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Note

First Description of Crown Gall Disease on Ginseng

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In March of 2003, tumors (galls) were observed on ginseng seedling roots in ginseng seedbeds at Yeoju, Gyeonggi province, Korea. Symptoms were spherical or galls with about 0.5-1.0 cm in diameter formed on the upper through middle parts of the primary roots. Bacterial isolates obtained from the root galls were Gram-negative, rod-shaped with peritrichous flagella, aerobic, not forming yellow or orange colonies on nutrient glucose agar, yeast extract-dextrose $CaCO_3$ agar and nutrient-broth yeast extract agar, non-fluorescent on King's B agar, and non-spore forming, which were identical to characteristics of the genus *Agrobacterium*. They were identified as *Agrobacterium tumefaciens* with 0.732-0.993 similarities in 100% probability by the Biolog analyses. The 16S rRNA gene partial sequences of the six isolates tested (Genbank Accession EF486308~EF486313) were 100% homologous to those of other *A. tumefaciens* strains (GenBank accession AF501343, AY701900, AY701898, AY701899). The above results confirmed that this bacterium is *A. tumefaciens*. Pathogenicity of the bacteria was proved by the inoculation test on carrot root discs and tomato seedlings. This is the first description of *A. tumefaciens* causing root gall in ginseng seedling. The disease occurred locally and sparsely, but considering its appearances in seedbeds suggests that the ginseng root gall may become a threat to ginseng in Korea.

Keywords **:** *Agrobacterium tumefaciens*, ginseng, identification, tumor

Ginseng (*Panax ginseng* C. A. Meyer), a deciduous perennial plant belonging to Araliaceae family, is cultivated mostly in Korea and China. The roots of ginseng are highly prized for medicinal properties and used for the development of commercial products, for which plants are cultivated under shade for 4-6 years. Ginseng plants are grown in seedbeds for about a year prior to transplanting in main fields.

Root diseases caused by various pathogens including fungi, bacteria and nematodes are one of the most limiting factors for ginseng cultivation both in seedbeds and main fields. For fungal diseases, *Cylindrocarpon destructans* is most responsible for ginseng root rot and is also known one of the main causes of replant problem (Chung, 1975; Lee, 2004; Park, 2001; Yu, 1987; Yu and Ohh, 1993). Some fungi such as *Botrytis cinerea* and *Fusarium solani* are also involved in the root rot of ginseng somewhat saprophytically (Chung and Kim, 1978; Chung et al., 1989). As for nematodes, root-knot nematodes, *Meloidogyne hapla*, and potato-rot nematode, *Ditylenchus destructor*, have been found commonly occurring in ginseng fields, of which the later is responsible for the root-rot disease of ginseng (Ohh et al., 1983, 1986). Currently root-lesion nematode, *Pratylenchus subpenetrans*, was firstly found in ginseng nursery in Korea (Kim et al., 2006). However, little has been known about the occurrences of the bacterial diseases in field conditions even though *Erwinia* and *Pseudomonas* spp. are listed as causal agents of root rots (Yu and Ohh, 1993).

During the survey of ginseng diseases in 2003, a typical crown gall disease was found on ginseng seedling roots at ginseng seedbeds in a major ginseng-growing area of Korea. The causal organism was isolated and identified as the crown gall-inducing bacterium, *Agrobacterium tumefaciens*. Its pathogenicity was confirmed. Herewith we report the occurrence of the tumor of ginseng seedling root, and the identification and characterization of the bacterial pathogen for the first time.

Disease occurrence and symptoms. In March of 2003, tumors (galls) were observed on Korea ginseng seedling roots in loamy plain ginseng seedbeds in Yeoju, Korea. Spherical to oval tumors of about 0.5-1.0 cm diameter were observed on the middle to upper regions of the primary roots (Fig. 1). Affected roots became longer and more slender than healthy ones, sometimes attenuated abruptly at

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Fig. 1. Tumors of Korea ginseng seedling roots collected from a seedbed at the harvest time of seedling roots.

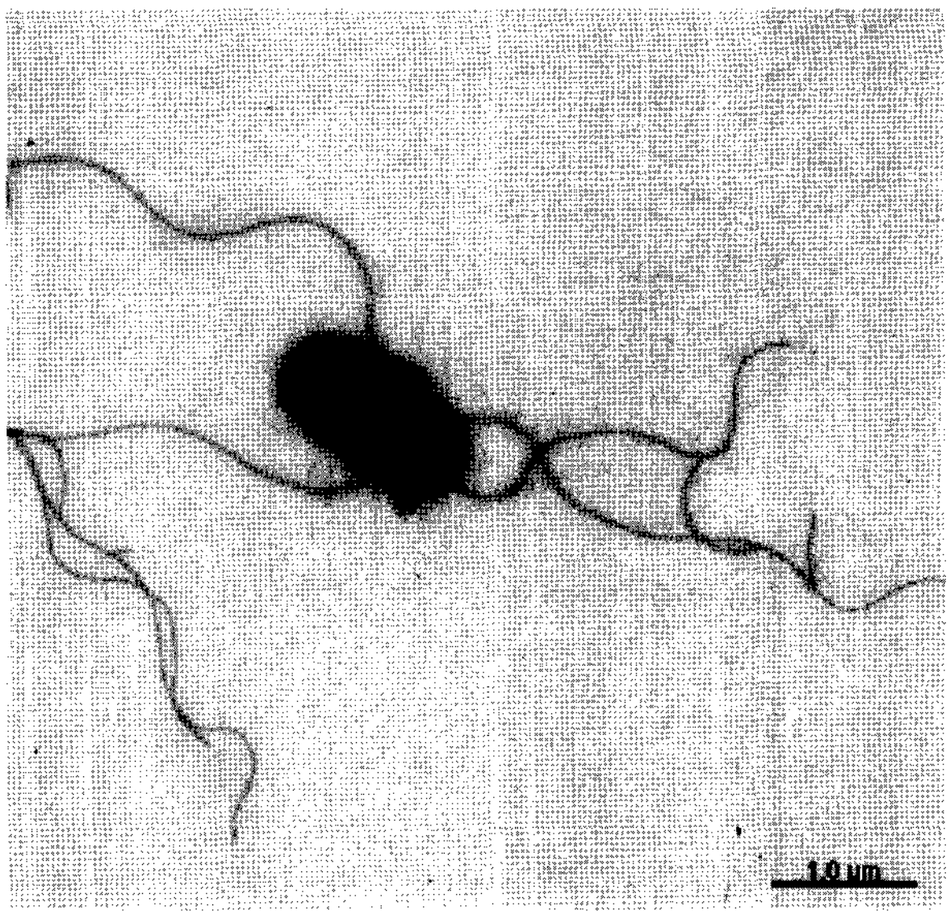

Fig. 2. Electron microscopy of *Agrobacterium tumefaciens*, showing a rod-shaped bacterium with peritrichous flagella (bar = 1.0 μm).

the galls. Such seedlings (transplants) were not transplanted to the main fields and had to be discarded.

Bacterial isolation. Pathogen isolation was conducted using the tumors of infected ginseng seedling roots. After surface sterilization of the gall tissues with 70% ethanol for 30 sec and 1% sodium hypochlorite for 1 min, followed by rinsing water in sterilized distilled water three times, inner gall tissues of about 5 mm in size were placed in 0.5 ml sterilized distilled water in an Eppendorf tube and cut into pieces (macerated) using a sterilized mess. Plant detritus was fully precipitated for 30 min at room temperature, and the supernatant was plated on *Agrobacterium*-selective medium (D1M agar) (Perry and Kado, 1982). *Agrobacterium*-looking colonies formed on the agar medium after 2-3 days of incubation at 28°C were isolated and used for further studies. Totally six bacterial isolates were obtained from the root galls.

Identification of the bacterial isolates. Bacterial characteristics of the 6 isolates from the root galls were investigated to the genus level by the method of Schaad et al. (2001), including Gram staining, growth on D1M agar, colony color on nutrient glucose agar (NGA), yeast extract-dextrose $CaCO_3$ agar (YDC) or nutrient-broth yeast extract agar (NBY), fluorescent pigment production on King's B (KB) agar, anaerobic or aerobic growth, flagella type, and spore and aerial mycelium formation. The morphology of the bacterial isolates including flagella type was examined using electron microscopy after negative staining of bacterial cells with 2% phosphotungstic acid (pH 7.0), followed by observing them under a JEM-1010 electron microscope (JEOL, Ltd., Japan) at 80 kV.

The 6 bacterial isolates obtained from the root galls on D1M agar were Gram-negative, rod-shaped with peritrichous flagella (Fig. 2), aerobic, not forming yellow or orange colonies on NGA, YDC or NBY, non-fluorescent on KB agar, and non-spore forming, which showed the bacterial characteristics identical to those of Genus *Agrobacterium* (Table 1) (Schaad et al., 2001).

Carbon source assimilation of the 6 bacterial isolates was also examined using the Biolog GN test kit (Biolog Inc., USA) according to the manufacturer's specifications as in the previous study (Kim et al., 2007). The 6 *Agrobacterium* isolates commonly utilized 58 carbon sources including *N*-acetyl glucosamine but not 15 carbon sources including α-cyclodextrin in the GN 96 well microplate (Biolog Inc., USA) (data not shown). There were also variations in the utilization of 21 carbon sources among the 6 isolates. The Biolog data gave the *Agrobacterium* isolates tested high similarities to *A. tumefaciens* (similarities of 0.732-0.993% with a match probability of 100%). Thus, based on the results of the above cultural and physiological characteristics of the bacterial isolates and their Biolog analyses, the 6 bacterial isolates tested were confirmed to be *A. tumefaciens*.

Genetic sequences of 16S ribosomal RNA genes of the bacterial isolates were analyzed to further support their species identification. For preparation of genomic DNAs from the six bacterial isolates cultured on nutrient agar, the amplification of 16S rRNA genes using the primers of 27mF <5'-AGAGTTTGATCMTGGCTCAG-3'> and 1492

Table 1. Comparison of characteristics of the present bacterial isolates from ginseng root galls with those of the known genera

Characters	Present isolates	Reference genera[a]			
		Ag	Er	Ps	Xa
Gram staining	–[b]	–	–	–	–
Growth on D1M agar	+	+	–	–	–
Yellow or orange colonies on NGA, YDC or NBY media	–	–	V–	–	+
Fluorescent pigment on KB agar	–	–	–	V+	–
Grows anaerobically	–	–	+	–	–
Grows aerobically	+	+	+	+	+
More than four peritrichous flagella	V	–	+	–	–
Spore formed	–	–	–	–	–
Aerial mycelium	–	–	–	–	–

[a]Data from Schaad et al. (2001). Ag, *Agrobacterium*; Er, *Erwinia*; Ps, *Pseudomonas*; Xa, *Xanthomonas*.
[b]+, positive; –, negative; V, variable.

mR <5'-GGYTACCTTGTTACGACTT-3'> (Brosius et al., 1978; Weisburg et al., 1991) and PCR buffer followed the methods described by Jeon et al. (2003). Direct sequencing of the amplified PCR products was done using ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Model ABI 3700). The sequences obtained from the isolates were used for sequence database searches in GenBank and sequence similarities to the known *A. tumefaciens* strains were measured using the BLAST program. Sequences were assembled using the SeqMan program and analyzed with the MegAlign programs (DNASTAR, Inc., USA).

The GenBank accession numbers for 16S rRNA gene sequences of the 6 bacterial isolates in this study are EF486308-EF486313. The partial sequences of 16S rRNA gene of all isolates tested contained the same 409 bases (data not shown), and BLAST analyses showed that they had 100% homology to those of other *A. tumefaciens* strains (GenBank accession numbers AF501343, AY701900, AY701898, and AY701899) that had been deposited in the GenBank database. These results may also confirm that the bacterial isolates are all *A. tumefaciens*.

Pathogenicity. Carrot discs and tomato plants were used for pathogenicity test instead of ginseng roots because it is difficult to maintain any forms of ginseng seedling roots physiologically active for 2-3 weeks in experimentally controlled conditions for inducing galls. The bacterial isolates grown on nutrient agar for 2-3 days were collected in sterilized distilled water (SDW), and the concentration was adjusted to 1.0×10^8 colony forming units (CFU)/ml as inoculum for pathogenicity test. Fully-grown root discs (0.5 cm in thickness) of carrot (*Dacus carota* var. *sativa*) were inoculated with a drop (50 μl) of the bacterial suspension on the xylem region at the center of the root discs. The same amount of SDW was used as control. The inoculated root discs were placed on moistened filter paper in Petri dishes at 22°C in an incubation chamber, replicating three times. For pathogenicity of the bacterial isolates to tomato, tomato seedlings (*Solanum lycopersicum* cv. Seokwang) at 8-10 true leaf stages were inoculated at the basal stems with the bacterial suspension (20 μl) after wounding with a sterilized toothpick. SDW was used as control. After inoculation, the wounding sites were sealed with Parafilm, and the inoculated plants were placed at 25°C in a green-

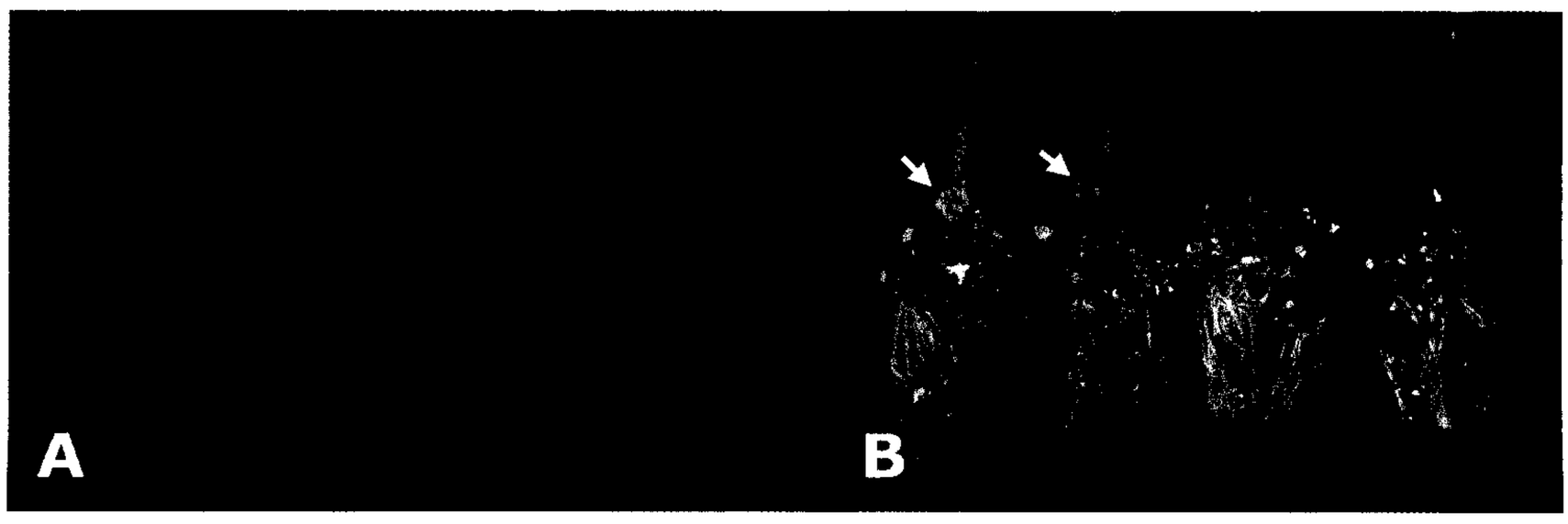

Fig. 3. Healthy (right) disc and diseased (left) carrot disc showing callus-like swellings (arrows) around vascular bundle 2 weeks after inoculation (**A**), and crown gall formation (arrow) on tomato (left two) (right two plants: healthy control) 4 weeks after inoculation (**B**).

house. The experiment replicated three times. Symptom developments on the inoculated carrot discs and tomato plants were examined visually.

The symptoms produced were callus-like swellings on carrot discs with the infection sites turning greenish and tumefacient 7 days after inoculation, which developed more with time after that (Fig. 3A). No such symptoms were produced in the uninoculated control carrot discs. In the inoculated tomato plants, small swellings appeared on the basal stems 10 days after inoculation, and enlarged with time to become typical crown gall tumors (Fig. 3B). The same bacterium was reisolated from the affected carrot discs and tomato gall tissues, fulfilling the Koch's postulates.

The bacterial pathogen isolated from the galls of ginseng seedling roots was identified as *A. tumefaciens*. It had pathogenicity to other host carrot and tomato, and therefore, it may also have pathogenicity to ginseng root from which it was isolated, although its virulence is still not known yet. Therefore, the crown gall disease of ginseng seedling root is caused by *A. tumefaciens*. There is a report showing the crown gall symptoms on the aged ginseng root (Cho et al., 1999), but no seedling root disease has been reported yet, and this is the first description of *A. tumefaciens* from the tumor of ginseng root. The bacterial disease occurred locally and sparsely in the ginseng seedbeds during the survey, suggesting that this disease may not be widely spread in ginseng fields in Korea. This bacterium, however, is a soil-inhabitant, perpetuating in soil for a long time and hardly eliminated from the soil once infested (Agrios, 2005; Cho et al., 1999), and has a wide host range, distributing more than 15 species of woody and herbaceous plants in Korea (Lee et al., 2006a, 2006b; The Korean Society of Plant Pathology, 2004), which suggests a high potential to be wide spread. Also, considering its appearances in seedbeds suggests that the ginseng root gall may become a threat to ginseng in Korea.

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