Bacterial Leaf Spot of *Amaranthus cruentus* L. Caused by *Burkholderia andropogonis* (Stapp) Gills et al.

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Key words : new bacterial disease, *Amaranthus cruentus*, *Burkholderia andropogonis*.

This paper deals with a new bacterial disease of *Amaranthus cruentus* L. (Japanese common name: Amaransasu or Sen Nin Koku) that was introduced from South America and grown in Japan locally either as a grain crop for white seeds, a vegetable for green leaves or an ornamental for yellow to red, upright flowers. This is the first record of bacterial leaf spot caused by *Burkholderia* (*Pseudomonas*) *andropogonis*³⁾ on the plants of Amaranthaceae.

Although the disease occurred from June through October, the most severe incidence was observed in July and August. The earliest symptom was dark green, water-soaked dots on leaf blades. They gradually enlarged to form circular spots a few to several mm in diameter and light brown to reddish brown in color. The spots were invariably surrounded by distinct yellow haloes 1 to 2 mm in diameter. The undersurface of the lesions was often greasy in appearance. The spots were usually scattered over leaf blades, but sometimes formed along veins. The disease development usually began from lower leaves, gradually spreading to upper leaves (Fig. 1A).

From diseased leaves collected at different fields in Shizuoka Pref., 6 strains (Am1-Am6) were isolated on nutrient agar plates by conventional method. A strain of *B. andropogonis* (Pa1) was isolated from sorghum which was affected by bacterial stripe disease in the experimental farms of MOA Nature Farming School.

By spray and prick inoculation methods, strains Am1-Am6 and Pa1 produced light brown to brown spots with yellow haloes on leaves of *A. cruentus*, black spots on leaves of white clover and alfalfa (cv. Natsuwakaba) and red stripes on sorghum (Fig. 1B), respectively. The symptoms on these plants except for alfalfa were identical with those produced by the infection under natural conditions. No difference was observed between strains Am1-Am6 and strain Pa1 in the symptoms and the disease severity produced on these plants. Both bacteria did not produce any symptoms on the following plants: Italian millet (cv. unknown), proso millet (cv. unknown), rice (cv. Koshihikari), oat (cv. unknown), radish (cv. Hatsukadaikon), *Brassica campestris* (cv. Jikyona), *Amaranthus tricolor* L. (cv. Morten-

fire), *A. mangostanus* L., *Celosia cristata* var. *childsii* Hort (cv. Yachiyo keito), kidney bean (cv. Eburon), carrot (cv. Kuroda-gosun-ninjin), tobacco (cv. Bright yellow 4), tomato (cv. Momotaro), spinach (cv. Sanraitto), welsh onion (cv. Akihiko), *Chrysanthemum coronarium* (cv. Satoyutaka), lettuce (cv. Shisuko), Calendula and *Corchorus olitorius*. All strains were negative in potato tuber rot and tobacco hypersensitive reaction.

Unless otherwise mentioned, the methods described by Cowan²⁾ were applied for the tests of physiological and biochemical properties. Strains Am1-Am6 and Pa1 were identical in the bacteriological properties with an exception of sorbitol utilization. Growth was relatively slow on nutrient agar plates, reaching to about 1 mm in diameter in 3 to 4 days at 28°C. Colonies were white, smooth, circular, convex, translucent with entire margins, and became very sticky when aged. Cells were Gram-negative, nonsporing, straight rods with round ends and motile with one to rarely two polar flagella. Poly- β -hydroxybutyrate granules were readily detected when grown on a synthetic basal medium of Ayers *et al.* with glucose as a sole source of carbon. All strains metabolized glucose oxidatively, turned litmus milk blue without coagulation and digestion, and grew in nutrient broth containing 3% NaCl but not 4%. Catalase reaction was positive. All strains were negative in the following tests: hydrolysis of casein, esculin, gelatin, pectin, starch and Tween 80, activities of arginine dihydrolase, lecithinase, urease and oxidase, production of pyoverdine on King's B medium, levan, acetoin, hydrogen sulfide, indole, and reducing substances from sucrose. All strains either produced acid from the following substances or used as a sole source of carbon: arabinose, ribose, fructose, galactose, glucose, mannose, rhamnose, adonitol, glycerol, inositol, mannitol, acetic acid, citric acid, folic acid, fumaric acid, galacturonic acid, gluconic acid, lactic acid, malic acid, malonic acid, succinic acid, alanine, tryptophan and tyrosine. Strains Am1, Am4, Am6 and Pa1 were positive in utilization of sorbitol, but Am2, Am3 and Am5 were negative. The following substances were not utilized as a sole source of carbon: xylose, cellobiose, melibiose, maltose, trehalose, sucrose, melezitose, raffinose, dextrin, inulin, starch, α -

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Fig. 1. A. Symptoms of bacterial leaf spot of *Amaranthus cruentus* in nature. B. Symptoms produced on sorghum by artificial inoculation of strain Am2.

methyl-D-glucoside, arbutin, esculin, salicin, dulcitol, erythritol, ethanol, geraniol, propanol, benzoic acid, glycolic acid, maleic acid, oxalic acid, propionic acid, tartaric acid, histidine and leucine.

In literature, four pathogenic bacteria have been listed on *Amaranthus* spp.: *Agrobacterium tumefaciens*, *Pseudomonas syringae* pv. *tabaci*, *Burkholderia solanacearum* and *Xanthomonas campestris* pv. *amaranthicola*¹⁾. Among them, *P. s.* pv. *tabaci* and *X. c.* pv. *amaranthicola* are discernible as the leaf spot pathogens. However, the present bacterium was clearly differentiated from these bacteria in many phenotypic properties including production of pigments, capacity to hydrolyse high molecular substances, and ability to use carbohydrates as a sole source of carbon. Thus, the bacterium under consideration was determined to be a new pathogen that have never been reported on *Amaranthus* spp.

From the bacteriological characteristics and host ranges, strains Am1-Am6 isolated from *Amaranthus cruentus* were identical with strain Pa1 isolated from bacterial stripe disease of sorghum. Therefore, we identified the amaranthus strains as *Burkholderia andropogonis*, and propose bacterial leaf spot (Japanese common name: Hanten-saikin Byō) as the common name of the disease.

This bacterium has been known by wide natural hosts including 33 species of 21 genera belonging to 12 families of Caryophyllaceae, Commelinaceae, Ericaceae, Graminae, Leguminosae, Liliaceae, Musaceae, Nyctaginaceae, Orchidaceae, Plumbaginaceae, Polygonaceae and Rubiaceae^{1,7)}. In addition, other 14 species of 13 genera have been reported to be susceptible by inocula-

tion tests¹⁾. In this paper, *Amaranthus cruentus* of the family Amaranthaceae was added to the list of natural hosts, suggesting that the host range of this bacterium may be further expanded in future. Some strains of *B. andropogonis* have been reported to be nonpathogenic to white clover^{5,7)}, implying the possible existence of the host specialization in this bacterium. In *Amaranthus* spp., the disease susceptibility greatly varied among species. Similar phenomenon has already been observed in *Sorghum* spp⁶⁾. *B. andropogonis* produces rhizobitoxine⁴⁾ and another toxin that is similar to tabtoxin in respect to the bioassay with *E. coli*⁵⁾. It is an open question which toxin could be involved in the development of yellow haloes on the leaves of *A. cruentus*.

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和 文 摘 要

村井雄一朗・後藤正夫: *Burkholderia andropogonis* による *Amaranthus cruentus* の斑点細菌病 (新称)

Amaranthus cruentus (和名: アマランサス; 仙人穀) の葉に黄色の暈を伴う褐色ないし赤褐色、直径数 mm の円形斑点をつくる細菌病が発生した。病原細菌は普通寒天平板上で比較的生育の遅い、粘性の白色集落を形成した。この細菌は *A. cruentus* の葉に自然発病と同じ病斑を形成してコッホの原則を満たしたほか、モロコシに赤色条斑を、またシロクローバおよび

アルファルファ上に黒色病斑を生じた。一方、アマランサス科のハゲイトウ、ヒユナ、ケイトウをはじめ17種の植物には病原性を示さなかった。本細菌の細菌学的性状は、ソルビトール分解能で陽性と陰性を示す菌株が存在したほかは、モロコシ条斑細菌病菌 (*Burkholderia* (*Pseudomonas*) *andropogonis*) のそれと完

全に一致した。これらの性状から本細菌を *B. andropogonis* と同定し、病名をアマランサス斑点細菌病と呼称することを提案する。

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