New Disease Reports

First report on the occurrence of *Enterobacter* sp. causing leaf dieback and wilt of potato in Madagascar

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In September 2010, a wilt and leaf dieback of potato (*Solanum tuberosum*) was observed in two fields located at Ambatolampy, Vakinankaratra region (Madagascar). The average temperature range during this time was 5°C to 22°C. Leaves of some stems had large brownish to black necrotic lesions covering about half of their surface. Plants wilted but brown vascular discolouration was not observed. The disease incidence was about 40-50% with a severity ranging from 70 to 100%. Symptom-bearing sections of the collar were surface-sterilised by immersing sections of the tissue for one minute in 1% NaOCl, rinsing the sections three times with sterilised distilled water, then tissues were crushed in sterilised 0.85% NaCl. The macerated tissue was left to soak for 30 minutes, a loopful of suspension was streaked onto plates of nutrient agar (NA) supplemented with nystatin (50 µg/ml). Gram negative, motile and rod-shaped bacteria forming light yellow, smooth and circular colonies with entire margins were observed following incubation for two to four days at 25°C. Physiological tests showed that the isolates were catalase positive, oxidase negative and facultatively anaerobic, which are characteristics of Enterobacteriaceae (Barrow & Feltham, 1993). In addition, they were non-pectolytic on potato. Molecular characterization of the strain B24 was done by amplifying and sequencing the *rpoB* gene and *gyrB* gene using primer pairs rpoBCM7-F/rpoBCM31b-R and gyrB01-F/gyrB02-R respectively (Brady *et al.*, 2008). The sequences of the *rpoB* gene and *gyrB* gene of the strain B24 (Genbank Accession Nos. KT356870 and KT356871) analysed on BLAST showed 99% similarity to the sequence of the *rpoB* gene and *gyrB* gene of *Enterobacter mori* LMG 25706. Pathogenicity of the strain was tested on three-week-old potato plants at the four-to-five true leaf stage. Potato stems at the axils of the fourth or fifth fully expanded leaves were inoculated with a drop of 10^8 cfu/ml bacterial suspension in 0.85% NaCl. Five plants were inoculated with the bacteria and five control plants were inoculated with 0.85% NaCl. After incubation in a greenhouse at 25°C with a 12 h photoperiod, inoculated plants developed symptoms of leaf dieback in the two older leaves within 14 days, and wilting within 21 days (Fig. 1). Non-inoculated plants remained symptomless. Bacteria were re-isolated from symptomatic plants on nutrient agar as described above and showed similar characteristics to the original strain based on morphological observation and amplification and sequencing of the *gyrB* and *rpoB* genes. To our knowledge, this is the first report of *Enterobacter* sp. on potato. *Enterobacter mori* is the pathogenic agent of *Morus alba* wilt (Zhu *et al.*, 2011). However, *Enterobacter* species have never been reported on potato plants.

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Figure 1

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