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Epidemiology of Bacterial Grain Rot of Rice Caused by *Pseudomonas glumae*

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Abstract

The mode of development of bacterial grain rot of rice caused by *Pseudomonas glumae* Kurita and Tabei in the field was studied using a selective medium in relation to factors associated with the disease. Host susceptibility, inoculum density, and climatic factors played a major role in the infection of grain. Studies on the population dynamics of the pathogen in pot and field experiments revealed that P. *glumae* on flag leaf sheaths was the inoculm source for the primary infection and that diseased panicles played an important role in the formation of foci leading to secondary infection. Pot and field experiments revealed that the pathogen showed a lognormal distribution on individual leaf sheaths, indicating that bacterial populations on the uppermost leaf sheaths in the field may be estimated from lognormal values of population size on each leaf sheath sample more accurately than on bulked leaf sheath samples, and that although the frequency of uppermost leaf sheaths harboring P. *glumae* drastically decreased with internode growth, the population size on rice plants before at least 30 days prior to heading time affected the disease incidence. As for the forecasting of the disease, the frequency of flag leaf sheaths harboring P. *glumae* (FFP) was an important indicator of the primary infection because the FFP was closely related to the disease incidence at the early stage just after heading and the presence of severely affected panicles at an early stage enabled to predict disease development after secondary infection.

Discipline: Plant disease
Additional key words: population dynamics, primary infection, secondary infection, selective medium, forecasting

Introduction

Pseudomonas glumae Kurita and Tabei 1967[4] causes seedling rot of rice in nursery boxes[25] and grain rot in the field after heading. Grain rot, especially, results in serious yield loss in the southern part of Japan. The incidence of the disease has increased with the dissemination of transplanting machinery because nursery boxes promote the increase in bacterial population[6]. However, no practical method of control has been developed due primarily to the lack of epidemiological data on the pathogen.

Selective medium (S-PG) for detection and colony type

A selective medium (S-PG) was developed to isolate the pathogen from rice plants[11]. The composition of the medium is shown in Table 1. *P. glumae* formed two different types of colonies, A and B, depending upon the isolate (Plate 1). Among other bacterial species tested, only *P. avenae* produced colonies similar to type B ones. All of the 37 isolates of *P. glumae* tested produced a specific precipitin band against the antiserum of *P. glumae* isolate Kyu82-34-2 in agar gel diffusion tests, but none of *P. avenae* isolates reacted, suggesting that *P. glumae* could be detected from natural specimens with S-PG

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Table 1. Selective medium (S-PG) for detection of *Pseudomonas glumae*

Compounds	Amount (/l)
A. Basal medium	
KH_2PO_4	1.3 g
Na_2HPO_4	1.2 g
$(NH_4)_2SO_4$	5.0 g
$MgSO_4 \cdot 7H_2O$	0.25 g
$Na_2MoO_4 \cdot 2H_2O$	24 mg
EDTA-Fe	10 mg
L-cystine	10 μg
Agar	15 g
B. Other compounds	
D-sorbitol	10 g
Pheneticillin potassium	50 mg
Ampicillin sodium	10 mg
Cetrimide	10 mg
Methyl violet	1 mg
Phenol red	20 mg

Basal medium was added to 970 ml of distilled water and then autoclaved. To make solution B, D-sorbitol was mixed with 30 ml of distilled water and filtered and, then, mixed with other compounds.
Methyl violet (10 mg) was dissolved in 2 ml of ethyl alcohol and added to 8 ml of water, and then, 1 ml of the solution was added.
Phenol red (0.2 g) was dissolved in 10 ml of NaOH (1/20 N) and then 1 ml of the solution was added.
The mixture was added to the basal medium which was cooled to about 50°C.

medium combined with the serological method. With S-PG medium, some ecological studies were carried out in Japan[1,2,7,24]. Type A was isolated mainly in the southern part of Japan while type B all over Japan[13], suggesting allopatric differentiation of both types in Japan.

Factors associated with infection of spikelets

Experiments under controlled conditions showed that host susceptibility, inoculum density and climatic factors played important roles in the infection of the spikelets.

1) Host susceptibility

Flowering of the spikelet was considered to be involved in the susceptibility of the spikelets[2,9,23]. Changes in the susceptibility of the spikelets (cv. Koshihikari, Koganebare and Asominori) to the disease with time were examined in detail by spraying a bacterial suspension on panicles at different stages of flowering[20]. Spikelets were most susceptible on the day of flowering, remained comparatively susceptible 1–3 days after flowering, but became resistant 2 days before and 4 days after flowering, even though the spikelets were incubated under high humidity (RH>95%) conditions for 24 h after inoculation. Analyses on the relationship between the susceptibility of a whole panicle and the flowering rate within a panicle showed that the susceptibility of the panicle depended on the daily flowering rate, indicating that the susceptibility of rice plants in the field may be estimated by the heading rate of panicles in the field and by the extent of the daily susceptibility of each panicle. The relative value for cumulative susceptibility of rice plants (Tt) in the field on the day t after first heading was calculated based on the value for the daily susceptibility of all panicles and the heading rate of panicles on days 0–t after first heading. Changes in Tt values showed that rice plants in the field were most susceptible 4 to 5 days after heading time, which was defined as the day when more than 40% of the panicles had headed, and more susceptible to the disease during a short period of time, i.e. from heading time to about 11 days after.

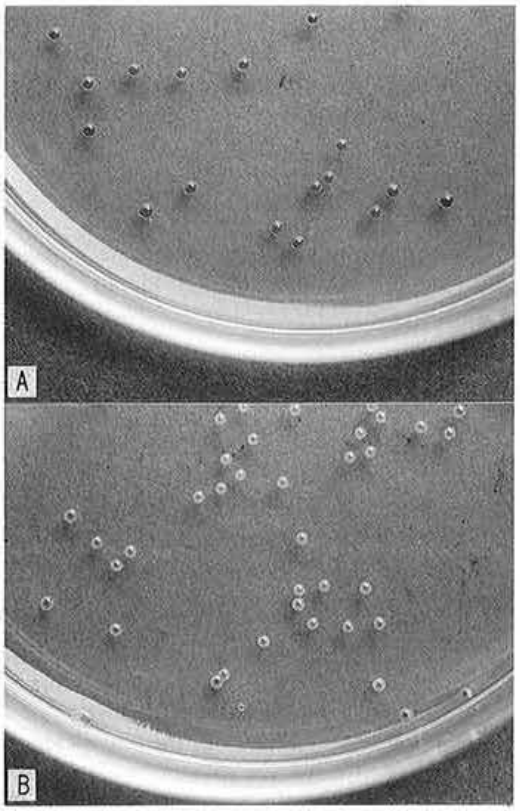

Plate 1. Colony types of *P. glumae* on S-PG medium
A: A-type, B: B-type.

2) Inoculum density

Pot experiments in which bacterial suspensions were sprayed revealed that the disease incidence increased linearly with the lognormal values of inoculum density[10]. Based on these results, the minimum inoculum density of the pathogen was estimated to be 10^2 to 10^4 cfu/ml in the field, although it was considered to be less than 10^2 cfu/ml under high temperature conditions (Tsushima, unpublished). Hikichi[2] reported that the disease occurred by the injection of a bacterial suspension at 1 cfu/ml. Inoculum density may decrease at the time of injection.

3) Humidity and temperature conditions

High humidity level at the flowering stage was conducive to the infection of the spikelets[20]; spikelets inoculated with a bacterial suspension 2 to 4 days before flowering did not show symptoms under low humidity conditions (RH<70%) unlike under high humidity conditions (RH>95%). The disease occurred at 20–32°C in pot experiments and the severity increased with increasing temperature[10]. In addition, the temperature during the critical period for infection affected the duration of incubation (Tsushima, unpublished).

Population dynamics on rice plants

A general model of the life cycle of *P. glumae* is schematically represented in Fig. 1 in relation to the growth of rice plants. The pathogen occurred on rice plants during the growing season[1,5,7] and on rice seeds stored at room temperature in winter[14]. However, its persistence in other environments, e.g. weeds, soil, etc., has not been verified.

1) Primary infection

The pathogen on leaf sheaths played an important role in the primary infection of bacterial grain rot[15]. When potted plants were inoculated by spraying bacterial suspensions 27 days before heading time, the pathogen was found on leaf sheaths until heading but was not detected on leaf blades and, later plants harboring *P. glumae* within the leaf sheaths were found to contain the pathogen on panicles, too. Bacterial populations on individual leaf sheaths varied from undetectable levels, i.e. less than 10^3 cfu/g, to ca. 10^6 cfu/g, but the rate of flag leaf sheaths with the pathogen (FFP) was very low even though the pathogen was detected on all the lower leaf sheaths. The pathogen showed a lognormal distribution on

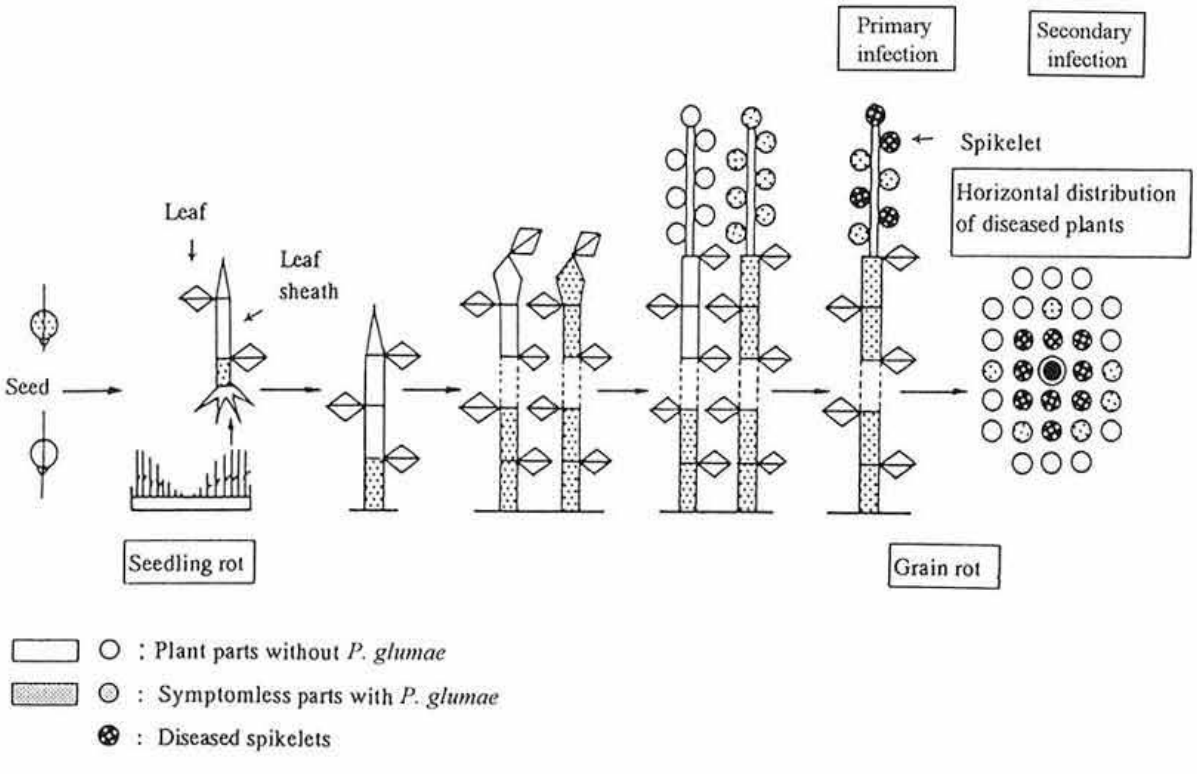

Fig. 1. Schematic representation of life cycle of *P. glumae* on rice plants during growing season in the field

individual leaf sheaths, suggesting that, in the case of this particular disease, the bacterial populations on the uppermost leaf sheaths in the field may be estimated from lognormal values of population size on each of the leaf sheath samples more accurately than on bulked leaf sheath samples as previously described by Hirano et al.[3].

The total population of *P. glumae* on spikelets was less than ca. 10^7 cfu/g at heading, but increased to 10^8-10^9 cfu/g during the 6-day period after heading and, then, remained at a level of more than 1×10^9 cfu/g on all the diseased spikelets[12]. *P. glumae* colonized the surface of the basal part of the lodicule and inner surface of lemma based on scanning electron microscopic observation[12]. Multiplication of the pathogen on the panicles was also studied in pot experiments[2] and in field experiments[2,24].

Population dynamics of the pathogen and relationship between bacterial population on leaf sheaths and disease incidence in the field were analyzed based on the results from pot experiments. When the pathogen was inoculated to 4 varieties (cv. Koganebare, Asominori, Hinohikari or Reiho) 28–31 days before heading time, the pathogen exhibited a clear lognormal distribution on individual leaf sheaths and the frequency of the leaf sheaths with the pathogen decreased drastically during the growth of internode[22]. When the pathogen was inoculated to rice plants at different times, the correlation coefficient between FFP and the disease incidence 1 week after heading time was high ($r=0.78$, $P=0.01$) but was not significant 2 and 3 weeks after, indicating that the extent of FFP was closely related to the disease incidence soon after heading. These results suggest that FFP can be used for the estimation of bacterial populations in relation to the incidence of grain rot in the field.

2) Secondary infection

Spatial distribution patterns of the disease were determined by Morishita's index I_B of distribution in the field and the dissemination of the pathogen was examined by setting an inoculated rice plant as a primary infection source at heading time or 1 week after heading time[16]. The results showed that severely diseased hills at an early stage after heading time were important for the formation of foci in the field.

The disease incidence in 62 fields was analyzed in terms of the rate of severely diseased panicles (SDPs) with >30% diseased spikelets which were observed within 7 days after heading time in an area of 5,000 hills selected randomly[21]. Disease incidence increased with increasing number of SDPs, and the occurrence of SDPs within a week after heading played an important role in disease development by secondary infection and was considered to be useful to predict the disease incidence.

Conclusion

The results, as previously indicated by Tsushima et al.[12] suggest that the inhibition of bacterial multiplication in spikelets during a short period after flowering is the clue to disease control by antagonists. An antagonistic bacterial strain, KyuA891, suppressed the development of the disease in the field by application of a bacterial suspension only at heading time[17], suggesting that the antagonistic bacterium may enable to control bacterial grain rot. *P. glumae* was reported to overwinter in rice plants buried in soil based on the antiserum technique[8] but its survival in other environments, e.g. soil, weeds, etc., has not been verified. Recently molecular techniques using polymerase chain reaction (PCR) have enabled to detect microorganisms at a low level in soils. Tsushima et al.[19] reported the development of a simple detection method using PCR by which 10 cfu of bacterial cells per gram of paddy soil could be detected. A specific DNA probe[18] for *P. glumae* developed recently should further facilitate the detection when combined with PCR. Such new approaches should enable to promote epidemiological studies of the disease.

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