Xanthomonas oryzae pv. *oryzicola*

Scientific Name
Xanthomonas oryzae pv. *oryzicola* (Ishiyama, 1922) Swings et al. (1990)

Synonyms:
Xanthomonas campestris pv. *oryzicola*, *Xanthomonas oryzicola, Xanthomonas translucens* f.sp. *oryzicola*

Common Name(s)
Bacterial leaf streak

Type of Pest
Plant pathogenic bacterium

Taxonomic Position
Class: Gammaproteobacteria, **Order:** Xanthomonodales, **Family:** Xanthomonodaceae

Reason for Inclusion in Manual
CAPS Target: AHP Prioritized Pest List – 2006 through 2012 (as *Xanthomonas oryzae)*

Pest Description
Xanthomonas oryzae pv. *oryzicola* is a gram-negative, non-sporeforming rod, 1.2 x 0.3 to 0.5 µm, with a single polar flagellum. Colonies on nutrient agar are pale yellow, circular, smooth, and convex. Bacteriological tests useful in distinguishing *X. oryzae* pv. *oryzicola* from *X. oryzae* pv. *oryzae*, which causes bacterial blight of rice, are listed in Table 1 below (Vera Cruz et al., 1984; Bradbury, 1986; Mew, 1992; Mew and Misra, 1994). Isolates of the bacterium that differ in virulence (probable races) have been identified. In the rice host, the bacterium is present in the parenchyma tissue (Mew, 1992).

A pathovar is a bacterial strain (or set of strains) with similar characteristics that are usually distinguished by a different host range. In this case, *Xanthomonas oryzae* has two pathovars (*Xanthomonas oryzae* pv. *oryzicola* and *X. oryzae* pv. *oryzae)* that affect the same host but have strong differences in symptomatology on the same host, which allows for different pathovar designations.

Table 1: Discriminating bacteriological tests for preliminary identification and differentiation of pathovars of *Xanthomonas oryzae*.

	X. oryzae pv. *oryzae*	*X. oryzae* pv. *oryzicola*
Gram staining	-	-
Oxidase test	-[a]	-[a]

2-ketoglucontate production	-	-
Fluorescence on King's B medium	-	-
Nitrate reduction	-	-
Acetoin production	-	+
Oxidation-fermentation of glucose	O/-	O/-
Gelatin hydrolysis	-/v	-/v
Starch hydrolysis	-	+
Sensitive to 0.001% cupric nitrate (w/v)	+	-
Utilization of L-alanine as carbon source	-	+/v
Growth on 0.2% vitamin-free casamino acids	-	+
Strong peptonization of litmus milk	-	+
Phenylalanine deaminase	-	+[b]

+ = positive; - = negative; O = oxidative; v = variable
[a] Weak positive reactions can be observed.
[b] Positive response in 50% of strains.

Biology and Ecology
Xanthomonas oryzae pv. *oryzicola* causes bacterial leaf streak by colonizing the parenchyma and is limited to the vascular regions of the plant (Niño-Liu et al., 2006). *X. oryzae* pv. *oryzicola* is seedborne and seed-transmitted. This bacterium is spreading geographically. *X. oryzae* pv. *oryzicola* survives largely on infested seed and straw and is known to infect all wild species of the genus *Oryza.* These wild rice species may also serve as reservoirs of inoculum. In addition, this bacterium may be able to survive in irrigation water.

The bacterium enters the host through stomata or wounds and multiplies. It then spreads to the parenchymatous tissue in its early stages. In the parenchyma, more multiplication takes place until the parenchymatous tissues are substituted with the bacterium (Ou, 1985).

Severe cases of bacterial leaf streak are found in earlier stages of growth, because younger rice plants are more susceptible to the disease. During moist conditions bacterial exudates are found on the surface of the leaf lesions. During dry conditions, however, yellow beads are found on the surface of the leaf lesions (Ou, 1985). The bacterial exudates tend to spread rapidly from plant to plant via irrigation water, which also contains the bacterial exudates. Field-to-field spread of the bacterium is primarily from wind, rain and leaf to leaf contact. Seeds are able to carry the bacterium as well (Mew, 1992).

Disease development is favored by rain, high humidity, and high temperatures (28 to 30°C, 82 to 86°F) (Mew, 1992). Bacterial leaf streak is more severe when excessive amounts of nitrogen are applied to the crop (IRRI, 2004; 2010).

Symptoms/Signs

Bacterial leaf streak can occur at any plant growth stage, but tends to be more severe on younger plants. The disease initially appears as small, interveinal (between leaf veins), water-soaked streaks. The streaks are at first dark green in color but later become translucent (Fig. 1). The streaks enlarge and coalesce and eventually become light brown. Numerous tiny yellow beads of bacterial exudates are common on the surface of lesions (Fig. 1). Eventually, entire leaves turn brown and then grayish white and dies. The latter stage of the disease can mimic bacterial blight (Mew, 1992). There are no symptoms on seeds with bacterial leaf streak.

Figure 1: Symptoms of bacterial leaf streak caused by *Xanthomonas oryzae pv. oryzicola* in the field (Africa). Note: Dried bacterial exudates on bottom image. Photos courtesy of Valerie Verdier, Institut de Recherche pour le Developpement (IRD).

Pest Importance

Bacterial leaf streak is thought to be first spotted in the Philippines in 1918, but the bacterium was not positively identified at that time. It was not until 1957 in China that the bacterium causing bacterial leaf streak was properly named (Ou, 1985). Bacterial leaf streak is widely distributed in tropical Asia and in West Africa in both lowland and upland rice-growing areas. Recently, the disease has been reported in epidemic proportions in southern China (Mew, 1992). Yield losses have been estimated from 5% to 30% (Soto-Suárez et al., 2010)

Known Hosts

Major Hosts: *Oryza sativa* (rice), *Oryza* spp.

Minor/Other Reported Hosts: *Alopecurus* aequalis (shortawn foxtail), *Echinochloa crus-galli* (barnyardgrass), *Leersia hexandra* (southern cut grass), *Leptochloa filiformis* (mucronate sprangeltop), *Murdannia nudiflora* (reported as *Commelina nudiflora*) (nakedstem dewflower), *Oryza barthii* (barth's rice), *Oryza latifolia* (broadleaf rice), *Oryza malampuzhaensis, Oryza perennis, Oryza officinalis*, *Paspalum scrobiculatum* (reported as *Paspalum orbiculare)* (kodomillet), Poaceae (grasses), *Zizania aquatica* (annual wildrice), *Zizania caduciflora* (Manchurian wildrice), *Zizania palustris* (northern wildrice), and *Zoysia japonica* (Korean lawngrass) (Reddy and Nayak, 1974; Chang et al., 1977; Bradbury, 1984; Leyns et al., 1984; Zhong et al.,1998; CABI, 2011).

Note: The level of susceptibility and symptom expression varies significantly among hosts tested by artificial inoculation.

Known Vectors (or associated insects)

Bacteria may be disseminated in irrigation water, as well as by humans, insects and birds (Niño-Liu et al., 2006).

Known Distribution

Bacterial leaf streak is widely distributed in tropical Asia and in West Africa in both lowland and upland rice-growing areas (Mew, 1992). It is also known to occur in tropical and subtropical Asia, including southern China, Thailand, Malaysia, India, Vietnam, the Philippines, Indonesia, parts of West Africa, and northern Australia.

Asia: Bangladesh, Cambodia, China, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Thailand, and Vietnam. **Africa:** Burkina Faso, Mali, Madagascar, Nigeria, and Senegal. **Oceania:** Australia (Niño-Liu et al., 2006; Gonzalez et al., 2007; CABI, 2011; Wonni et al., 2011).

Pathway

Outbreaks of bacterial leaf streak (BLS) are more likely to occur during the monsoon season of the south-east Asian and Indian oceans (from June to September) than at other times of the year (Niño-Liu et al., 2006; Mew, 1992). Wind and rain, as well as contaminated rice stubble from previous crop seasons, spread the bacterium from infected rice plants and other hosts. This is the most important source of primary inoculum.

Severe epidemics often occur following typhoons. The fierce winds, wind-blown rain and hail wound rice plants and disperse bacteria. Irrigation water and leaf-to-leaf contact can spread the bacterium locally (Niño-Liu et al., 2006).

Potential Distribution within the United States

Surveys should be focused where the greatest risk for pest establishment occurs. A recent risk map developed by USDA-APHIS-PPQ-CPHST (Fig. 2) indicates that most states have a low risk rating for *X. oryzae* pv. *oryzae* establishment based on host availability and climate within the continental United States. Establishment is precluded from portions of most western states. Rice growing areas of the southern United States showing low to moderate risk include portions of Texas, Louisiana, Arkansas, and Missouri.

Survey

CAPS-Approved Method: Visual survey is the approved survey method for *Xanthomonas oryzae pv. oryzicola.* For visual survey, collect symptomatic leaf samples. For a preliminary indications of seed infection, look for bacterial streaming (Singh and Rao, 1977).

*For the most up-to-date methods for survey and identification, see Approved Methods on the CAPS Resource and Collaboration Site, at http://caps.ceris.purdue.edu/.

Literature-Based Methods: Survey for *Xanthomonas oryzae* pv. *oryzae* consists of visual inspection for symptoms, tissue sampling, and pathogen isolation.

Key Diagnostics/Identification

CAPS-Approved Method: Morphological and Serological methods are approved methods for *Xanthomonas oryzae* pv. *oryzae*.

Morphological:

Colony morphology: The pathogen is difficult to isolate directly from plant material and seed due to slow growth of bacterium and overgrowth by other organisms. Contaminants may also be of a yellow color (*e.g., Pantoea agglomerans* and *Xanthomonas*-like saprophytic bacteria). Isolation from symptomatic material should be completed as soon as possible after collection of samples. Plant parts showing fresh symptoms preferably with bacterial exudates should be selected for isolation if available. See EPPO (2007) for additional detail about pathogen isolation from plant material and seeds.

Isolation of *Xanthomonas* from symptomatic material can be performed using peptone sucrose agar (PSA), nutrient broth yeast extract medium (NBY), growth factor (GF) agar, modified Wakimoto's agar (without the ferrous sulfate), and various semi-selective media (Agarwal et al., 1989; Mew and Mistra, 1994; Sakthivel et al., 2001; EPPO, 2007). Growth is very slow on nutrient agar (NA) (EPPO, 2007).

Ming et al. (1991) developed a semi-selective medium, called XOS, to isolate both *Xanthomonas oryzae* pathovars from rice seed.

Gnanamanickam et al. (1994) tested three strains for growth on TZC, WF-P, YCM, YAT, MXO, and XOS semi-selective media. Results varied for each isolate used, but worked best when using monoclonal antibodies to confirm the genus and pathovar.

<u>Serological:</u> Monoclonal antibodies: Genus and pathovar specific antibodies can be used in an ELISA reaction on presumptive positives (Alvarez et al., 1985; Benedict et al., 1989).

<u>Literature-Based Methods:</u>
<u>Pathogenicity:</u> Isolates can be tested for pathogenicity on susceptible rice cultivars. For *X. oryzae* pv. *oryzicola* use 30-45 day old IR24 or IR50 (International Rice Institute) or local popular varieties with known susceptibility to bacterial leaf streak. Leaf clipping and spray inoculation methods are available for inoculations (Kauffman et al., 1973; Cottyn et al., 1994; EPPO, 2007). Niño-Liu et al. (2005) inoculated plants by dipping them in bacterial mixture and incubating in a growth chamber. Symptoms developed a 6-day period.

Xie and Mew (1998) used inoculum that came from seed and leaves sediments that had the bacterium using a washing procedure. The leaves were then placed on water agar and pricked with a needle that was dipped in the tissue sediments.

<u>Fatty Acid Profiles:</u> Fatty acid profiles allow identification at the genus level only (Swings et al., 1990), so this analysis is not recommended a diagnostic method.

<u>Molecular:</u>
<u>PCR:</u> Leach et al. (1990) used a repetitive DNA sequence (pJEL 101) to distinguish *X. oryzae* pv. *oryzae* from other pathovars and species of *Xanthomonas*.

Kang et al. (2008) developed a specific PCR detection system (targets a membrane fusion protein gene) for *X. oryzae* pv. *oryzicola*.

<u>Real-time PCR:</u> Zhao et al. (2007) developed a real-time PCR to detect *X. oryzae* pv. *oryzae* and can distinguish it from *X. oryzae* pv. *oryzicola*.

Liao et al. (2003) developed a real-time PCR that can distinguish the two pathovars.

<u>Computational Genomics/Multiplex PCR:</u> Lang et al. (2010) used a computational genomics pipeline to compare sequenced genomes of *Xanthomonas* species to identify regions for development of highly specific diagnostic markers. A suite of primers were selected to monitor diverse loci and to distinguish the rice bacterial blight and leaf streak pathogens. A subset of primers were combined into a multiplex PCR to accurately

distinguish the two rice pathogens in a geographically diverse collection from other xanthomonads and other plant pathogenic and plant-or seed associated bacteria.

Easily Confused Pests

In the early stage of disease, the symptoms are similar to narrow brown leaf spot. Physiological disorders can also cause similar symptoms to bacterial leaf streak.

Xanthomonas oryzae pv. *oryzicola* (bacterial leaf streak) and *Xanthomonas oryzae pv. oryzae* (bacterial blight) symptoms are easily differentiated in the early stages of disease and reflect the different modes of infection by each pathogen. Foliar symptoms of bacterial blight usually become evident at the tillering stage as small, green water-soaked spots at the tips and margins of fully developed leaves. The spots expand along the veins, merge, and become chlorotic and then necrotic, forming opaque, white to gray colored lesions that typically extend from the leaf tip down along the leaf veins and margins. Bacterial leaf streak symptoms, by contrast, begin with small, water-soaked lesions anywhere along the leaf between the veins. Veins act as barriers as infected areas expand and coalesce lengthwise, resulting in the symptom for which the disease is named. Streaks are translucent and typically yellow. At later stages infected leaves turn grayish white and die. When infection results from entry through breaks in the leaf as might occur due to high wind, symptoms may extend across the leaf break and expand lengthwise killing most or all of the leaf (Niño-Liu et al., 2006)

At the later stage, when the streaks have coalesced, symptoms of bacterial blight and bacterial leaf streak are similar. The shape of the edges of the lesions differs; straight in leaf streak and wavy in leaf blight. *X. oryzae* pv. *oryzicola* may be distinguished from *X. oryzae* pv. *oryzae* by colony morphology in typical isolates, strong starch and gelatin hydrolysis, and by biochemical and molecular methods.

Glossary

Parenchyma (adjective: parenchymatous): The primary tissue of higher plants, composed of thin-walled cells and forming the greater part of leaves, roots, the pulp of fruit, and the pith of stems.

Stomate (also known as: stoma plural: Stomata): A small opening on the surface of a leaf through which gaseous exchange takes place.

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This datasheet was developed by USDA-APHIS-PPQ-CPHST staff. Cite this document as:

Sullivan, M., Daniells, E., and Southwick, C. 2011. CPHST Pest Datasheet for *Xanthomonas oryzae* pv. oryzae. USDA-APHIS-PPQ-CPHST.