

IDENTIFICATION AND PATHOGENICITY OF ISOLATE OF BACTERIUM CAUSED LEAF BLIGHT DISEASE ON *Maranta arundinacea*

Supriadi, Nildar Ibrahim, and N. Karyani

Research Institute for Spice and Medicinal Crops, Jalan Tentara Pelajar No. 3, Bogor 16111, Indonesia

ABSTRACT

Arrowroot (*Maranta arundinacea* L.) is a multi-functional plant used as a source of medicinal, carbohydrate (especially the green leaf type) and also as ornamental plant (the streaked white leaf type). A leaf blight disease is recently found on the streaked white type in Bogor. Preliminary observation indicated that the disease was associated with bacterial infection. The cause of the disease has not been studied. This study was aimed to identify the cause of bacterial leaf blight disease. Experiments were conducted in the laboratory of Research Institute for Spice and Medicinal Crops in Bogor. Suspected bacteria were isolated from diseased leaves. The results showed that the bacterium produced white to brownish colonies on rich agar media containing peptone or cassamino acid, 3-5 mm in diameter, circular, and did not yield fluorescent pigment on King's B medium. The bacterium formed rod cells, Gram negative, accumulated poly β hydroxybutyrate, utilized glucose under aerobic condition, not hydrolyse arginine and starch, positive catalase, insensitive to tetrazolium salt (0.1%), and grew at 35°C. The bacterium neither produced xanthomonadin pigment nor reduced nitrate to nitrite. The pathogen was tolerant to penicillin and oxolinic acid, but sensitive to streptomycin and oxytetracycline at high concentration (1,000 ppm). These characteristics met to those of *Pseudomonas cepacia*. Pathogenicity test on detached leaves showed that the typical symptom of blight was similar to that of natural infection on arrowroot. This is the first report on occurrence of *P. cepacia* on arrowroot plant.

[Keywords: *Maranta arundinacea*; arrowroot, bacterial leaf blight; *Pseudomonas cepacia*]

INTRODUCTION

Arrowroot (*Maranta arundinacea* L.) is one of multi-functional crops grown as sources of medicine, carbohydrates, and ornament. Rhizomes of the plant contain protein (1-2.2%), fat (0.1%), starch (19.4-21.7%), fibers (0.6-13%), and ash (1.3-1.4%). Its starch is high quality, easily digested, and multi-

purpose such as to make cakes, dressings, and foods. The starch is also used for treating stomach problems such as diarrhoea. Arrowroot is rather tolerant to low light intensity, therefore it can be cultivated as intercrop in certain forests and plantations. However, cultivation techniques of the plant are still limited, including information on diseases.

Two types of leaves, i.e., green and streaked white (Villamayor and Jukeme, 1996) are of important characteristics of arrowroot. Both types produce rhizomes, however only the green type commonly grown for starch production, whereas the streaked white is for ornaments. Purseglove (1972) reported that the pulp from the fresh rhizomes was used by Indians in the Caribbean to treat wounds inflicted by poison arrows, it is still used by Caribs in Dominica to make poultices for healing wounds and ulcers.

Some diseases already reported to suffer arrowroot plant are burning disease caused by *Rosselinia bunodes* and banded leaf blight caused by *Pellicularia filamentosa* (Purseglove, 1972), bacterial wilt caused by *Ralstonia* (synonym *Pseudomonas*) *solanacearum* (Bradbury, 1986; Adhi *et al.*, 1999), and wilt caused by *Xanthomonas marantae* (Zagattos and Pereira in Bradbury, 1986). Diseases of arrowroot could cause serious losses in rhizome production (Villamayor and Jukeme, 1996).

A disease of leaf blight symptom is found attacking the streaked white type of arrowroot plant in several locations in Bogor, West Java. The disease quickly spreads to surrounding the affected plants and caused most of leaves become dry. Its existence is thought a serious consideration to include in development program, while information on causal agents, occurrence and economic loss is still not known. This study was aimed to identify the causal agent of the leaf blight disease and other characteristics related to it.

MATERIALS AND METHODS

Isolation of Pathogen

Leaves of the streaked white types of arrowroot showing blight symptoms were collected from Cimanggu Experimental Garden in Bogor, West Java. The leaves were cleaned with tap water, cut into small pieces (5 mm), immersed in 1% commercial solution of Na_2SO_3 for 1-2 minutes, then transferred into sterile distilled water (SDW) in aseptic isolation box. Leaf sample was crushed in few drops of SDW on a flamed-sterilized glass slide. Plant extract was then streaked using isolation loop on plates containing sucrose peptone agar medium (Lelliott and Stead, 1987). The plates were then incubated for 3 days at 29°C.

Single colony of the round and white to brownish bacteria was then subcultured on the same medium agar. Purified isolate of bacteria was suspended in SDW in screw-caped bottles for further study.

Pathogenicity Test

Pure culture of the isolated bacteria (isolate T961) was inoculated by using wound methods of detach leaves of white streaked type arrowroot. Leaf samples were taken from healthy plants, and washed thoroughly by tap water.

The leaves were cross-wounded by using a fine needle and carborundum powder. Excess of carborundum was washed before the leaves were inoculated. Following of needle wounding, the wounds were covered with a drop of bacterial suspension containing about 10^8 cell ml^{-1} . In the case of carborundum wounds, the wounded leaves were sprayed with suspension of the bacteria as described before. Control treatment was using wounded leaves but treated with SDW instead of bacterial suspension. Leaves of green type arrowroot were also included in the pathogenicity tests.

All treated leaves were then laid down on a platform placed inside a plastic box (34 x 26 x 6 cm). The bottom of the container was filled with water about 2 cm high to maintain high air humidity. The plastic box was then covered and kept at room temperature. Development of disease symptoms on inoculated leaves was assessed every day.

Characterization of Pathogen

Isolate of bacteria which proved pathogenic following inoculation described above was tested for

identification according to the standard methods of Lelliott and Stead (1987) and Schaad (1990). The tests were Gram reaction (staining and solubility in KOH 3%), poly β hydroxy butyrate accumulation, catalase, arginine hydrolysis, starch hydrolysis, oxidase using tetramethylphenile diamine dihydrochloride, oxidation of glucose, reduction of nitrate to nitrite, xanthomonadin pigment, production of fluorescent pigment, serological reaction, and potato soft test described as follows:

- The pathogen was tested for Gram stain using the standard staining methods. Solubility of bacterial suspension in KOH 3% was also performed to confirm the Gram staining test.
- Accumulation of poly β hydroxy butyrate was also tested by growing the bacterium on SPA medium containing β hydroxy butyrate (0.5%); assessed by staining cells with solution of Sudan B Black 0.3% in ethanol 70%, then observed under a light microscope.
- Catalase was tested by mixing culture of the bacterium in few drops of H_2O_2 3%; production of bubbles indicated positive catalase.
- Arginine dihydrolase test was performed by growing the bacterium on the arginine dihydrolase medium in test tubes, and immediately sealed with sterile liquid paraffin. Changing of color of the medium from orange to cherry red during 3-day incubation indicated arginine positive.
- Capability of the bacterium to utilize D-glucose as sole carbon under aerobic and anaerobic (fermentative) conditions was performed by growing it in two tubes containing oxidation and fermentation medium. One of them was immediately sealed with sterile liquid paraffin for fermentative condition, while the other tube was left unsealed. Changing of color of the unsealed medium from dark blue to yellow within 3-5 days indicated oxidative reaction, whereas yellowing in both sealed and unsealed tubes indicated oxidative and fermentative reactions.
- Urease activity of the bacterium was tested by growing the isolate on urease medium containing 2% urea. Development of a pink-red coloration within 7 days of incubation indicated positive reaction for urease.
- Reduction of nitrate to nitrite by the bacterium was tested by growing it in the nitrate medium in the test tube. The presence of nitrite was tested by Follet and Ratcliff reagent. Development of an orange to orange-brown color after addition of the reagent indicated positive reaction of nitrate reduction to nitrite.

- Xanthomonadin pigment is only produced by *Xanthomonas* spp. The test was performed by diluting bacterial culture on nutrient agar medium in 3-5 ml of methanol; development of yellow color was indication of xanthomonadin pigment.
- Production of fluorescent pigment on King's B medium was tested by using illuminated culture of the bacterium with ultra violet light.
- Potato soft test was conducted by stab inoculating bacterium on a piece of sterile potato. Production of intensive yellow growth and softened potato formation indicated positive results.
- Sensitivity of the bacterium to tetrazolium chloride and several antibiotics such as penicillin, oxolinic acid, streptomycin, and oxytetracycline was tested.
- Serological reaction of isolate T961 was tested against polyclonal antisera raised for different isolates of *R. solanacearum* (antisera numbers: R 230, 283, 303, 608, B II) following ELISA procedure (Supriadi et al., 1997).
- Starch hydrolysis was tested by growing the bacterium on nutrient agar medium complemented with 0.2% soluble starch in the plates. After at least 7 days, scrape away some of the growth and flood with Lugol's iodine. The presence of clear zones in the black stain medium around or under the colonies indicated the presence of starch hydrolysis.

In all identification tests, three reference isolates of bacterium, i.e., *R. solanacearum* (T952) isolated from ginger, *Xanthomonas campestris* pv. *malvacearum* (T873) from cotton leaf, and *Pseudomonas cepacia* (R044) from onion, were included as the standard bacteria.

RESULTS AND DISCUSSION

Disease Symptoms

In naturally infected plants, the first symptom of the disease was small transparent spots (about one mm in diameter), usually found at tip of leaf margins (Fig. 1). The spots appeared very clearly during early morning. Few spots coalesced to become a large spot. Under exposure to the sunlight, disease spots turned to necrotic then became a blight. In severe cases few leaves or the whole leaves of a plant were full of blights, and the affected leaves became dry to course leafless plants. Disease incidence was more severe during wet season. New shoots may grow from the diseased plants, however, they were also infected afterwards, although the plants still produced rhizomes. Assessment was not made on the effect of the disease on rhizome yield.

Characteristics of the Bacterium Colony

The bacterium, freshly isolated from diseased leaf, produced 3-5 mm of colonies, white, circular with sharp edge on rich agar medium on sucrose peptone agar at 28°C. On King's B medium, the bacterium produced diffusible yellow colonies, but did not produce fluorescent pigment (Fig. 2).

Pathogenicity Test

Inoculation of isolate T961 on detached leaves of white-streaked type yielded first clear symptoms which were characterized by a transparent spot. The disease symptoms developed fast when infection



Fig. 1. *Maranta arundinacea* plant showing bacterial leaf blight symptoms (left). Left-natural infection; middle-carborundum inoculation, right-healthy leaves (right).

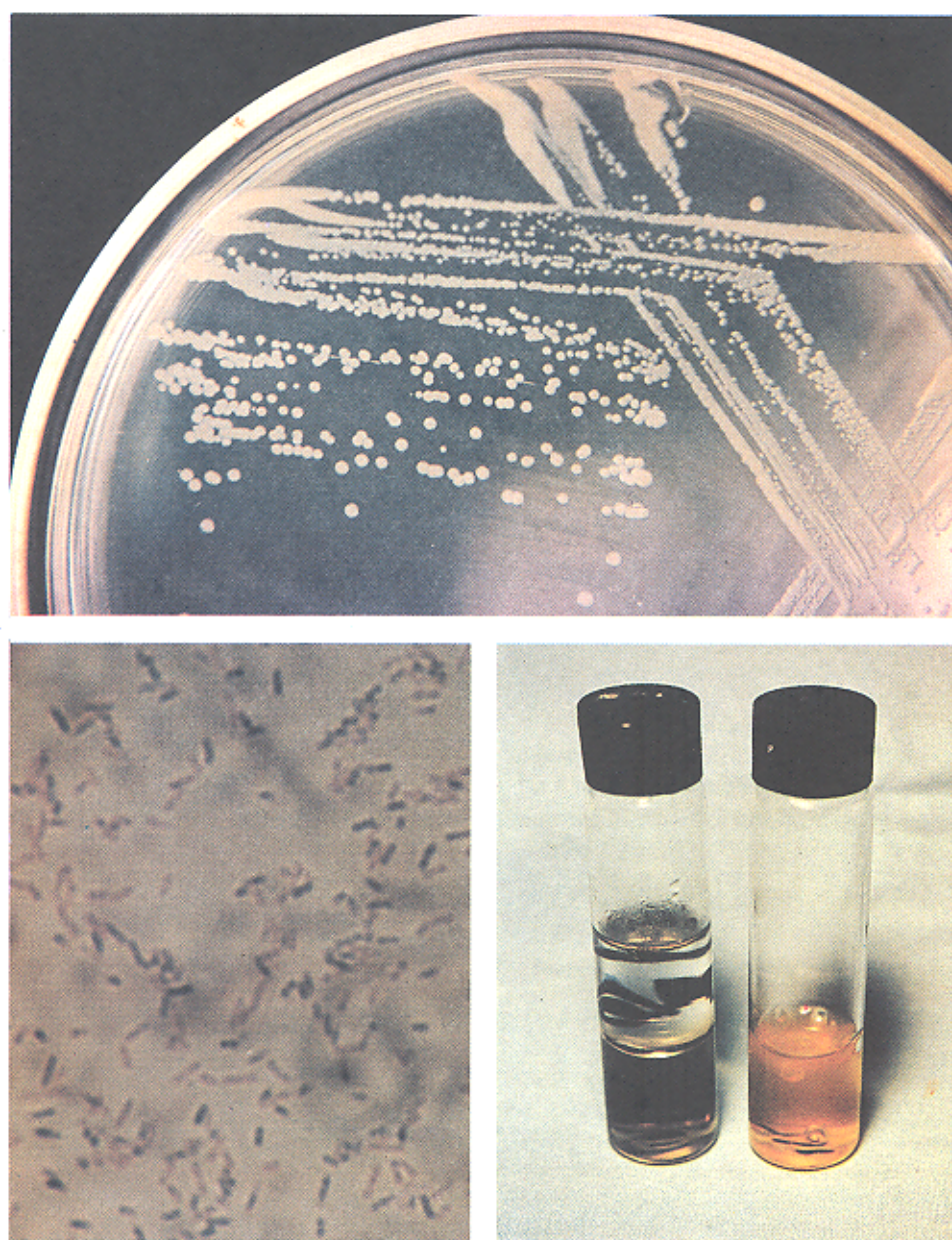


Fig. 2. Few characteristics of *Pseudomonas cepacia* caused bacterial leaf blight on *Maranta arundinacea*. Colony formation on sucrose peptone agar (top), accumulation of poly β hydroxybutyrate seen as a blue black particle within the cell (bottom, left), and oxidation of glucose under aerobic condition seen as yellow coloration of the medium (bottom, right).

reached vascular systems in the leaves. Within 4-5 days of incubation, whole surface area of the leaf completely changed into blight. Disease symptoms in artificially inoculated leaves were similar to those of naturally infection in the fields.

Isolate T961 caused severe damage only on leaves of the white-streaked type. Natural infection on the green type arrowroot was very light or almost none, however under artificial condition of inoculation in laboratory showed that the green type was also infected. In the artificial inoculation test, epidermis

layer (surface) of the inoculated leaves of the green type was deliberately wounded, therefore cells of the inoculated bacteria directly entered tissues and caused infection. However, in natural condition epidermis did not wound which become first mechanical barrier of the plant to prevent infection of the pathogen. Furthermore, observation on the leaf morphology of both green and white-streaked leaves indicated differences. Leaf blades of the green type were more firm (strong), thicker and hairy, but the white-streaked type were smooth and thinner.

Identification of the Bacterium

Results of all identification tests of bacterial isolate T961 from leaf of arrowroot are shown in Table 1. Compared to the characteristics of the reference isolates included in the test, i.e., *R. solanacearum* (T952), *X. campestris* pv. *malvacearum* (T873), and *P. cepacia* (R044), isolate T961 is identified as *P. cepacia*. Based on the description of *Pseudomonas* as described by Hayward (1983), Bradbury (1986), and Palleroni (1986), isolate T961 is categorized in the genus *Pseudomonas* based on the morphological characteristics, i.e., rod cell, Gram negative, and accumulated poly hydroxy butyrate. Physiological tests also supported the above conclusion, especially the results of strictly aerobic (or oxidative), positive oxidase, and positive catalase. Inability of isolate T961 to produce fluorescent pigmentation on King's B medium agar and xanthomonadin pigmentation, indicated that T961 was neither fluorescent *Pseudomonas* nor *Xanthomonas* (Fahy and Lloyd, 1983). Further identification results justified that the inclusion of isolate T961 as *P. cepacia* are based on the characteristics of not hydrolyse arginine and starch (Hayward, 1983; Bradbury, 1986). It was interesting to note that isolate T961 has certain

serological relationship with *R. solanacearum* as shown from ELISA result that positive reaction with a polyclonal antiserum B11 prepared for *R. solanacearum* (Supriadi et al., 1997). However, the isolate did not react with other polyclonal antisera also prepared for *R. solanacearum* isolates such as R230, R283, R303, and R608 (Robinson-Smith et al., 1995). They found that one out of five antisera to *R. solanacearum* also cross reacts with *P. cepacia* (isolate R044).

The present study is the first report on the occurrence of *P. cepacia* causing leaf blight disease on arrowroot in Indonesia and may be in the world since it is not mentioned in the list of plant pathogenic bacteria described by Moffett and Dye (1983) and Bradbury (1986). *P. cepacia* has been known for several years to cause sour skin of onion in USA and Italy, but it also occurred in soil, water, and clinical material as an opportunistic animal pathogen (Hayward, 1983; Bradbury, 1986). However, Bradbury (1986) stated that *P. cepacia* did not seem to be a strong invasive pathogen, rather attacked weakened plant. Certain strain of *P. cepacia* is also known as a potential biological control agent against damping-off pathogens such as *Rhizoctonia solani* and *Pythium* spp. on cotton seedlings (Zaki et al., 1998).

Table 1. Characteristics of isolate T961, the cause of bacterial leaf blight on *Maranta arundinacea*.

Test	Isolate ¹			
	R044	T961	T952	T873
Colony characters	White, circular	White, circular	Fluidal, white	Yellow, circular
Gram stain	-	-	-	-
Solubility in KOH 3%	+	+	+	+
Arginine dihydrolase	-	-	-	-
Poly hydroxy butyrate accumulation	+	+	+	-
Catalase	+	+	+	+
Oxidative metabolism of glucose	+	+	+	+
Nitrate reduction to nitrite	-	-	+	-
Starch hydrolysis	-	-	-	+
Potato soft rot	-	-	-	-
Growth at 40°C	+	+	+	+
Growth on tetrazolium salt:				
0.02%	+	+	+	-
0.1%	+	+	+	-
Fluorescent pigment production	-	-	-	-
Xanthomonadin pigment production	-	-	-	+
Reaction to polyclonal antibody prepared to <i>R. solanacearum</i>	+	+	+	-

¹R044 = *Pseudomonas cepacia*; T952 = *Ralstonia solanacearum*;

T873 = *Xanthomonas campestris* pv. *malvacearum*

 + = positive reaction

 - = negative reaction

Field observation on spread of the disease in limited area in Cimanggu Experimental Garden, Bogor indicated that the disease development was favored by humid condition during wet season. The pathogen spread very quickly from diseased leaf to the healthy ones in the same bunch and the same period. It was also spreading from diseased plant to the others. Rain splashed seems to play important role for disease spread. In dry season, progress of disease spread was slower or ceased. Further study is necessary to examine more intensively on aspects such as host plant and survival as well as economical damage (yield lost of rhizome) caused by the pathogen. However, it is sure that leaf performance of affected arrowroot plants was damage and cosmetic value of these plants was loss. Therefore, as an ornamental plant, the occurrence of bacterial leaf blight on the streaked-white of arrow root should be seriously considered as a threat.

Result on the sensitivity of isolate T961 to several antibiotics showed that the pathogen was tolerant to penicillin and oxolinic acid, but sensitive to streptomycin and oxytetracycline at high concentration (1,000 ppm). Further study is required to understand epidemiological factors that can be used as strategy to control the disease.

CONCLUSION

Based on the laboratory test it was concluded that:

1. Leaf blight disease on arrowroot (*M. arundinacea*) of the white streaked type was caused by *P. cepacia*.
2. The pathogen was tolerant to penicillin and oxolinic acid, but sensitive to streptomycin and oxytetracycline at high concentration (1,000 ppm).
3. The green type of leaves of arrowroot was tolerant to the natural infection by the pathogen, but susceptible to artificial inoculation by wounded.

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