little effect on the growth of the seedlings.

2) Tillering stage

After the infected seedlings were transplanted to the paddy fields, the symptoms were masked. Consequently, natural occurrence of the disease could not be seen at this stage. However, it was confirmed that if a needle prick inoculation was done on the leaf sheath, the brown stripe appeared around the inoculated spots.

Plate 3. Symptoms of the rice plant infected by *P. avenae* at the panicle formation stage

3) Panicle formation stage

Based on the observation of the masked symptoms at the tillering stage as mentioned above, it was presumed that the symptoms of the disease might occur only at the seedling stage of rice plants. However, the symptoms also emerged at the panicle formation stage when the paddy fields were submerged by flood[11].

In June 1985, there were converged heavy rains in the Joetsu district of Niigata Prefecture. The rice plants just at the panicle formation stage were submerged for 1-3 days. Several days after the water drained away, a bacterial disease, resembling the bacterial foot rot of rice by *Erwinia chrysanthemi*[4-6], came out on the rice plants (Plate 3). Sixty percent or more stems of the submerged plants were damaged by the disease. Water-soaked and dark-green spots were seen on the leaf sheaths of the plants. Young unfolding leaves turned dark-brown color and eventually died. On the lightly infected plants, a long and slender brown stripe came out along the midrib of the new leaf. The plants demonstrated normal growth in appearance, but the panicles were deformed and were sterile in most cases. Deformation of the panicles included: 1) abnormal elongation of the 1st and 2nd internodes; 2) curving of rachis; 3) reduction of rachis-branch and turning brown color; 4) abnormal elongation of palea and lemma to the long axis; and 5) no flowering of the glumous flower. In the paddy fields all these incidences took place at the same time.

A number of bacteria were isolated from the infected rice plants and had pathogenicity to rice. Based on their bacteriological properties, they were identified as *Pseudomonas avenae*. From the results of inoculation test and identification of pathogenic bacteria, it was clearly confirmed that the pathogenic bacterium (*P. avenae*) related to the occurrence of the symptom on the mature rice plants.

4) Heading stage

(1) Symptom of the glume

Since infection with *P. avenae* on the seed and a relevant symptom of the disease was not known, these matters were confirmed under the following experiments: bacterial suspensions of *P. avenae* were sprayed to the rice panicles immediately after they came out from the sheaths. Four or five days after inoculation, a dark green and water-soaked symptom appeared slightly on the palea and lemma only when high density suspensions (more than 10^8 CFU/m*l*) were sprayed. When these seeds were planted in nursery boxes, brown stripes came out on the seedlings, and the rate of the infected seedlings was about 80% or occasionally more. Causal bacteria were usually isolated from the seeds and the seedlings as well. They were identified as *P. avenae*.

On the other hand, this bacterium could not be isolated from the seeds which showed the similar symptoms in the paddy fields. When these seeds were sown in the nursery boxes, however, this disease usually appeared on the seedlings and the causal bacterium could be easily isolated.

These findings suggest that the causal bacteria invade the seeds without any symptoms, and that a small quantity of the causal agent increases rapidly during the germination period and becomes the source of infection in the nursery boxes in the following year[9].

(2) Invasion period on rice seeds

In order to confirm the above-stated observation that the causal bacterium was likely to invade the seeds, an additional experiment was undertaken as described hereafter: Rice plants (cultivar: Koshihikari and Ishikari-shiroke) were grown in the pots in a greenhouse. Bacterial suspensions (*P. avenae* H 8201 isolate, 10^2–10^8 CFU/m*l*) were sprayed at an interval of 3 days on the rice plants at the heading stage. The seeds of the inoculated plants were harvested and planted in the nursery boxes. The incubation degree of the causal bacterium was investigated as a disease index. The following formula was adopted to estimate the index:

Disease index = (A + 25B + 50C + 100D) / N where A, B, C and D are the number of seedlings with the coleoptile, incomplete leaf, the 1st leaf, and the 2nd leaf infected, respectively; and N is the total number of the seedlings under testing.

The highest disease index was seen on the seed inoculated at the flowering day on both cultivars, and gradually declining before and after away from the flowering date. The density of suspension for the disease incidence was over 10^4 CFU/m*l* (Fig. 1).

From this inoculation test, it was recognized that the invasion period of the causal bacterium on rice seeds was several days around the flowering date, and that there was a close relationship between the

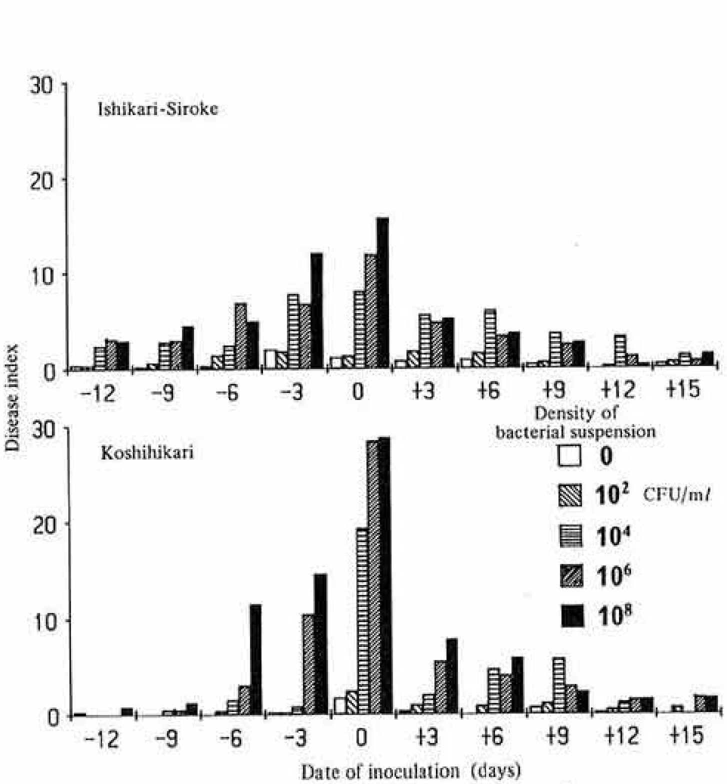

Fig. 1. Distribution of the disease indexes on different dates of inoculation to rice panicles
0: The flowering date, +: Days after the flowering date, −: Days before the flowering date.

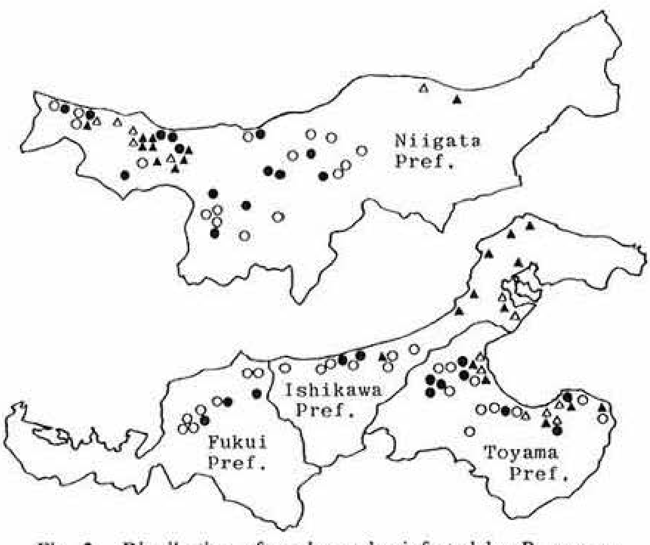

Fig. 2. Distribution of seed-samples infected by *P. avenae*
●, ○, ▲ and △ are the locations of sampling of the seeds with and without pathogen produced in 1982 as well as in 1984, respectively.

density of bacterial suspensions for spraying and the disease indexes[10].

(3) Incubation degree of the causal bacterium

To identify the variation of infected seed samples in the Hokuriku area, 102 samples were collected from various parts of Hokuriku Prefecture in 1982 and 1984. These seed samples were planted in nursery boxes and the rates of the infected seedlings were surveyed in each sample. The disease incidence was seen in 48 of the 102 samples (Fig. 2). The rates of the infected seedlings varied among the samples; the majority was less than 10% with the exception of more than 10% in 8 samples. There was no relationship among the rates of infected seedlings, the cultivars of rice, the heading dates and the fields for sampling.

It is therefore concluded that the rice seeds produced in the Hokuriku area generally have a potential to incubate the causal bacterium[10].

Control

Under general conditions for rice cultivation, the symptoms of the bacterial brown stripe are only seen at the seedling stage. Very limited information are presently available regarding the dynamics of the pathogen on the host plants. For this reason, it is difficult at this moment to identify an effective method for controlling the bacterial disease. In this connection, however, some experiments indicate that the disease incidence is associated with temperature and humidity of the environment. Causal bacterium grows at 41°C with an optimum temperature of 34–36°C, and thrives vigorously under aerobic and wet conditions. Such conditions are also favorable for the seedlings, particularly for germination, in a nursery box. It would therefore be effective to maintain an adequate level of temperature and humidity each in raising rice seedlings.

The most essential in controlling the disease is cutting-out of the disease life-cycle. The experimental results indicate that a primary source of infection on rice seedlings is the seeds with the pathogen. However, it is difficult to separate the seeds with and without the pathogen. It is therefore necessary to identify whether the seeds have the pathogen with a germination test before planting. In case where the seeds are contaminated with the pathogen, it is important to control this disease by a bactericide of Kasugamycin[17].

References

1) Azegami, K. et al. (1987): *Pseudomonas plantarii* sp. nov., the causal agent of rice seedling blight. *Int. J. Syst. Bacteriol.*, **37**, 144–152.
2) Doudoroff, M. & Palleroni, N. J. (1974): Bergey's manual of determinative bacteriology (8th ed). Williams & Wilkins Co., Baltimore.
3) Goto, K. & Ohta K. (1961): Bacterial stripe of rice. *Spec. Public. Coll. Agr. Taiwan Univ.*, **10**, 49–57.
4) Goto, M. (1965): A comparative study of the sheath rot bacteria of rice. *Ann. Phytopath. Soc. Jpn.*, **30**, 42–45.
5) Goto, M. (1979): Bacterial foot rot of rice caused by a strain of *Erwinia chrysanthemi*. *Phytopathology*, **69**, 213–216.
6) Goto, M. (1983): Nomenclature of the rice strain of *Erwinia chrysanthemi*, the causal agent of bacterial foot rot of rice. *Ann. Phytopath. Soc. Jpn.*, **49**, 576–579.
7) Kado, C. & Heskett, M. G. (1970): Selective media for isolation of *Agrobacterium, Corynebacterium, Erwinia, Pseudomonas,* and *Xanthomonas*. *Phytopathology*, **60**, 969–976.
8) Kadota, I. & Ohuchi A. (1983): Symptoms of bacterial brown stripe of rice seedlings in nursery boxes. *Ann. Phytopath. Soc. Jpn.*, **49**, 561–564 [In Japanese].
9) Kadota, I & Ohuchi A. (1987): Symptom of rice glume inoculated with *Pseudomonas avenae*. *Proc. Assoc. Pl. Prot. Hokuriku*, **35**, 17–20 [In Japanese].
10) Kadota, I & Ohuchi A. (1987): Seed-transmission of causal bacterium (*Pseudomonas avenae*) and its invasion period in rice plants. *Proc. Assoc. Pl. Prot. Hokuriku*, **35**, 21–23 [In Japanese].
11) Kadota, I & Ohuchi A. (1988): Symptoms of rice in tillering stage caused by *Pseudomonas avenae*. *Proc. Assoc. Pl. Prot. Hokuriku*, **36**, 8–13 [In Japanese].
12) Manns, T. F. (1909): The blade blight of oat — a bacterial disease. *Ohio Agr. Exp. Sta. Bull.*, **210**, 91–167.
13) Schaad, N. W. (1980): Laboratory guide for identification of plant pathogenic bacteria. Bacteriol. Commit. Amer. Phytopath. Soc., Minnesota.
14) Tominaga, T. et al. (1983): Bacterial brown stripe of rice in nursery box, caused by *Pseudomonas avenae*. *Ann. Phytopath. Soc. Jpn.*, **49**, 463–466 [In Japanese].
15) Uematsu, T. et al. (1976): Pathogenic bacterium causing seedling rot of rice. *Ann. Phytopath. Soc. Jpn.*, **42**, 464–471 [In Japanese].
16) Wilson, E. E. et al. (1967): Bacterial phloem canker, a new disease of Persian walnut trees. *Phytopathology*, **57**, 618–621.
17) Yaoita T. et al. (1984): Control of rice bacterial brown stripe disease multiplied in Hatomune auto-sproutor with Kasugamycin. *Proc. Assoc. Pl. Prot. Hokuriku*, **32**, 86–90 [In Japanese].

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