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First Report of Soft Rot by *Pectobacterium carotovorum* subsp. *brasiliense* on Amaranth in Korea

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Amaranth has the potential for good materials related to nutrients and health benefits. There are several diseases of amaranth such as leaf blight, damping-off, and root rot. As a causal agent of soft rot disease, *Pectobacterium* spp. could infect various plant species. In this study, we isolated the bacterial pathogen causing soft rot of amaranth in South Korea. In Gangneung, Gangwon province during 2017, amaranth plants showed typical soft rot symptoms such as wilting, defoliation and odd smell. To isolate pathogen, the macerated tissues of contaminated amaranth were spread onto LB agar plates and purified by a single colony subculture. One ml bacterial suspension of a representative isolate was injected to the stem of five seedlings of 2-week-old amaranth with a needle. Ten mM magnesium sulfate solution was used as a negative control. 16S rDNA gene and *recA* gene were sequenced and compared with the reference sequences using the BLAST. In the phylogenetic tree based on 16S rDNA gene and *recA* gene, GSA1 strain was grouped in Pcb.

Keywords: Amaranth, Pcb, Soft rot, South Korea

As one of the oldest crops, amaranth (*Amaranthus* spp.) is domesticated in the Andean region of Latin America about 8,000 years ago (Rastogi and Shukla, 2013). The amaranth belongs to the genus *Amaranthus* and family *Chenopodiaceae*, and is classified to three types of grain, vegetable and ornamental type (Caselato-Sousa and Amaya-Farfan, 2012; Pandey, 2013; Tang and Tsao, 2017). Amaranth is recognized as good materials related with nutrients, and has received attention in recent years because of their potential health benefits (Tang and Tsao, 2017). There are several diseases of amaranth such as leaf blight, damping-off, and root rot (Celine et al., 2013; Rastogi and Shukla, 2013). *Pectobacterium* spp. cause soft rot and blackleg on various hosts (Charkowski, 2018). New species had been classified in recent studies such as *P. aroidearum, P. parmentieri, P. peruviense*, and *P. polaris* (Dees et al., 2017; Khayi et al., 2016; Nabhan et al., 2013; Waleron et al., 2018). In this day, *Pectobacterium* spp. are considered as species complex because *Pectobacterium* species showed diverse characteristics. Of old collection of *P. carotovorum* subsp. *carotovorum*, several isolates were re-classified as *P. carotovorum* subsp. *brasiliense* (Pcb) in South Korea (Lee et al., 2014). In this study, we aimed to isolate and identify the soft rot pathogen of amaranth in South Korea and identify.

Showing typical symptoms of soft rot disease, amaranth (*Amaranthus caudatus* L.) plants were collected from Gangneung, Gangwon province during 2017. Wilting and defoliation were observed on amaranth plant (Fig. 1A). Inner part of stem had macerated tissue and foul smell (Fig. 1B). To isolate pathogen, partial stem of amaranth was surface-sterilized for 90 s in 1% hypochlorite solution and rinsed in sterile distilled

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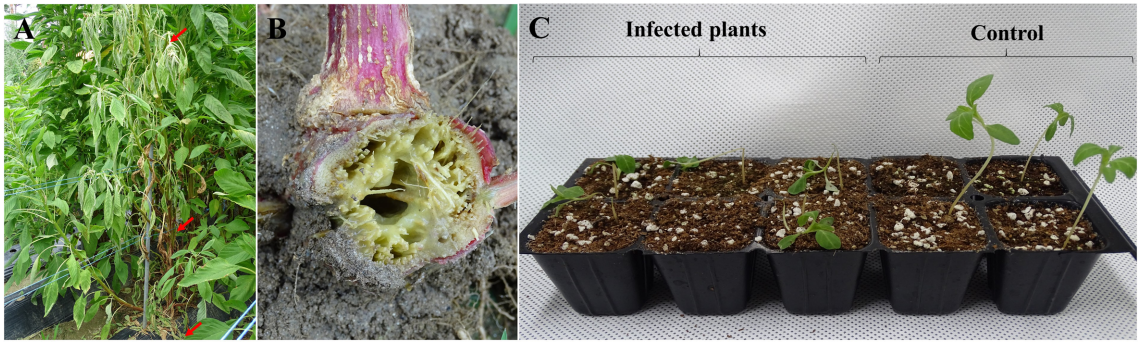

Fig. 1. Soft rot symptoms of amaranth caused by *Pectobacterium carotovorum* subsp. *brasiliense* on field (A, B), and artificial symptoms by inoculation (C). On third day after infection, infected plants showed soft rot symptoms and control was not affected by infection of 10 mM $MgSO_4$.

water. Samples were macerated in 1.5 ml tube and were streaked on Luria-Bertani (LB) agar medium. The single colony was purified by subculture for two times. Bacterial isolates were stored at -72°C with 20% glycerol. To test pathogenicity, a representative isolate was grown on LB agar for 16 h at 28°C and suspended in 10 mM magnesium sulfate ($MgSO_4$) solution with an optical density of 0.5 at 600 nm wavelength using spectrophotometer (DS-11, DeNovix, USA). 1 ml bacterial suspension was injected to the stem of five seedlings of 2-week-old amaranth with a needle. As negative control, 1 ml of 10 mM $MgSO_4$ solution was inoculated to three seedlings of 2-week-old amaranth. Inoculated seedlings were incubated in a chamber with 28±1°C, 95% relative humidity for 16 h. Inoculated amaranth seedlings were evaluated after disease symptom developed (Fig. 1C_left). The control plants didn't show the symptoms (Fig. 1C_right) of soft rot. To confirm Koch's postulates, the bacterium was re-isolated from amaranth seedling. To identify isolated bacterium, 16S rDNA gene and recombinase A (*recA*) gene were sequenced by Macrogen, Inc. (Korea). Sequences of 16S rDNA (1,390 bp, Accession No. MH886389) and *recA* (668 bp) were compared with the reference sequences using the BLAST software in the GenBank database (http://www.ncbi.nlm.nih.gov/). With 16S rDNA sequence of Pcb GSA1 strain, we found 99% similarity to those of *P. carotovorum*. In the phylogenetic tree (Fig. 2), isolated bacterium was located in a group comprising a reference strain of Pcb. Based on symptoms, pathogenicity, and molecular analysis of 16S rDNA and *recA*, we concluded

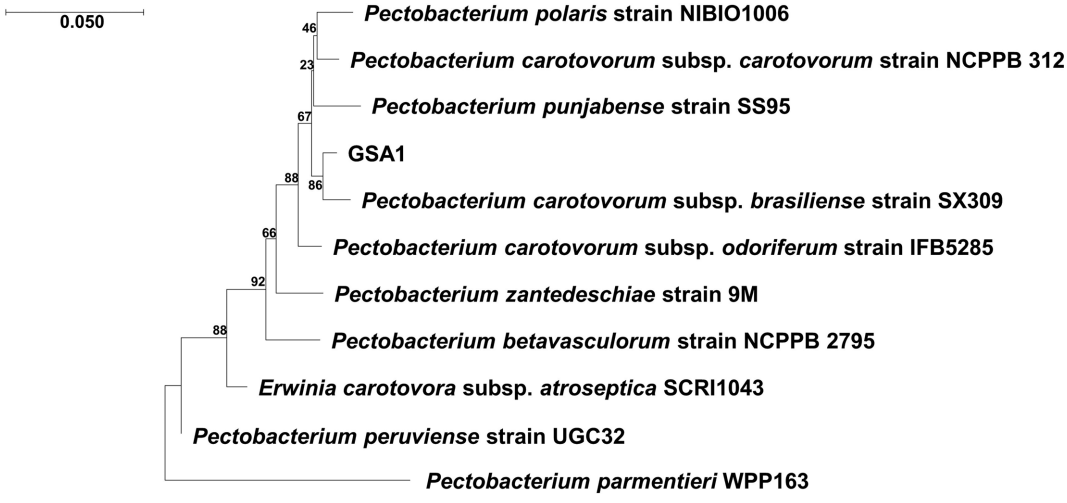

Fig. 2. Phylogenetic tree based on 16S rDNA sequences and recombinase A sequences of *Pectobacterium* strains. DNA sequences from the NCBI database were aligned using ClustalW and phylogenetic trees were constructed using the neighbor-joining method and visualized with MEGA7. Isolated bacteria is GSA1.

that the isolated bacterium from soft rot symptom of amaranth is Pcb. To our knowledge, this is the first report of soft rot disease by Pcb in amaranth plant.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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