First report of *Pectobacterium carotovorum* subsp. *brasiliense* causing soft rot and blackleg of potatoes in Kenya

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During the 2012/2013 growing season, potato tubers and stems showing rotting tissue and black discoloration, respectively, were obtained for analysis from Nyandarua and Mau Narok areas of Kenya where potatoes are widely grown. During this period, more than 50% of the farms across Kenya reported cases of soft rot and blackleg diseases. Soft rot and blackleg diseases account for as much as 1/4 of the annual potato losses in Kenya (1). Bacteria from infected potato tuber and stem samples were isolated on nutrient agar then transferred to crystal violet polypectate medium (CVP) according to established standard procedures (2). All pit-forming (48) strains were purified on nutrient agar and stored in 30% glycerol at -80°C for further use. All strains grew at 28°C and 37°C. PCR with *pel* gene specific primers (Y1/Y2) produced a 434 bp product and confirmed that all 48 strains have the gene sequence coding for pectate lyase specific for *Pectobacterium* spp. (3). Primers (Br1f/L1r) identified 1/3 of these strains as *Pectobacterium carotovorum* subsp. *brasiliense* based on their characteristic 322 bp (4). The other *Pectobacterium* spp. are currently undergoing further characterization. To further identify these pectolytic strains, a multi locus sequence typing (MLST) approach was employed (5, 6). To this end, partial nucleotide sequences of the housekeeping genes, *mdh* and *gapA* (accession number; KF72004-KF72009), showed 92% similarity to the *Pcb*1692 reference strain in GenBank. These results were in agreement with those obtained by species-specific primers. Phylogenetic analysis of the 679 bp concatenated partial gene sequences grouped strains collected in this study together with *Pectobacterium* subsp. *brasiliense* strains identified in other parts of the world with a 98% bootstrap support value. Three randomly selected Kenyan strains and *Pcb*1692 reference strain were inoculated into potato tubers in our research laboratory by making 1 cm holes into the tubers using a sterile pipette tip and thereafter injecting 10 µl (at 1.0×10^6 cfu/ml) into the tuber for pathogenicity assays. A negative control of 10 mM $MgSO_4$ was included and all the inoculated holes sealed with petroleum jelly to avoid contamination. This experiment consisted of five potato tubers per strain in three independent assays. All the three representative strains induced water soaked soft symptoms similar to the symptoms previously observed on infected

potato tubers. Furthermore, when bacterial suspensions of 1.0 $\times 10^6$ cfu/ml isolated strains and the *Pcb*1692 reference strain were inoculated onto potato stems maintained at 28°C, blackleg and wilting of the stems occurred within a period of 3-21 days. No symptoms were observed in potato tubers or stems inoculated with the negative control ($MgSO_4$). PCR with Br1f/L1r primers confirmed that the re-isolated bacteria were *Pectobacterium carotovorum* subsp. *brasiliense*. To our knowledge, this is the first occurrence of *Pectobacterium carotovorum* subsp. *brasiliense* on potatoes in Kenya.

References: (1) J. Muthoni et al. J. Agric. Sci. 5:182-197, 2013 (2) L. J. Hyman et al. Potato Research. 44: 265-270, 2001 (3) A. Darrasse et al. Appl. Environ. Microbiol, 60: 1437-1443, 1994 (4) V. Duarte et al. J. of Appl. Microbiol. 96: 535-545, 2004 (5) I. K. Toth et al. Mol. Plant Pathol.4: 17-30, 2003 (6) Ma et al. Phytopathol.97: 1150-1163, 2007.