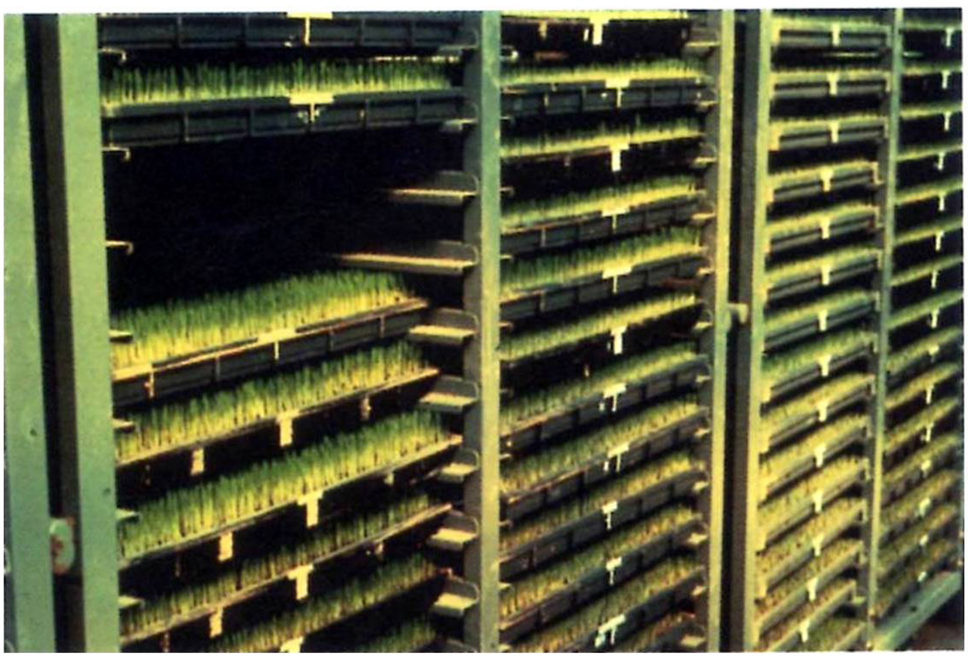
Section 2. Bacterial diseases

Chapter 1. Seedling diseases

The incidence and severity of bacterial seedling diseases have increased since adaption of new cultural methods in rice cultivation, such as mechanization of rice planting. Machine driven transplanting requires that seedlings be raised in nursery boxes indoors instead of the traditional outdoor field nursery (**SD Figure 1**). The conditions for seedling production using the seedbox method include high seeding rate, high temperature and high humidity, which are conducive to occurrence of seedling diseases especially seedborne pathogens. Because of fluctuation in temperatures and humidity, and field soil moisture saturation, few seedling diseases would develop in traditional outdoor nursery.

Some of the common bacterial seedling diseases are described in the following.



SD Fig. 1. Seedlings grown in nursery boxes for mechanical transplanting.

1. Seedling blight (SdB)

SdBl is a relatively new disease of rice first reported in Japan. The causal bacterium, *Burkholderia plantarii* (formerly *Pseudomonas plantarri*), was detected in 1982 (Azegami et al 1987b). Its occurrence is a consequence of the change in rice seedling production from the traditional outdoor nursery to the indoor nursery boxes intended for machine transplanting.

1.1. Symptoms

The early symptom may be characterized by a conspicuous chlorosis and withering of the second or third leaves. In severely infected seedlings, the root growth weakens and seedling dumping-off occurs (Azegami et al 1987a,b). When the growth of infected seedlings exceeds to certain height, chlorosis occurs at the basal parts of the leaves (**SdB Figure 1**).

The seedlings then wither and turn brown, but never rot, which differentiates it from seedling rot caused by *B. glumae*. Infected seedlings later become reddish brown and desiccated but the leaf tissues do not rot. With severe infection, root growth is retarded and seedlings easily lodge (**SdB Figure 2**).

1.2. Causal organism

The causal bacterium of SdB was identified as *Pseudomonas plantarii* by Azegami et al (1985). The bacterium is a gram-negative, nonspore-forming, nonencapsulated rod, measuring 0.7-1.0 x 1.4-1.9 µm, with one to three polar flagella. On plates of potato-peptone-glucose agar, colonies are round with tan centers and translucent margins and produce a weak, reddish brown pigment. It grows poorly on nutrient agar. The optimum and maximum temperatures for thebacterial growth were 32-35^{o}C and 38^{o}C, respectively. The bacterium produces no fluorescent pigment on King's medium B, is oxidase positive and arginine dihydrolase negative, and accumulates poly-β-hydroxybutyrate. The bacterium produces a compound tropolone, which is responsible for the retar-

SdB Fig. 1. Seedling blight chlorosis at the basal parts of the leaves.
Source: National Institute for Agro-Environmental Sciences, Japan.

SdB Fig. 2. Patches of diseased seedlings infected with seedling blight.
Source: National Institute for Agro-Environmental Sciences, Japan.

dation of root growth and leaf chlorosis observed in infected rice seedlings (Azegami et al 1985). Tropolone, which causes seedling dumpling but not rotting, is produced in both culture medium and in infected rice seedlings (Azegami et al 1985).

Plant-associated bacteria belonging to *Pseudomonas* rRNA group II were separated from members of the genus *Pseudomonas* and transferred to the genus *Burkhoderia* on the basis of plant-associated characteristics, chemotaxonomic characteristics, DNA-DNA hybridization data, rRNA-DNA hybridization data, and the sequences of 5S and 16S rRNAs (Urakami et al 1994). The seedling blight bacterium, the bacterium of seedling rot, and *P. gladioli* are renamed as *B. plantarii*, *B. glumae*, and *B. gladioli*, respectivley Taxonomically, *B. plantarii* is very close to *B. glumae* and *B. gladioli*, and these species are closely related to the members of the *Burkholderia cepacia* complex, which are known to colonize several environmental niches and to cause chronic infection of humans (Coenye and Vandamme 2003). It is reported that both quorum sensing and *RpoS*, which are global regulatory systems, are involved in the pathogenicity of *B. plantarii* (Solis et al 2006), and are very similar to that of *B. glumae* and *B. gladioli*.

1.3. Disease cycle

SdB occurs only on seedlings in nursery boxes. In Japan, the incidence and severity have increased rapidly after machines have been employed for rice transplanting. Seedlings in nursery boxes are grown under high temperature and humidity conditions that favor the occurrence of SdB. Outbreaks occur often in commercial seedling centers causing severe damage to seedling production (Miyazawa and Inoue 2002).

B. plantarii is seedborne, thus, the primary source of inoculum is from infested or infected rice seeds (Azegami et al 1987b). The causal bacteria persist in the basal parts of the plants after transplanting in the field. However, *B. plantarii* has been isolated from several graminaceous weeds growing in the levees of rice fields where bacteria may be released from these weeds into the paddy water (Miyazawa 2000). Indeed, the bacterial pathogen has been detected in paddy water and its populations have increased during July and August. In paddy water, however, its concentration is always higher along the levees than at a distance away from the levees, indicating an additional pathway for the bacteria from weed to paddy water that may serve as inoculum for primary infection (Miyazawa and Inoue 2002).

1.4. Control measures

Agrochemicals such as kasugamycin, oxilinic acid, copper bactericide, Ag containing bactericide, and biocontrol agents, such as *Trichoderma atroviride*, have been registered and used in Japan (K. Aegami, pers. comm.). Because of its seedborne nature, using healthy seed is an important approach to control the disease. Likewise, field sanitation, which avoids weeds from growing in the levees, is an additional measure of seedling blight management.

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2. Bacterial brown stripe (BbS)

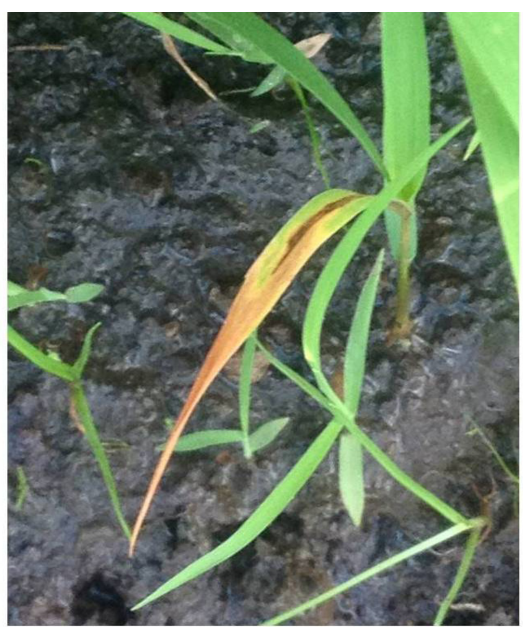
BbS, also known as bacterial stripe, is caused by *Acidovorax avenae* subsp. *avenae* (Manns) Willems et al. (formerly *Pseudomonas avenae* Manns) (Willems et al 1992). The disease occurs in rice plants in many countries at different stages of rice growth but it is most common at the seedling stage under upland conditions and sometimes in lowland nurseries.

BbS, one of the bacterial diseases that strikes at the seedling stage, has become very prominent on rice seedlings in indoor nursery boxes (Tominaga et al 1981). In the literature, *A. avenae* subsp. *avenae* (*P. avenae* Manns) and *P. syringae* pv. *panici* (Elliott) are reported to cause brown stripe (Young et al 1988). However, *A. avenae* subsp. *avenae* is more common than *P. syringae* pv. *panici* as as the causal agent of brown stripe and is distributed worldwide. Interestingly though, the disease has been reported only in a limited number of countries. Seed health testing has detected *A. avenae* subsp. *avenae* in rice seedlots from all major rice-growing countries (Shakya et al 1985). In Japan, rice seeds that carry the bacteria have produced seedlings in nursery boxes with typical brown BbS symptoms. BbS caused by *P. syringae* pv. *panici*, on the other hand, has been reported only in Japan and China (Bradbury 1985, Goto and Ohata 1961).

2.1. Symptoms

The symptoms of BbS consist of water-soaked stripes on the leaves and leaf sheaths, which turn brown as the lesion ages. On the leaves, the stripes occur between the veins of the leaf blades, along midrib or leaf margins. The stripes or lesions measure up to 10 cm x 1 mm but may coalesce to form wider lesions. The pathogen can also attack and rot young, unfolded leaves, which result in stunting or death of the seedlings. If infection occurs on primary leaves only, the affected seedlings generally outgrow the disease and new leaves appear to be normal (**BbS Figure 1**).

A. avenae subsp. *avenae* has been involved in grain discoloration of mature plants (Zeigler and Alvarez 1990)



BbS Fig. 1. Symptoms of bacterial brown stripe.

2.2. Causal organism

Pseudomonais avenae together with *P. cattleyae* were previously included in section V of the genus *Pseudomonas* in Bergey's Manual of Systematic Bacteriology (Palleroni 1984). However, data from rRNA-DNA hybridization demonstrated that the two belonged to the *Pseudomonas acidovorans* rRNA complex in rRNA superfamily III (De Vos et al 1989). Willems et al (1992) later proposed the transfer of *P. avenae* and *P. cattleyae* to the new genus *Acidovorax* (Willems et al 1992) and were renamed as *A. avenae* subsp. *avenae* and *A. avenae* subsp. *cattleyae,* respectively.

A. avenae subsp. *avenae* is a gram-negative, nonspore-producing, nonencapsulated rod, measuring 0.92-2.4 x 0.5-0.7 µm, with one or two polar flagella. The bacterium produces no fluorescent pigment on King's medium B, and is negative for oxidase, nitrate reduction, and starch hydrolysis.

P. syringae pv. *panici* (syn. *P. panici* (Elliott) Stapp) is also a gram-negative, non-spore-forming, nonencapsulated rod. The bacterium, however, produces a fluorescent pigment on King's medium B and is negative for oxidase, nitrate reduction, and starch hydrolysis. A BIO-PCR detection assay for *A. avenae* ssp. *avenae* in rice seeds has been developed (Song et al 2004). Molecular primers designed from a fragment of the internal transcribed spacer (ITS) region of *A. avenae* ssp. *avenae* strain were specific at the subspecies level for detection by producing the expected DNA product from 58 rice strains tested but not with DNA from 27 strains of *A. avenae* ssp. *avenae* from maize and other hosts.

2.3. Disease cycle

Both *A. avenae* subsp. *avenae* and *P. syringae* pv. *panici* are seedborne. There is little to no information about their epidemiology and infection process on rice plants. Since both are seedborne, it is assumed that seedborne inoculum is likely the most important source of inoculum for primary infection. *A. avenae* subsp. *avenae* has been reported to cause disease on *Panicum miliaceum* L., *Hordeum vulgarize* L., and *Setaria italica* (L.) (Tominaga 1968). Despite the fact that *A. avenue* ssp. *avenue* has a wide natural host range of monocotylydonus crop plants, strains from rice (Kadota 1996) and millet (Nishiyama et al 1979) apparently infect only rice and millet.

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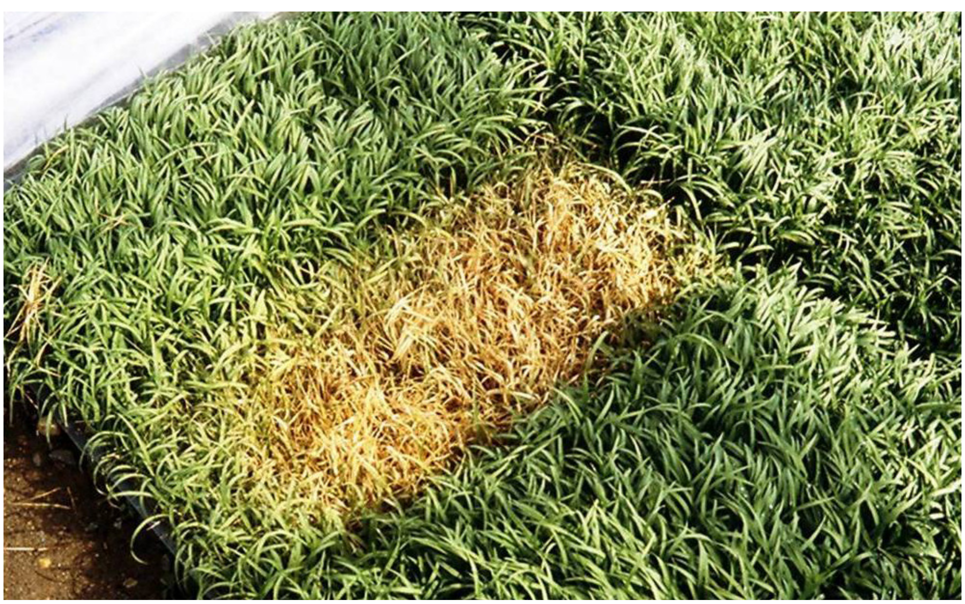
3. Seedling rot (SdR)

SdR is another seedling disease reported quite some time ago (Goto and Ohata 1956) but has only become a serious problem after the nursery box technique was employed to produce seedlings for machine transplanting (Uematsu et al 1976), first in Japan and later in other countries (**SdR Figure 1**). *Burkholderia (formerly Psudomonas) glumae* is the causal bacterium for both grain rot at the heading stage and seedling rot at the seedling stage.

In Japan, SdR was first observed in Fukushima and Okayama Prefectures in 1974 and 1975 (Uematsu et al 1976) on young seedlings grown in the nursery boxes. From these infected seedlings, the bacterium was isolated and shown to reproduce the seedling rot. The bacterium was initially identified as *Pseudomonas glumae* (Kurita et Tabei) Tominaga, based on its morphological, bacteriological,physiological and biochemical properties.



SdR Fig. 1. Seedling rot in nursery boxes.
Source: National Institute of Agrobiological Science, Japan.

P. glumae was earlier known to cause grain rot of rice. Although the bacterium was shown to also cause browning on seedlings, the disease does not further intensify. Uetmatsu et al. (1976) later reported that the bacterium was the causal agent of severe seedling rot on young rice seedlings. Henceforth, SdR has been widely observed in seedling centers and, once it occurs, it often causes severe damage to seedlings in the nursery boxes. The inoculum carried by the seeds likely comes from the same seedlots.

The bacterium was renamed as *Burkholderia glumae* (Urakami et al 1994) and is discussed in detail under grain rot in Chapter 4 below.

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