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First report of pathogenicity of *Pantoea ananatis* in sorghum (*Sorghum bicolor*) in Brazil

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Abstract. Bacterial isolates from sorghum plants showing leaf spot symptoms were identified through molecular and phenotypic traits, showing that the isolates belong to *Pantoea ananatis*. Sorghum plants inoculated with those isolates showed a pathogenic reaction. The causal agent was reisolated and Koch's postulates were fulfilled. This is the first report of *P. ananatis* causing leaf spots on sorghum plants in Brazil.

The bacteria *Pantoea ananatis* is reported to survive in nature on different hosts as an epiphyte, a saprophyte and a pathogen. Natural infections occur in pineapple, corn, onion, Sudan grass (*Sorghum sudanense*), eucalyptus, rice, tomato and melon (Azad *et al*. 2000; Coutinho and Venter 2009). Artificial inoculations have shown this organism to be pathogenic to sugarcane, oats, cotton and sorghum (*Sorghum bicolor*) (Azad *et al*. 2000; Coutinho and Venter 2009). On maize *P. ananatis* was identified as the causal agent of white spot (Paccola-Meirelles *et al*. 2001; Bomfeti *et al*. 2008) where it caused more than 60% yield losses on susceptible genotypes (Pinto 1999). Sawazaki *et al*. (1997) has also shown a correlation between the incidence of white spot on maize and a reduction in corn kernel weight. In Mexico, maize is an important cereal for human nutrition and *P. ananatis* has been detected causing leaf spots on corn (Pérez-y-Terrón *et al*. 2009). This disease has also been reported in two other South American maize-producing countries: Argentina (Alippi and López 2010) and Brazil (Paccola-Meirelles *et al*. 2001).

In April 2010, near Embrapa, at the Maize and Sorghum Research Center Experiment Station in Sete Lagoas, Minas Gerais, Brazil, sorghum plants were observed with leaf spot symptoms, which were ellipsoid to irregular in shape, had reddish brown borders and chlorotic centers (Fig. 1). Infected tissue, collected from these plants, was examined under a stereomicroscope and revealed no fungal structures on the lesions. Tissue segments from the lesion borders were excised, surface sterilised, and plated out on TSA (soybeans casein digest agar – tryptone soya agar) media. Two days later, bacterial colonies were observed (Fig. 2), from which one

Fig. 1. Sorghum plants with leaf spots occurring on field-grown plants (*a*–*c*): (*a*) sorghum plant; (*b*) leaf; (*c*) detailed lesion, new lesion with necrosis starting at the centre (black arrow), lesion with necrosis covering ~50% of the lesion area (white arrow), old lesion with most of the lesion area necrotic (yellow arrow).

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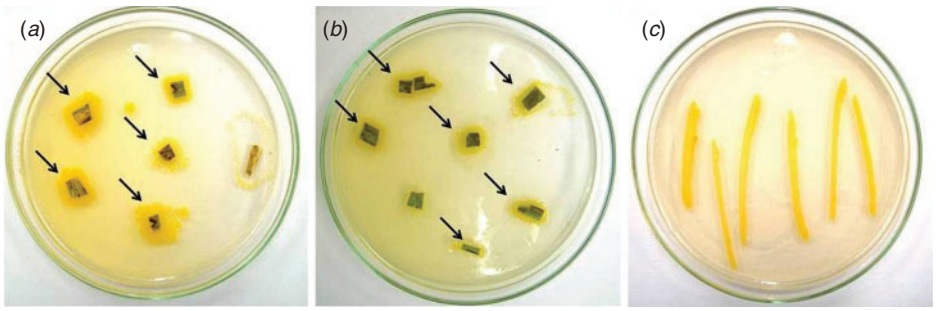

Fig. 2. Colonies of *Pantoea ananatis* on tryptone soya broth – soybean casein digest (*a*–*c*): (*a*) leaf fragment from the field with natural infection; (*b*) leaf fragment from the greenhouse with artificial infection; (*c*) colony of *P. ananatis*.

yellow colony was subcultured and purified for pathogenicity testing and DNA extraction.

For the pathogenicity test, yellow colonies were transferred to 100 mL TSB (tryptone soya broth – soybean casein digest) media, and grown on a shaker for 16 h at room temperature (25–27°C). Subsequently, 1 mL of this suspension was transferred to fresh 100 mL TSB media and shaken for 4 h. The bacterial concentration of this suspension was measured in a spectrophotometer at OD600 and adjusted to a final concentration of 10^8–10^9 cells/mL (Lelliot and Stead 1987; Romeiro 2001), using 0.85% saline in a 1 : 1 ratio. Healthy leaves were inoculated by spraying both leaf surfaces of 30-day-old sorghum plants with this suspension. After inoculation, plants were kept for 18 h in a dew chamber at a temperature of 28°C and 80% humidity. After the incubation period, plants were transferred to a growth chamber at a temperature of 28°C and 40% humidity, where they remained until evaluation. First symptoms were observed 24 h after inoculation and were characterised by the presence of red spots scattered on the leaf surface. After 3–4 days, these spots had progressed to necrotic lesions similar to the symptoms observed on sorghum plants in the field (Fig. 3). Tissue

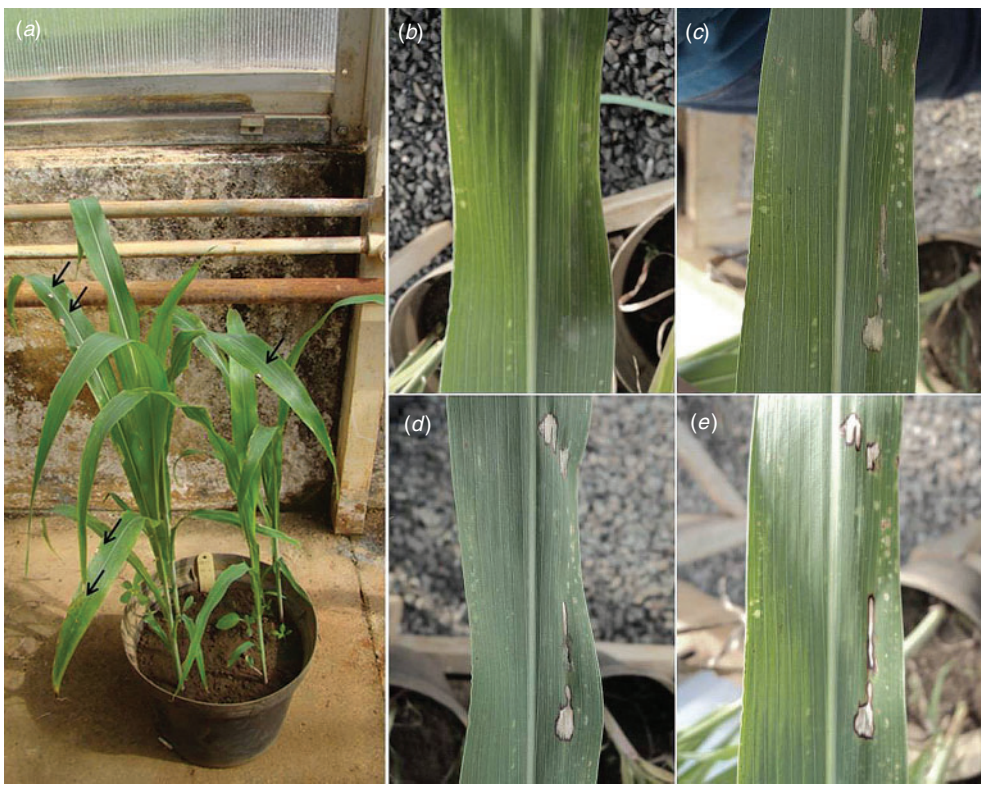

Fig. 3. Progression of sorghum symptoms on artificially infected plants grow in the greenhouse (*a*–*e*): (*a*) sorghum plant with leaf spots (black arrows); (*b*) first symptoms of light green leaf spots 2 days after inoculation; (*c*) lesions with centre whitening and thin red-brown border, 3 days after inoculation (necrotic) and red brown border (*d*) lesions well delimitated with red brown border, four days after inoculation (*e*) old lesions eighteen days after inoculation.

segments of infected leaves were collected and used for reisolation of the bacterium, following the above described procedures. Typical yellow colonies similar to the original colony obtained from infected material collected in the field were produced on TSA (Fig. 2).

Molecular identification of the bacteria isolated both from the original material and the greenhouse inoculations were conducted. Genomic DNA was extracted from bacterial colonies according to Sambrook *et al*. (1989). The recombinant DNA (rDNA) fragments were amplified using primers F968 and R1401, targeting the 16S rDNA gene (Nübel *et al*. 1996). The polymerase chain reaction consisted of 20 ng DNA, 50 mmol/L of each deoxynucleotide triphosphate (dNTP), 2.5 mmol/L $MgCl_2$, 2 mmol/L TRIS-HCl (pH 8.4), 50 mmol/L KCl, 0.2 mmol/L of each primer and 1 unit of Taq DNA polymerase (Invitrogen, Carslbad, CA, US) in a final volume of 50 mL. The samples were denatured at 94°C for 2 min, followed by 30 amplification cycles (94°C for 1 min, 55°C for 1 min, 72°C for 2 min) and a final extension (72°C for 10 min). The amplification products were analysed by electrophoresis on 1.5% agarose gel using TAE buffer (40 mmol/L TRIS-acetate, 1 mmol/L ethylenediaminetetraacetic acid, pH 8.0). The gel was stained with ethidium bromide (0.5 mg/mL), viewed under ultraviolet light and images were captured and stored in a photo documentation system (Gel Logic 200 Kodak, Rochester, NY, US). The amplification products were removed from the gel, purified using the kit 'QIAquick Gel Extraction', according to the manufacturer's instructions (Qiagen, Hilden, Germany) and sequenced using 'Big Dye Terminator v3.1.Cycle Sequencing' (Applied Biosystems, Foster City, CA, US). The samples were analysed in an automatic sequencer (ABI Prism 3100, Applied Biosystems) and nucleotide sequences were compared with sequences deposited in the GenBank database (http://www.ncbi.nlm.nih.gov/) using the program BlastN (Altschul *et al*. 1997). Both colonies most closely matched *P. ananatis*, showing greater than 99% similarity to an isolate deposited by Kido *et al*. (2008). The partial nucleotide sequence of 16S rRNA, used in the present work, was deposited in GenBank with the accession no. HQ333482, and a culture of the bacterial strain was deposited in the Collection of Plant Pathogens of Culture of Maize and Sorghum of Embrapa Milho e Sorgo, with the accession no. PA 033.10

This is the first reported occurrence of *P. ananatis* as a sorghum pathogen in Brazil, and the first report of natural infection of sorgham plants by *P. ananatis*. In the case of sorghum, there are no studies that show the extent or severity of occurrence of this pathogen in Brazil. However, given that sorghum is grown in areas that are adjacent to or used for maize production, identification of this pathogen on sorghum is important as this host may provide an alternative inoculum source for maize crops. White spot is currently a major disease of maize in Brazil and causes losses in virtually all maize-producing regions.

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