Elucidation of the taxonomy and pathological status of *Pyricularia* associated with banana blast in Australia

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Abstract In September 2010, banana blast symptoms were observed on leaves of tissue cultured banana plantlets cv. Dwarf Cavendish. The pathogen was identified as *Pyricularia angulata*, based on disease symptoms, fungal morphology, pathogenicity and ITS sequencing. Previous *Pyricularia* spp. samples and records were confirmed as being *P. angulata*. This report elucidates the identity and pathological status of *P. angulata* in Australia.

Keywords *Pyricularia angulata* · Banana blast · *Musa* · Cavendish

Approximately 80% of Australia's banana production occurs in the wet tropical coast region of northern Queensland near the towns of Innisfail and Tully. This region receives abundant annual rainfall of between 3570 and 4490 mm, most of which falls during the warm (22–31°C) summer months (Dec–Feb), providing favourable environmental conditions for the development and proliferation of many fungal diseases. Over the past decade, the pursuit of uniform banana plants free of diseases has increased the use of tissue-cultured plantlets in the establishment of new banana plantings. Dense populations of banana plantlets tend to be raised in plastic covered plant houses under overhead irrigation, which often creates a poorly ventilated and damp environment. Sequential plantings of banana plantlets throughout the year further encourage fungal

diseases as they provide an ongoing source of a susceptible host.

In September 2010, leaf symptoms were observed on young tissue cultured banana plantlets cv. Dwarf Cavendish (AAA; Cavendish subgroup) at two nurseries in north Queensland. Symptoms included severe spotting on all leaves, death of some leaves and in severe cases, death of the whole plant. Lesions started as small, light tan coloured spots 1 mm in diam. that developed into circular or elliptical tan coloured spots 3–4 mm in diam. with a yellow margin. Mature lesions were brown in colour, 8–10 mm in diam., with a slightly darker margin and zonate appearance (Fig. 1). Adjacent lesions coalesced to produce large areas of necrotic tissue.

Pieces of diseased leaves were surface-sterilised by immersion in 1.0% sodium hypochlorite solution (Northern Chemicals, Cairns, Australia) for 1 min., rinsed in sterile distilled water and dried between pieces of sterile paper in a laminar flow cabinet. Small sections of diseased tissue were taken from the margin of lesions and placed onto 2% potato dextrose agar (Bacto Laboratories, Liverpool, Australia) amended with streptomycin sulfate (40U/mL) (PDA+S) and incubated in the dark for 48 h at 27°C. Resulting colonies were subcultured onto PDA+S and incubated for 48 h in the dark at 27°C before being placed under near-UV light with a 12 h photoperiod for a further 5 day to induce sporulation. Similar fungal colonies were consistently recovered and identified on the basis of conidial morphology as belonging to *Pyricularia* sp. (Ellis 1971). Conidiophores were pale brown, darker towards the base, up to 120 µm long and 4 µm wide, sometimes branched, straight to flexuous. Conidiogenous cells were polyblastic, integrated, terminal, sympodial, geniculate, with denticles up to 1.5 µm long separated by a septum. Conidia were ovoid to obpyriform, 19–22×6.5–8 µm, subhyaline to pale brown, smooth,

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Fig. 1 *Pyricularia angulata* lesions of banana blast on Dwarf Cavendish banana. **(a)** early stage; **(b)** close-up of mature lesions, zonation apparent

thin-walled, 2-septate, hilum protuberant (Fig. 2). Monoconidial cultures were produced by gently brushing over a sporulating culture with a sterile camel hair paintbrush and tapping the brush over PDA+S culture plates. After 12 h, germinating conidia were transferred to fresh PDA+S culture plates. Two monoconidial cultures (BRIP 53746 and BRIP 53869) were subsequently deposited in the Plant Pathology Herbarium, Dutton Park (BRIP).

For DNA extraction, mycelium from isolate BRIP 53746 was placed in a 2.0 mL safe-lock tube (Eppendorf, Hamburg, Germany). The equivalent of 0.5 mL of 0.5 mm glass beads (Daintree Scientific, St Helens,

Australia) was added and the mycelium was lysed using the Tissue Lyser (Qiagen, Hilden, Germany) for 1 min at 30 hz/s. DNA was extracted from this mixture using the Gentra Puregene kit (Qiagen), following the manufacturer's instructions. PCR amplification of the internal transcribed spacer (ITS) region was conducted using the Phusion High-Fidelity PCR Master Mix (Finnzymes, Espoo, Finland), which consisted of 25 µl of 2× Master Mix with HF Buffer, 1 µl each of 10 mM of primers ITS1 (5'-TCCGTAGGT GAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGA TATGC-3') (White et al. 1990), and 1 µl of DNA template. PCR products were amplified in a Bio-Rad C1000 thermal

Fig. 2 *Pyricularia angulata*. **a** denticulate conidiophores; **b** conidiophore bearing conidia Bars=10 µm

cycler (Bio-Rad, Hercules, USA) using the following conditions: 98°C for 30 s, 30 cycles at 98°C for 10 s, 55°C for 30 s, 72°C for 30 s, followed by a 5 min final extension at 72°C. The products were purified using the QIAquick PCR Purification Kit (Qiagen). Purified PCR products were sequenced by Macrogen Incorporated (Seoul, Korea) using the AB 3730xl DNA Analyser (Applied Biosystems, Foster City, USA). The sequence obtained (Genbank accession JF719830) was compared to the nucleotide sequence database (GenBank) using the nucleotide query BLASTn accessed via the National Centre for Biotechnology Information website (<http://www.ncbi.nlm.nih.gov/>). The ITS sequence of BRIP 53746 was identical to GenBank accession AY265322 *Pyricularia angulata* strain NBRC9625.

Conidia from a sporulating monoconidial culture (BRIP 53746) were removed with a camel-hair paintbrush and dispersed across V8 agar (Campbells, Silverwater, Australia) (V8) by tapping the brush gently on the side of the petri dish. The culture was then incubated in the dark for 48 h at 27°C and placed under near-UV light for a further 5 day at 26°C. To test for pathogenicity, a conidial suspension was obtained by flooding a culture plate with 1 mL sterile distilled water and using a glass hockey stick to dislodge the conidia. A further 1 mL of sterile distilled water was used to rinse the plate into a sterile beaker. The spore suspension was diluted to 10 mL with sterile distilled water before being passed through sterile muslin and the spore concentration quantified with a haemocytometer. The spore suspension was estimated to be 9×10^4 conidia/mL. A Preval portable atomiser (Precision Valve Corporation, New York, USA) was then used to apply the spore suspension to both abaxial and adaxial leaf surfaces of 14-wk-old tissue cultured 'Dwarf Cavendish' banana plantlets. Control plantlets were sprayed with sterile water only. To replicate growing conditions often found in nursery plant houses during disease outbreaks and minimise the risk of natural infection, plantlets were grown in a Perspex humidity chamber for 6 week prior to and after inoculation at 26°C under artificial light [1× Philips TLD 36W 850 fluorescent tube (Koninklijke Philips Electronics N.V., Amsterdam, Netherlands), 1× Hagen Aqua-Glo 40W fluorescent tube (Rolf C Hagen Inc., Montreal, Canada)] with a 14:10 photoperiod. Humidity was maintained at >90% with a Faran H-15 humidifier (Faran, South Korea) programmed to run for 2 min, 15 times per day. Inoculated plants developed typical symptoms of banana blast within 5 day and the control plants remained healthy. Following the same procedure described earlier, fragments of diseased tissue were placed on PDA+S culture media and the resulting fungal colonies were identified morphologically as *P. angulata* thereby fulfilling Koch's postulates.

The growth rate and colony characteristics of *P. angulata* were determined by taking a single 5 mm mycelial/agar

plug from the growing margin of a 10-d-old single spored culture growing on PDA+S (BRIP 53746) and placing it onto V8, PDA, corn meal agar (Bacto Laboratories) (CMA) and potato carrot agar (PCA) under near-UV light at 26°C. Twelve replicate plates were used per treatment. Colonies were measured and the mean colony diameter calculated after 13 day. Colonies on PDA and V8 agar were circular, up to 60 mm diam. white to greyish, darker towards the centre, flat to loosely floccose with irregular tufts of white mycelium; margin smooth, entire; abundant sporulation towards the centre of colonies on V8, sparse on PDA. Colonies on PCA were circular up to 72 mm diam., uniformly grey to hyaline; flat, aerial mycelium absent; slight, regular radial furrows; margin smooth, entire; sporulation almost entirely absent. Colonies on CMA were circular up to 57 mm diam.; highly variable growth characteristics, white to grey to hyaline, flat to loosely floccose, some colonies tightly floccose with dense spongy tufts of white mycelium; regular radial furrows present in some colonies, absent in others; margin smooth, entire; sporulation sparse to absent.

Pyricularia species cause the disease known as 'blast' on a wide range of mostly monocotyledonous plant species worldwide. The most economically important species being *P. grisea*, the causal agent of blast disease in rice (e.g. Hashioka 1972). *Pyricularia* spp. have been reported on bananas (*Musa* spp.) in various parts of the world including Australia (Hashioka 1971; Hoette 1936; Kim et al. 1987; Meredith 1962, 1963).

The description of the organism given in this paper is very similar to the descriptions of *P. grisea*, found to be responsible for pitting disease in bananas in Australia (Hoette 1936) and Central America (Meredith 1963). Hashioka (1971) observed morphological differences between three isolates of *Pyricularia* and reassigned *P. grisea* occurring on Musaceae to its own species, *P. angulata*. Further, Kim et al. (1987) found *P. angulata* to be pathogenic only on banana plants whereas *P. grisea* isolated from *Digitaria sanguinalis* was not. More recently, molecular research has demonstrated the differences between *P. angulata*, *P. grisea* and 39 other *Pyricularia* species and allied genera (Bussaban et al. 2005). This suggested that the original identifications from Hoette (1936) in Australia and Meredith (1963) of *P. grisea* were most likely *P. angulata*.

Two other specimens of *Pyricularia* sp. isolated from *Musa* sp. and lodged with BRIP were examined. One of these, BRIP 15526 from leaf spot on banana cv. Cavendish was collected at Tully, Qld in 1986 and was morphologically identical to *P. angulata*. The other, BRIP 5641, deposited in 1950 consisted of two dried microscope slides that could not be reconstituted. This report elucidates the identity and pathological status of *P. angulata* in Australia.

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