Biological Control of Gom-chwi (*Ligularia fischeri*) Phytophthora Root Rot with *Enterobacter asburiae* ObRS-5 to Suppress Zoosporangia Formation and Zoospores Germination

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Gom-chwi (*Ligularia fischeri*) is severely infected with *Phytophthora drechsleri*, the causal organism of Phytophthora root rot, an economically important crop disease that needs management throughout the cultivation period. In the present study, Phytophthora root rot was controlled by using bacterial isolates from rhizosphere soils collected from various plants and screened for antagonistic activity against *P. drechsleri*. A total of 172 bacterial strains were isolated, of which, 49 strains showed antagonistic activities by dual culture assay. In the seedling assay, six out of the 49 strains showed a predominant effect on suppressing *P. drechsleri*. Among the six strains, the ObRS-5 strain showed remarkable against *P. drechsleri* when treated with seed dipping or soil drenching. The ObRS-5 strain was identified as *Enterobacter asburiae* based on 16S ribosomal RNA gene sequences analysis. The bacterial cells of *E. asburiae* ObRS-5 significantly suppressed sporangium formation and zoospore germination in *P. drechsleri* by 87.4% and 66.7%, respectively. In addition, culture filtrate of *E. asburiae* ObRS-5 also significantly inhibited sporangium formation and zoospore germination by 97.0% and 67.6%, respectively. Soil drenched bacterial cells, filtrate, and culture solution of *E. asburiae* ObRS-5 effectively suppressed Phytophthora root rot by 63.2%, 57.9%, and 81.1%, respectively. Thus, *E.*

asburiae ObRS-5 could be used as a potential agent for the biological control of Phytophthora root rot infecting gom-chwi.

Keywords : antagonism, biological control, *Enterobacter asburiae* ObRS-5, *Ligularia fischeri*, *Phytophthora drechsleri*

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Ligularia fischeri, locally known as gom-chwi, is a perennial wild vegetable found in shady and humid environments of forest understories in Korea. It is an edible vegetable with high consumer preference because of its bitter taste and rich flavor (Cho and Kim, 2005). In the 1990s, the plant started being cultivated in greenhouses and forest fields in Gangwon-do, Gyeongsang-do, Jeollabuk-do, and Jeollanam-do (Rural Development Administration, 2018). Although the cultivation of gom-chwi plants in greenhouses has facilitated the supply of fresh products to consumers throughout the year, the environmental characteristics of greenhouses have promoted the occurrence of many plant diseases (Nam, 2001). It has been reported that various problems such as Phytophthora root rot, Sclerotium stem rot, and powdery mildew occur during cultivation in greenhouses with environmental characteristics that differ from the crop's native habitat, such as high temperature and low humidity (Korean Society of Plant Pathology, 2009; Kwon et al., 1999; Moon et al., 2015). Cultivation in greenhouses adversely affects gom-chwi growth because underground and above-ground temperatures are higher than that of the native habitat, and organic matter content is lower (~19-55 g/kg) than that of the native habitat (110 g/kg) (Kim et al., 2016; Rural Development Administration, 2018).

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One of the major biotic problems in gom-chwi cultivation is Phytophthora root rot caused by *Phytophthora drechsleri*. This fungal pathogen spreads quickly in the field through infected plants and wind-blown rain, as well as contaminated irrigation water, soil, or gravel substrates, making it difficult to control (Kwon et al., 1999). Many studies have been conducted on the use of microorganisms as biological control agents against plant diseases to increase agricultural productivity, as an alternative to chemical pesticides (Bhardwaj et al., 2014; Borriss, 2015; Fravel, 2005; Gupta et al., 2015). Generally, farmers and consumers prefer eco-friendly cultivation of gom-chwi. However, the use of microbial biocontrol agents is not available yet. Moreover, seedling production is difficult, and the cost of seedlings is higher than other crops because of the low germination rate. Therefore, only a few farmers are willing to plant seedlings every year, leading to a high prevalence of Phytophthora root rot caused by serial cultivation of gom-chwi. In the last two years, some gom-chwi fields in Jeollado were infected by Phytophthora root rot at 24.1–43.1% infection rate, leading to reduced yields; therefore, farmers replaced gom-chwi with other crops such as butterbur, *Aralia elata* and *Capsicum annuum*.

Many biocontrol agents can interfere with the growth and infection of phytopathogens inhabiting the plant rhizosphere (Doran and Zeiss, 2000; Kim et al., 1999; Liu et al., 2015; Ryan et al., 2008). Biocontrol agents use direct and indirect antagonistic and supportive mechanisms for achieving disease control. These mechanisms include antimicrobial activity, parasitic activity, space or nutrient competition, antibiotic production, and disease resistance induction. Another mechanism is the provision of biological fertilizer through phosphoric acid solubilization, nitrogen fixation, and plant growth-promoting hormone production (Haggag and Timmusk, 2008; Mahaffee and Backman, 1993; Park et al., 2010; Szczech and Shoda, 2006).

Studies have been conducted on the biological control of Phytophthora root rot using microorganisms. *Paenibacillus polymyxa* AC-1 strain, which effectively controls red pepper blight, has been commercialized in Korea. In addition, in Europe and the United States, *Bacillus subtilis* QST 713, *Streptomyces lydicus* WYEC 108, *Trichoderma atroviride* CHS 861, and *Gliocladium virens* GL-21 are already commercialized as biocontrol agents against *Phytophthora* disease (Elliott et al., 2009). These microorganisms produce various secondary metabolites and antibiotics that inhibit *Phytophthora* spp. and let the expression of defense-related genes increase in treated plants to which it is applied (Bae et al., 2016; Shahidi Bonjar et al., 2006; Shirzad et al., 2012). *Trichoderma atroviride*, which establishes in the

plant root, changes the carbohydrate composition of the root exudates in order to control the disease by predominating rhizosphere colonization over *Phytophthora cinnamomi* and competing for space and nutrients (Macías-Rodríguez et al., 2018). Rhamnolipids and cyclic lipopeptides produced by *Pseudomonas* spp. can inhibit the formation of the zoosporangia membrane of *Phytophthora* spp. (De Bruijn et al., 2007; Maleki et al., 2011).

Biological control agents for the management of *P. drechsleri* have not yet been reported, indicating that biocontrol agents still need to be developed for the successful control of Phytophthora root rot of gom-chwi. The objectives of this study were to (1) isolate rhizobacteria from various plants, (2) describe a screening approach with *in vitro* and seedling bioassays by seed dipping and soil drenching treatments to select bacterial strains with antagonistic activities towards *P. drechsleri*, (3) investigate the suppression mechanisms of a selected bacterium, *E. asburiae* ObRS-5, on *P. drechsleri*, including effects on zoosporangia formation and zoospore germination in plants treated with ObRS-5 bacterial cells, filtrate and culture solution, and (4) evaluate the biocontrol efficacy of ObRS-5 on Phytophthora root rot of gom-chwi in potted plants under glasshouse conditions.

Materials and Methods

Pathogen isolate and preparation of inoculum. The *Phytophthora drechsleri* Gcbs-5 strain was isolated from gom-chwi in greenhouse fields in Geochang in Korea following Meszka and Michalecka (2016). It was then stored on a potato dextrose agar (PDA; Difco, Detroit, MI, USA) slant in the dark at 15°C (Kwon et al., 1999). Identification of *P. drechsleri* was done by comparing its cultural and morphological characteristics with descriptions published in the literature and by phylogenetic analysis (Rural Development Administration, National Institute of Agricultural Science and Technology, Plant Pathology Division, 2000).

Zoospores probably function as the primary inoculum for infecting gom-chwi plants (Biles et al., 1995), however, a major problem is production of the large quantities of similar-aged zoospores of *P. drechsleri* required for inoculation (Mansoori and Banihashemi, 1982) according to existing methods (Barash et al., 1965; Cother and Griffin 1973; Mehrotra 1970). Thus, the mycelium of *P. drechsleri* were used as a pathogen inoculum for seedling assay and tests on potted plants in the glasshouse. Forty grams of barley was added to 45 ml of distilled water in a 1 l Erlenmeyer flask and then it was autoclaved twice for 20 min at 121°C. The flask was inoculated with five mycelial plugs

(5 mm diameter) from a 5-day-old culture of *P. drechsleri*, that was incubated at 25°C. The inoculated barley medium was shaken daily to break up particles so that the pathogen and medium were well mixed. For pathogen inoculation, a granule of *P. drechsleri* infected barley was attached to the upper part of gom-chwi roots (Pieterse et al., 1996).

Field sampling and isolation of rhizobacteria. Bacteria were isolated from the rhizosphere soil collected from seven kinds of plants: gom-chwies, strawberries, celeries, kales, red beets, chicories, and red lettuces from Damyang, Iksan, Milyang and Sunchang regions in Korea. A three-gram soil sample was added to 27 ml of sterilized distilled water and shaken at 180 rpm and 28°C, for 30 min. Subsequently, 1 ml suspension was taken and diluted to 9 ml of sterilized distilled water, 5 times in a row, and 1 ml of each final suspension was taken and spread onto tryptic soybean agar (TSA; Difco), 1/10 TSA and nutrient agar (Difco) medium. After incubation at 28°C for 48 h, single colonies with different colors and shapes were isolated and stored at -70°C in 20% glycerol.

Screening of antagonistic bacteria *in vitro* and in seedling assays in the glasshouse

Dual culture assay. Inhibition of mycelial growth of *P. drechsleri* in the bacterial isolates was tested by dual culture assay. The bacterial isolates were equidistantly streaked on the margins of the PDA plates and incubated at 28°C for 24 h. Sterile distilled water was used for the control. The agar disc with 5 mm of *P. drechsleri* was placed at the center of the PDA plate and incubated at 25°C for 5 days. The growth inhibition length of mycelium was measured in order to screen the antagonistic bacterial strains against *P. drechsleri*, using the method of Idris et al. (2007).

Seedling assay. Forty-nine bacterial strains, resulting from dual culture assays, were evaluated for the suppression of Phytophthora root rot using gom-chwi seedlings. For the seed dipping treatment, 10^8 cfu/ml ($OD_{600} = 0.25$) of bacterial suspension in 0.1% peptone water (Becton Dickinson Biosciences, San Jose, CA, USA) was prepared. The seeds (cv. Aram) were dipped in a bacterial suspension for 3 h. For the pathogen-treated control (control) and pathogen non-treated control (mock), seeds were dipped in 0.1% peptone water without any bacterial treatment. The gom-chwi seeds were sown on a 32-hole tray filled with soil (Green Soil, Seoul Bio, Eumseong, Korea). A set of 16 individual gom-chwi plants was used for each treatment. To introduce the pathogen inoculum, one granule of barley infected with *P. drechsleri*, as described above, was inoculat-

ed on the upper part of the root of a 5-week-old gom-chwi after the soil was slightly removed. Pathogen non-treated control plants were mock-inoculated with a granule of non-infected barley (Maleki et al., 2011; Pieterse et al., 1996). All plants were irrigated regularly. The severity of diseased plants was evaluated 7 days after pathogen inoculation and the onset of symptoms. Disease severity (modified from Irabor and Mmbaga, 2017) was evaluated on a scale of 0-3 (Supplementary Table 1): 0 = no visible symptoms; 1 = slightly diseased, 20% of total leaves were wilted with brownish lesions beginning to appear on the stem; 2 = stem lesions extending to cotyledons or petioles and 50% of total leaves were wilted; and 3 = entire plant diseased or dead.

Six bacterial strains from the seedling assays with seed dipping treatment were evaluated using gom-chwi plants using a soil drenching treatment. The bacterial strains were incubated in the tryptic soybean broth medium (TSB; Difco) at 28°C for 24 h. The culture solutions of bacterial strains were adjusted to a concentration of 10^8 cfu/ml ($OD_{600} = 0.25$). Five-week-old gom-chwi seedlings growing in a 32-hole tray were drenched once with 15 ml bacterial culture solution, and a set of 32 individual gom-chwi plants was used for each treatment. The control and mock-control were treated with 15 ml of TSB medium. Pathogen inoculum was conducted as described above and, disease severity were also evaluated as described above. All experiment were repeated three times. The control efficacies indicate the differences in the disease incidence rates, as a percentage, of between bacterial treatments and untreated control, which was calculated by the method of Tsuda et al. (2016).

16S rRNA gene sequencing for the identification of the ObRS-5 strain. The total genomic DNA of the ObRS-5 strain was extracted using a Genomic DNA Prep kit (Nanohelix, Daejeon, Korea) following the user manual. A 1,356-bp of 16S rRNA gene fragment was amplified from genomic DNA of strain ObRS-5 using the universal primers, 27F (forward) 5'-GAG TTT GMT CCT GGC TCA G-3' and 1492R (reverse) 5'-ACG GYT ACC TTG TTA CGA CTT-3' (Weisbrug et al., 1991). Amplification of the 16S rRNA region was performed in a total volume of 25 µl containing 50 ng of DNA template, 0.25 µM of each primer, 0.25 µM of dNTP (Inclone, Daejeon, Korea), 2.5 µl of 10× buffer (Inclone) and 1.25 U of *Taq* DNA polymerase (Inclone), and 1.25 µl of 1.10 g/ml of dimethyl sulphoxide (Sigma-Aldrich, St. Louis, MO, USA). The PCR mixture was first heated at 95°C for 5 min, and 30 cycles were then performed at different temperatures: 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min 30 s. The reaction was finally incubated at 72°C for 10 min. The fragment size of

the amplified PCR products was confirmed by electrophoresis analysis, and afterward, sequencing was conducted by Genotech Co. (Daejeon, Korea). The raw sequences were aligned for a phylogenetic dendrogram, which was constructed using a neighbor-joining algorithm using MEGA software (ver. 6.06) and 1,000 replicates of bootstrapping (Felsenstein, 1985). The working reference sequences were downloaded using the EzBioCloud server at <http://www.ezbiocloud.net/>.

Antagonism of *Enterobacter asburiae* ObRS-5 against *P. drechsleri*

Effect on zoosporangium formation. The culture solution of the ObRS-5 strain was adjusted to 1.0×10^8 cfu/ml ($OD_{600} = 0.25$) and centrifuged at 8,000 rpm for 10 min. The supernatant was then filter-sterilized using a 0.22 μ m syringe filter (Millipore, Billerica, MA, USA) for 'filtered filtrate' or was autoclaved at 121°C for 15 min for 'sterilized filtrate'. The harvested cells were resuspended in 0.1% peptone water at a concentration of 1.0×10^8 cfu/ml ($OD_{600} = 0.25$). Mycelial discs of *P. drechsleri* from the peripheral growth of a 5-day-old culture on PDA were transferred to Petri dishes, containing 30 ml of 10% (v/v) clarified V8 medium with 0.3% $CaCO_3$, which were incubated at 25°C for 4 days (Lee et al., 2001). The medium was decanted and replaced by 30 ml of bacterial cell suspension, culture solution, and filtrate of *E. asburiae* ObRS-5, with the sterile soil extracts at a ratio of 1:6 (v/v) (De Cock and Lévesque, 2004). Soil extracts were prepared as described by Mansoori and Banihashemi (1982). The control was treated with only sterile soil extracts, and the medium-treated control was treated with TSB and sterile soil extracts at a ratio of 1:6 (v/v).

All plates were light-irradiated at 24 h per day at 25°C for 2 days. Microscopic observations (M205A, Leica, Jena, Germany) were conducted to count the number of zoosporangia formed (zoosporangia/5 mm²) from five random areas of the fungal growth, from each mycelial disc, because zoosporangium of *P. drechsleri* is non-caducous (Singh et al., 1992). Thus, the total number of zoospores was also investigated. In order to release the zoospores, all plates were treated at -20°C for 25 min and treated at room temperature for 3 h. The mycelium was filtered with sterile gauze and the released zoospores were counted using a hemocytometer (Incyto, Cheonan, Korea) under an optical microscope (DM2500, Leica).

Effect on zoospore germination. A zoospores suspension of *P. drechsleri* was prepared at a concentration of 1.0×10^4 zoospores/ml as described above. A prepared cell

suspension, filtrate, or culture solution of ObRS-5 strain was mixed with the sterile soil extract at a ratio of 1:1 (v/v) in a 96-well cell culture plate (SPL, Pocheon, Korea). Zoospore germination was observed under a microscope at 2-h intervals during incubation at 25°C. The result of the cell suspension treatment was compared to the soil extract-treated control and the result of the remaining treatments were compared to the medium treated control.

Experiments on potted plants in a glasshouse. Gom-chwi were sown in a 32-hole tray, they were grown for 4 weeks, and then gom-chwi plants were transplanted into plastic pots (10-cm-diameter) with soil. A set of 30 individual 12-week-old gom-chwi plants was used for tests under glasshouse conditions. Each plant was drenched once with 50 ml of cell suspension, filtrate, sterilized filtrate, culture solution of *E. asburiae* ObRS-5 or TSB medium for control and mock-control treatments. Pathogen inoculum was treated with two granules of barley infected with *P. drechsleri* as described above. Disease severity were also evaluated as described above.

Statistical analysis

Data were statistically verified whether it follows a normal distribution or not by Levene test, and analyzed using analysis of variance (ANOVA) in R software (ver. 3.4.1), and the significance of each value was determined using Duncan's multiple range test ($P < 0.05$).

Results

The effect of bacterial isolates on mycelial growth in *Phytophthora drechsleri*. A total of 172 isolates were finally obtained from rhizosphere soils from diverse crops. Among these bacterial isolates, 26 isolates had 10-20% and

Table 1. Number and percentage of bacterial isolates classified by degree of mycelial growth inhibition by dual culture assay against *Phytophthora drechsleri*

Mycelial growth inhibition rate (%)	No. of isolates	Percentage of isolates
- ^a	123	71.5
0-5	10 ^b	5.8
5-10	11 ^b	6.4
10-15	14 ^b	8.1
15-20	12	7.0
20-25	1	0.6
25-30	1	0.6

^a-, no inhibition observed.

^bSelected strains.

144 isolates had less than 10% inhibition effect on mycelial growth in *P. drechsleri* (Table 1, Supplementary Fig. 1). In addition, only 2 isolates showed more than 20% mycelial inhibition rate, which was 1.2% of the total bacterial isolates. Thus, the evaluation with the seedling assay was carried out on 49 bacterial strains (28.5% of isolates) having a varied range of mycelial growth inhibition abilities.

Selection of bacterial strains and seedling assay in glasshouse. Among the 49 antagonistic strains, in the seed dipping treatment of six bacterial strains, RIR9, ObRS-5, ObRS-6, ObRS-10, ObRS-15, and HRS-20 were the most efficient in reducing disease severity in gom-chwi seedlings compared to the bacterially untreated control (Supplementary Table 2). The value of disease control efficacy (%) of RIR9, ObRS-5, ObRS-6, ObRS-10, ObRS-15, and HRS-

Fig. 1. Biocontrol effect of soil drenching treatment of bacterial strains against *Phytophthora drechsleri* using 5-week-old gom-chwi seedlings. Values are means $\pm$ standard errors of disease severity. Asterisk designate mean, which are significantly different from the mean of control, using Duncan’s multiple range test (* $P < 0.05$).

Fig. 2. Morphological and phylogenetic characterization of *Enterobacter asburiae* ObRS-5. (A) Colonies morphology on tryptic soybean agar medium and (B) phylogenetic dendrogram constructed from a comparative analysis of 16S rRNA sequences showing the relationships between *E. asburiae* ObRS-5 and related species. Numbers at the branching points represent bootstrap values (>50%) for 1,000 replicates. The scale bar indicates a distance of 0.002 substitutions per site.

20 was 76.5, 76.5, 70.6, 70.6, 64.7, and 58.8, respectively. Whereas 22 strains showed a mildly suppressive effect with no significant differences compared to the control and 21 strains had no effect (0%) (Supplementary Table 2). Thus, six bacterial strains were selected as potentially effective antagonists for further tests.

To assess whether these six bacterial strains have suppressive effects in sterilized soils or not, we performed to shoot plate assay (Supplementary Fig. 2A). In the shoot plate assay, seed dipping treatment of RIR9, ObRS-5, ObRS-6, ObRS-10, ObRS-15, and HRS-20 strains were

showed means of infected rate at 15.6%, 12.5%, 31.3%, 34.4%, 34.4%, and 15.6%, respectively, and those of ObRS-6, ObRS-10, and ObRS-15 strains have no suppressive effects compared to water-treated control. Whereas RIR9, ObRS-5, and HRS-20 showed control efficacy at 29.2%, 45.8%, and 25%, respectively (Supplementary Fig. 2B).

In the seedling assay with soil drenching treatment, three strains, ObRS-5, ObRS-6, and ObRS-15, showed significant control efficacies of 90.5%, 77.8%, and 66.7%, respectively (Fig. 1, Supplementary Fig. 3). Thus, among the

Fig. 3. Inhibition of zoosporangia formation of *Phytophthora drechsleri* by *Enterobacter asburiae* ObRS-5. (A) Number of zoosporangia per mm² and zoospores per ml. (B) Inhibition on zoosporangia formation of treatment of ObRS-5 cell suspension (10⁸ cells/ml), filtered or sterilized filtrate, culture solution and control (treated with soil extract or medium). Values are means $\pm$ standard error of the number of zoosporangia (/mm²) or zoospores (/ml). Lower cases mean, which are significantly different from the mean of control, using Duncan's multiple range test ($P < 0.05$).

six antagonistic strains, the ObRS-5 strain was selected as potentially the most effective antagonist for further testing.

Identification of ObRS-5 strain with a biocontrol effect. Molecular phylogenetic analysis of the 16S rRNA sequences of ObRS-5 strain showed 99.9% homology with *Enterobacter asburiae* JCM 6052, and the bootstrap value was 86% indicating that it belongs to the subcluster of *E. asburiae* (Fig. 2). Consequently, the ObRS-5 strain was

finally identified as *Enterobacter asburiae* ObRS-5. The nucleotide sequences of ObRS-5 strain was submitted to Genbank as the accession number MT020371.

The antagonistic effect of ObRS-5 on *P. drechsleri* in vitro. Zoospore germination was reduced by treatment with cell suspension (87.4%), filtrate (97.0%), sterile filtrate (98.5%) and culture solution (100%) of *E. asburiae* ObRS-5 (Fig. 3). As a result of counting the number of zoospores,

Fig. 4. Suppression of zoospores germination of *Phytophthora drechsleri* by *Enterobacter asburiae* ObRS-5. (A) Germination rate of zoospores (%). (B) Inhibition effect on zoospores germination of treatment of ObRS-5 cell (10^8 cells/ml), culture solution, filtered or sterilized filtrate and control (treated with soil extract or medium). The arrows indicate that black is not suppressed, yellow is slightly suppressed and blue is significantly suppressed in zoospores germination. Values are means $\pm$ standard error of zoospores germination rate (%). Letters mean, which are significantly different from the mean of control, using Duncan's multiple range test ($P < 0.05$).

Fig. 5. Biocontrol effect of *Enterobacter asburiae* ObRS-5 on *Phytophthora* root rot in potted gom-chwi plants in the glasshouse. Gom-chwi plants was soil drenched with 1, culture solution; 2, cell suspension; 3, filtrate and 4, sterilized-filtrate. As controls without *E. asburiae* ObRS-5 pathogen-inoculated control (5, water and 6, medium) and uninoculated control (7, water and 8, medium). Values are means $\pm$ standard error of disease severity. Letters mean, which are significantly different from the mean of control, using Duncan's multiple range test ($P < 0.05$).

the inhibition rate compared to the control was highest for the sterile filtrate (94.6%), followed by the filtrate (91.7%), culture solution (87.1%) and cell suspension (31.5%) of *E. asburiae* ObRS-5 (Fig. 3).

Meanwhile, in the zoospore germination, treatment of cell suspension, filtrate, sterile filtrate, and culture solution of *E. asburiae* ObRS-5 showed a germination rate of 33.3%, 32.4%, 93.6%, and 9.6%, respectively. The germination rate of sterile filtrate did not show significant differences in either medium treated control (90.6%) or soil extract-treated control. There was also no statistical difference between the medium treated control and soil extract-treated control. As a result, the cell suspension, filtrate, and culture solution reduced zoospore germination at 66.7%, 90.4%, and 67.6%, respectively (Fig. 4). In particular, *E. asburiae* ObRS-5 culture solution effectively suppressed zoospore germination. Therefore, *E. asburiae* ObRS-5 or its products might be involved in the inhibition of zoosporangia formation of *P. drechsleri*.

The biocontrol effect of ObRS-5 on *Phytophthora* root rot in plants in the glasshouse. The *E. asburiae* ObRS-5 strain inhibited *P. drechsleri* infection in potted plants in the glasshouse. It was significantly antagonistic, and the control efficacies of gom-chwi plants were 80% (culture solution), 63.2% (cell suspension), 55.6% (filtrate) (Fig. 5). Gom-chwi plants treated with sterilized filtrate of *E. asburiae* ObRS-5 showed a disease severity of 1.08, of which the pathogen-treated controls were 1.50 (medium) and 1.58 (water), and the results revealed that there was not significantly suppression effect, which is similar to the result of the *in vitro* experiment.

Discussion

In this study, we describe selection of potential bacterial biocontrol agent and investigation of selected bacterial character related biocontrol of *Phytophthora* root rot in gom-chwi. *Phytophthora* root rot caused by the oomycete pathogen *Phytophthora drechsleri*, has already reached a major problem of gom-chwi cultivation farms in Korea, thus it is necessary to develop agents which could work as effective biocontrol (Rural Development Administration, Agricultural Sciences Institute, 1991).

As a result of initial screening bacterial strains of disease suppression against *P. drechsleri*, among 172 isolates, 49 bacterial isolates with antagonistic behavior were selected by a dual culture assay. Especially, only two strains (1.2% of the 172 strains) showed more than 20% mycelial inhibition rate. This result indicates that many of the isolates did

not have antagonistic activity against *P. drechsleri* on agar (Table 1). In case of previous studies, Syed-Ab-Rahman et al. (2018) selected only 6% of the total isolates showing antagonism against *Phytophthora* spp., and Tjamos et al. (2004) reported that 12% of the isolates produced antifungal compounds in dual cultures against fungal phytopathogens. Although the bacterial strain showed the strongest antagonistic action against fungal pathogens on the agar medium, antibiotic activity was not always a constant feature for good disease control efficacy in fields (Tjamos et al., 2004).

Dual culture techniques on agar plates have commonly been used as a useful screening method (Schroth and Hancock, 1982). However, this screening might be limited to only investigating antagonistic ability on agar and it cannot select biocontrol agents that provide disease control by other mode of action, such as hyperparasitism/predation, lytic enzymes, and niche competition (Bakker et al., 2003; Pang et al., 2009; Pliego et al., 2011). On the other hand, numerous environmental factors, which were not considered in the screening steps, could disturb the microbial control efficacy in field, such as soil temperature, humidity, and soil microbial composition (Kloepper and Schroth, 1981).

Thus, for successful selection of suitable strains for disease control in field conditions, we conducted repetitive bioassays in host plants by using strains having varied antagonistic ranges in the microbial screening steps. Among these 49 isolates, 6 isolates were initially selected (Fig. 1). Among these six bacterial strains, HRS-20 strain had a significant effect by seed dipping, but, had no effect on *Phytophthora* root rot by soil drenching. The ObRS-6 and ObRS-15 strains also showed significant results with control efficacies of 77.8% and 52.4%, respectively, in the seedling assay. However, the most effective strain, ObRS-5, reduced the percentage of disease severity by 90.5% with soil drenching as well as by 76.5% with the seed dipping treatment compared to the untreated control ($P < 0.05$). Finally ObRS-5 strain was finally selected using repeated experiments, which predominantly reduced *Phytophthora* root rot and showed a control efficacy (Fig. 1).

In all bio-assays conducted in this study, in ObRS-5 strain treated gom-chwi plants, control effect of the soil drenching treatment (90.5%) was better than the seed dipping method (76.5%) or the seed dipping applied for sterilized soil (shoot plate assay, 45.8%). Although there was a solely control effect by treatment with the ObRS-5 strain alone, it might underline that the higher effect by soil drenching is due to the complex interaction of microbiota in the root zone of the plant (Kim et al., 2019). Thus, the soil drenching could have been more effective than other

methods because the isolation sources of the strains were rhizosphere soil of crops. Therefore, further research is needed to determine how the soil drenched strains can defend against pathogen invasion, interact with native microbes and react to root exudate in the rhizosphere of plants (Pascale et al., 2020).

Many of previous studies have demonstrated that the interaction of seeds and microorganisms improves the biological stress tolerance of plants (Kim et al., 2015; Mahmood et al., 2016; Nelson et al., 1986; Nelson, 2018). When seeds are treated with *Enterobacter cloacae*, the strain binds the mycelium of *Pythium* spp. so as to inhibit the growth of the pathogen (Nelson et al., 1986). *Bacillus* spp. also have a control effect on seed infectious plant diseases (Kim et al., 2015). There has been no report on the control effect of *E. asburiae* on *Phytophthora* spp.; however, the taxonomic position of *E. asburiae* ObRS-5 supports its plant disease control capacity. During a control study using *Enterobacter* spp., lipopolysaccharides of *E. asburiae* RS83 was reported to induce early stages of plant defense enzymes against lettuce bacterial blight (Jetiyanon and Plianbangchang, 2013). The *E. cloacae* strain was reported to effectively control *Pythium* disease and *Fusarium* blight through chitinolytic activity (Chernin et al., 1995; Jetiyanon, 2015), and the *E. aerogenes* strain is known to control *Phytophthora* root rot by colonizing the plant rhizosphere by secreting antibiotics such as hydroxamate, siderophore, volatile and nonvolatile antagonist (Berner et al., 1988; Utkhede, 1986).

A striking feature of *Phytophthora* spp. is that it produces numerous zoospores, which rapidly result in plant infection (Erwin and Ribeiro, 1996). Thus, the control of *Phytophthora* root rot needs to effectively target the zoosporangia and zoospores. Treatment with cell suspension, filtrate, sterile filtrate, and culture solution of *E. asburiae* ObRS-5 resulted in inhibition of zoosporangia formation and zoospores germination (Figs. 3 and 4). Thus, *E. asburiae* ObRS-5 strain or its products might be involved in the suppression of reproduction and growth of *P. drechsleri*. Among these treatments, only sterile filtrate did not show a statistically significant difference to the control, which might be influenced of heat denaturation of yet-known, active compounds in culture filtrate. Meanwhile, in the medium treatment, the zoosporangia formation seemed to be inhibited (87.6%). However, as the total number of zoospores was similar to the soil extract-treated control, it was thought that the medium might promote the release of zoospores from zoosporangia, and hollow zoosporangia were not counted (Fig. 3).

Similarly to the above, in the result of the pot plant experiment, gom-chwi plants treated with cell suspension,

filtrate, and culture solution of *E. asburiae* ObRS-5 effectively suppressed the *P. drechsleri* infection with a biocontrol efficacy of 57.9-81.1%. The action of suppression of zoosporangia formation and zoospore germination by *E. asburiae* ObRS-5 might be an important contributor to the suppression of *Phytophthora* root rot in gom-chwi. Hence, the use of *E. asburiae* ObRS-5 as a biocontrol agent should be promoted in gom-chwi cultivation.

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Electronic Supplementary Material

Supplementary materials are available at The Plant Pathology Journal website (<http://www.ppjonline.org/>).

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