New Disease Reports

First report of *Pectobacterium betavasculorum* associated with bacterial vascular necrosis and root rot disease of sugar beet in Turkey

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During June and September of 2018, bacterial necrosis and root rot disease surveys were conducted in sugar beet (*Beta vulgaris*) fields in Yozgat province in the Central Anatolia region of Turkey. From 33 fields surveyed, 19 sugar beet plants exhibiting vascular necrosis with blackened petioles, root rotting varying from wet to dry and wilting symptoms were collected for bacterial isolation. Sap from the margins of affected root tissue and petioles was streaked on crystal violet pectate (CVP) media and incubated at 28°C for 48 hours. Ten individual cavity-forming bacterial colonies were obtained on CVP media from petiole and roots of infected plants and transferred to nutrient agar for bacterial identification and pathogenicity tests. All bacterial isolates were gram negative, catalase positive, oxidase negative, facultative anaerobe, non-fluorescent on King's B medium, utilising gelatine, capable of eliciting hypersensitive response on tobacco plants (*Nicotiana bentamiana*) and caused soft rot symptoms on potato tuber slices. They were able to grow at 37°C and in Luria broth containing 5% NaCl, but not at 39ºC.

Bacterial DNA was extracted from a representative isolate (P12) pathogenic to sugar beet. The isolate did not produce a specific PCR amplicon with taxon-specific primers such as Y1/Y2 for *Pectobacterium* spp., EXPCCF/EXPCCR for *P. carotovorum* subsp. *carotovorum* and Br1f/L1r for *P. carotovorum* subsp. *brasiliense* (Dees *et al*., 2017; Ozturk *et al*., 2018). Isolate P12 was further characterised by sequence analysis using *gap*A and *mdh* housekeeping genes with *gap*A-7-F/*gap*A-938-R and *mdh*86F/*mdh*628R primers, respectively (Ma *et al*., 2007; Cigna *et al*., 2017). Partial 701 and 452 bp nucleotide sequences (GenBank Accession Nos. MK689857 and MK689856) were subjected to BLAST analysis and had 96.0% sequence identity with the *gap*A gene of *P. atrosepticum* (SCRI1043; CP009125) and 100% identity with *mdh* gene of *P. betavasculorum* (CFBP2122^{T}; JN600343) isolated from sugar beet (Gardan *et al*., 2003). A phylogenetic tree based on the *mdh* gene was constructed using maximum likelihood method, and clustered isolate P12 with *P. betavasculorum* CFBP2122^{T} and Ecb1 strains (Fig. 1).

A pathogenicity experiment was performed on 40-day-old sugar beet plants (cv. Turbata) at the eight-leaf stage by puncturing a hole (diameter 3-5 mm) in three petioles per plant using a sterile pipette tip containing fresh single colonies grown on nutrient agar for 24 hours. After four days, vascular blackening of petioles including frothing occurred at the inoculation point. The leaves of infected petioles was half chlorotic seven days after inoculation (Fig. 2). Control petioles treated with sterile water remained symptomless. Re-isolated pectolytic bacterial colonies on CVP formed cavities and the isolates had the same biochemical and physiological features as the original culture.

To the best our knowledge, this is the first report of *P. betavasculorum* causing bacterial vascular necrosis and root rot disease on sugar beet in Turkey. Disease on sugar beet caused by *P. betavasculorum* is widespread in Iran (Nedaienia & Fassihiani, 2011) and control measures to prevent further spread of pathogen should be taken in the region.

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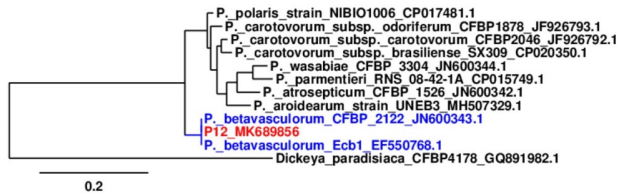

Figure 1

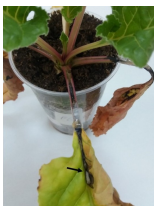

Figure 2

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