First record of *Xanthomonas axonopodis* pv. *manihotis* in Solomon Islands

R. K. Taylor¹ · R. L. Griffin¹ · L. M. Jones² · B. Pease² · F. Tsatsia³ · C. Fanai³ · B. Macfarlane³ · C. J. Dale⁴ · R. I. Davis²

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Abstract Cassava bacterial blight disease was detected for the first time in Solomon Islands on Malaita Island. The bacterium was isolated from diseased cassava plants exhibiting typical leaf symptoms. *Xanthomonas axonopodis* pv. *manihotis* was identified using biochemical and molecular tests and confirmed as the causal agent by fulfilling Koch's postulates. This is the first record of this pathogen in the South Pacific.

Keywords Cassava · *Xanthomonas axonopodis* pv. *manihotis* · Solomon Islands

In August 2015, Biosecurity Solomon Islands (BSI) and the Australian Department of Agriculture and Water Resources (DAWR) conducted a plant health survey on the islands of Guadalcanal and Malaita.

At six locations on Malaita, classic symptoms of cassava bacterial blight, caused by *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) were identified. These were angular brown lesions with water soaked margins (Fig. 1) and obvious droplets of bacterial ooze on the lower surface (Fig. 2). Larger

blight lesions at leaf tips were also found (Fig. 3), giving an overall appearance similar to that of scorching by fire (Fig. 4). Bacterial blight on cassava (*Manihot esculenta*) is a major disease with a devastating impact (Lozano 1986). It occurs in many parts of the world where cassava is grown, including Central and South America, the Caribbean, Africa and South East Asia (Anonymous 1977; CABI 2015). In the Pacific, this disease has reached only certain islands in the North West, close to South East Asia. These are Guam (Wall 1991), the Federated States of Micronesia and the Republic of Palau (EPPO 2015). It has never been recorded before in the South Pacific. CABI (2015) lists the presence of this disease in Fiji, citing Frison and Feliu (1991). However, there is no such reference to this disease in that document and there have been no observations or reports of this disease anywhere in the Fiji Islands in the following 25 years (M. Lomavatu-Fong, senior plant pathologist, Ministry of Agriculture, Fiji, personal communication, 2016). The most likely source of this error is a photograph of disease symptoms in the document, presumably taken in the northern Pacific, supplied by the then named plant protection service of the South Pacific Commission (SPC) which happened to be based in Fiji.

During the Malaita survey, bacteria were isolated from two leaf samples collected from Atori on the east coast. To do this, the leaf samples were surface sterilized and lesion edges were excised and macerated in sterile water and sterile phosphate buffered saline (PBS, pH 7.0). These suspensions were then streaked onto nutrient agar in the field. Circular, smooth grey-whitish colonies were obtained using both sterile water and sterile PBS. These were selected and sub-cultured onto nutrient agar after 48 h. DNA was extracted from these cultures in the Solomon Islands Ministry of Agriculture and Livestock plant pathology laboratory in Honiara using a DNeasy Plant Minikit (Qiagen) following the manufacturer's instructions, except that liquid nitrogen was not used and bacterial colonies

✉ R. I. Davis
richard.davis@agriculture.gov.au

¹ Plant Health and Environment Laboratory, Ministry for Primary Industries, PO Box 2095, Auckland 1140, New Zealand

² Department of Agriculture and Water Resources, Northern Australia Quarantine Strategy, PO Box 96, Cairns International Airport, Cairns, Qld 4870, Australia

³ Biosecurity Solomon Islands, Ministry of Agriculture and Livestock, PO Box G13, Honiara, Solomon Islands

⁴ Department of Agriculture and Water Resources, International Plant Health Program, GPO Box 858, Canberra City, ACT 2601, Australia

Fig. 1 Early angular brown lesions with water soaked margins on cassava leaves caused by *Xam* on Malaita Island, Solomon Islands

were macerated in microfuge tubes using micropestles. The extracts were returned to the Northern Australia Quarantine Strategy (NAQS) plant pathology laboratory in Cairns, Australia. Here they were tested using the nested PCR assay of Ojeda and Verdier (2000) which is specific for *Xam*. Strong bands for both products (first and second round amplifications) were obtained for nine out of ten bacterial DNA extracts obtained from the two Atori leaf samples. BLASTn analysis of the 898 bp amplicon showed that the region was a close match to *Xam* effector protein sequence (GenBank accession HQ113297.1).

On subsequent days of the survey, when symptoms were found in four other locations on Malaita, it was not possible to make further isolations. Instead, early lesion margins were excised, chopped finely and desiccated over anhydrous calcium chloride at 4 °C. These samples were returned to the NAQS Cairns laboratory, Australia after mandatory gamma irradiation at 25 KGy. They were then subject to the DNA

Fig. 2 Lower surface of a cassava bacterial blight lesion on Malaita Island, Solomon Islands, showing water soaked margins and droplets of bacterial ooze

Fig. 3 Lower surface of a *Xam* infected cassava leaf on Malaita Island, Solomon Islands, in which a larger blight lesion has developed in addition to four smaller angular lesions

extraction and nested PCR testing procedures described above. All DNA samples returned positive results for *Xam*.

For a secondary identification, diseased leaf samples collected from the town of Auki on the west side of Malaita were dispatched to the Plant Health and Environment Laboratory, Ministry for Primary Industries, Auckland, New Zealand, under relevant biosecurity protocols. Here, isolations were conducted in a biocontainment laboratory (Physical containment level 2 with additional controls). Suspensions of macerated diseased leaf tissue were streaked onto LPGA media containing cycloheximide (250 mg/L) (Verdier et al. 1998) and non-pigmented, circular, raised, convex, creamy-white bacterial colonies were consistently produced following 48 h incubation at 30 °C. All colonies were Gram negative, oxidase negative, catalase positive, hydrolysed starch and gelatin and tested positive for *Xam* by a PCR assay developed by Verdier

Fig. 4 Advanced leaf blight symptoms on Malaita Island, Solomon Islands, in which extensive lesions have developed on several cassava leaf palmate lobes, giving an overall appearance similar to that of scorching by fire

Fig. 5 Maximum likelihood tree illustrating the phylogenetic relationships between the Solomon Island *Xanthomonas axonopodis* pv. *manihotis* (Xam) strains (in bold type) and other related *Xanthomonas* strains (*X. axonopodis* pathovars - X.a). Phylogenetic analyses of the *gyrB* gene was performed via Geneious Pro 10.0.6 (Biomatters, Auckland, New Zealand) using RAXML 7.2.8 for tree construction. Topology was rooted with the type strain of *X. cassavae* and bootstrap values were 100 from 10,000 replicates. Sequences produced in this study are deposited in Genbank under accession numbers KY863500 – KY863503

et al. (1998). Three cultures were stored in the International Collection of Microorganisms from Plants (ICMP) under ICMP 22010, 22,011 and 22,012. The housekeeping genes *gyrB* and *rpoD* were amplified from the three isolates (ICMP 22010, 22,011 and 22,012) and the gene sequences were analysed as described by Parkinson et al. (2009). BLASTn analysis of the *gyrB* and *rpoD* sequences showed that they were 100% match to *Xam* pathotype strain (ICMP 5741) sequences (accessions EU499006 and EU499125) in GenBank. A phylogenetic tree based on comparison of *Xam gyrB* sequences showed that the Solomon Islands strains closely aligned with other strains of *Xam* present in Africa and South America (Fig. 5). Pathogenicity tests were performed with these three isolates by inoculating leaves and stems of 2-month old *M. esculenta* plants (wildtype original

Fig. 6 View of a stand of bacterial blight diseased cassava plants on Malaita Island, Solomon Islands, indicating how damage can become so extensive that it is readily visible from a distance

cassava, as grown in the New Zealand Northland climate). Each isolate was inoculated by spraying a bacterial suspension (10^7 cfu/ml) onto the leaf surface, pricking through the drops using sterile needles and into stems with a syringe. Plants were incubated in humid conditions at 25 °C. Four weeks later leaf lesions and wilting developed and bacterial colonies identified as *Xam* were re-isolated from the lesions, fulfilling Koch's postulates (data not shown).

It is possible that this is a new incursion of this disease in the country, confined to Malaita. Disease incidence and severity in affected plantings was high enough to be visible from a distance (Fig. 6) and growers had noticed the impact on their crops. Cassava bacterial blight symptoms are striking and unmistakable and were not seen on two previous surveys of Malaita conducted in 2007 and 2010, nor anywhere else in an extensive series of plant health surveys undertaken across the archipelago from 2007 to 2016 (BSI unpublished data). In 2015, two leaf samples from cassava plantings on the island of Guadalcanal were also tested in the way described above because they were showing some angular lesions, but no bacterial ooze. Both samples were negative for *Xam*. In a following survey conducted in 2016, seven more similar leaf samples (two from Guadalcanal, two from Isabel Province and three from Western Province) also tested negative in identical testing. As this pathogen is readily transmitted in planting material (Frison and Feliu 1991), a concerted containment effort will be required to prevent spread to other islands.

This finding is also of some relevance to Australia, where in the northern tropics, cassava is found in numerous small domestic plantings in towns and remote communities (DAWR unpublished data). Commercial cropping to supply gluten free starch markets is also a recent initiative (See <http://www.casstech.com.au>). In the broader Australasian region, interest in cassava's potential to be a major bioethanol crop is growing rapidly (Jansson et al. 2009). As it is also one of the most

drought tolerant crops in the world (El-Sharkawy 2007) and is capable of growing on marginal land, northern Australia could potentially benefit greatly from developing this crop.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

- Anonymous (1977) *Xanthomonas manihotis* (Arthaud-Berthet & Bondar) Starr. CMI Distribution Maps of Plant Diseases. No. 521. Commonwealth Agricultural Bureau, Slough
- CABI (2015) Crop protection compendium datasheet report for *Xanthomonas axonopodis* pv. *manihotis* (cassava bacterial blight). <http://www.cabi.org/cpc/datasheet/56952>. Accessed 24 March 2017
- El-Sharkawy MA (2007) Physiological characteristics of cassava tolerance to prolonged drought in the tropics: implications for breeding cultivars adapted to seasonally dry and semiarid environments. *Braz J Plant Physiol* 19:257–286
- EPPO (2015) PQR - EPPO database on quarantine pests (available online). <http://www.eppo.int>
- Frison EA, Feliu E (1991). FAO/IBPGR technical guidelines for the safe movement of cassava germplasm. Food and agriculture organization of the United Nations/International board for plant genetic resources, Rome
- Jansson C, Westerbergh A, Zhang J, Hu X, Sun C (2009) Cassava, a potential biofuel crop in (the) People's republic of China. *Appl Energy* 86:S95–S99
- Lozano JC (1986) Cassava bacterial blight: a manageable disease. *Plant Dis* 70:1089–1093
- Ojeda S, Verdier V (2000) Detecting *Xanthomonas axonopodis* pv. *manihotis* in cassava true seeds by nested polymerase chain reaction assay. *Can J Plant Pathol* 22:241–247
- Parkinson N, Cowie C, Heeney J, Stead D (2009) Phylogenetic structure of *Xanthomonas* determined by comparison of *gyrB* sequences. *Int J Syst Evol Microbiol* 59:264–274
- Verdier V, Mosquera G, Assigbetsé K (1998) Detection of cassava bacterial blight pathogen, *Xanthomonas axonopodis* pv. *manihotis*, by polymerase chain reaction. *Plant Dis* 82:79–83
- Wall GC (1991) Plant diseases of recent introduction to Guam. *Micronesica Suppl* 3:41–45