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Effect of *Bacillus mesonae* H20-5 Treatment on Rhizospheric Bacterial Community of Tomato Plants under Salinity Stress

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Plant growth-promoting bacteria improve plant growth under abiotic stress conditions. However, their effects on microbial succession in the rhizosphere are poorly understood. In this study, the inoculants of *Bacillus mesonae* strain H20-5 were administered to tomato plants grown in soils with different salinity levels (EC of 2, 4, and 6 dS/m). The bacterial communities in the bulk and rhizosphere soils were examined 14 days after H20-5 treatment using Illumina MiSeq sequencing of the bacterial 16S rRNA gene. Although the abundance of H20-5 rapidly decreased in the bulk and rhizosphere soils, a shift in the bacterial community was observed following H20-5 treatment. The variation in bacterial communities due to H20-5 treatment was higher in the rhizosphere than in the bulk soils. Additionally, the bacterial species richness and diversity were greater in the H20-5 treated rhizosphere than in the control. The composition and structure of the bacterial communities varied with soil salinity levels, and those in the H20-5 treated rhizosphere soil were clustered. The members of *Actinobacteria* genera, including *Kineosporia*, *Virgisporangium*, *Actinoplanes*, *Gaiella, Blastococcus*, and *Solirubrobacter*, were enriched in the H20-5 treated rhizosphere soils. The microbial co-occurrence network of the bacterial community in the H20-5 treated rhizosphere soils had more modules and keystone taxa compared to the control. These findings revealed that the strain H20-5 induced systemic tolerance in tomato plants and influenced the diversity, composition, structure, and network of bacterial communities. The bacterial community in the H20-5 treated rhizosphere soils also appeared to be relatively stable to soil salinity changes.

Keywords **:** *Bacillus mesonae*, bacterial community, rhizosphere, salinity, tomato

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Soil salinity is a critical environmental factor that limits plant development and growth. Low precipitation and high evaporation rates resulting from climate change and poor agricultural management have resulted in the expansion of salinized agricultural areas. It is expected that 50% of agricultural areas will be salinized by 2050 (Shrivastava and Kumar, 2015). Soil salinity levels indicate the electrical conductivity (EC) of the soil; higher levels of soil EC (>4 dS/m) reduce the growth and yield of most crop plants (Jamil et al., 2011; Munns, 2005). Soil EC is also a critical edaphic factor that shapes the bacterial community; Lee et al. observed a significant relationship between bacterial communities in the tomato rhizosphere and soil EC levels (Kim et al., 2016; Lee et al., 2019). Using microorganisms to enhance crop tolerance to salinity stress has been considered an alternative strategy to improve crop production. The physiological effects of plant growth-promoting bacteria (PGPB) on plants under abiotic stresses have been studied. However, there is limited understanding on how PGPB treatment affects the soil microbial composition under abiotic stress conditions.

Soil harbors a high microbial diversity, whose rhizospheric composition is influenced by plants through their root exudates (Bulgarelli et al., 2013; Compant et al., 2019). A subset of the microorganisms in the rhizosphere also penetrates the plant tissues and colonizes the endosphere depending on the plant's innate immune system (Bulgarelli et al., 2013). Hence, plants have distinct microbial communities depending on their rhizo-compartments; additionally, the composition and structure of the microbial communities affect plant phenotypes such as flowering time, disease suppression, and drought tolerance (Panke-Buisse et al., 2015; Wei et al., 2019; Zolla et al., 2013). The manipulation of plant rhizosphere microbiome can improve plant performances (Toju et al., 2018).

PGPB are beneficial microorganisms that colonize the rhizosphere and endosphere. These bacteria increase plant nutrient acquisition, suppress plant pathogens, and alleviate biotic and abiotic stress damage (Backer et al., 2018; Glick, 2012). PGPB modulate environmental stresses in plants by reducing ethylene levels using 1-aminocyclopropane-1-carboxylate deaminase and producing indole-3-acetic acid, cytokinin, and volatile organic compounds. PGPB also reduce the reactive oxygen species levels using antioxidants and accumulate osmolytes in plant cells (Glick, 2012; Yang et al., 2009). Yang et al. (2009) proposed the term induced systemic tolerance (IST) for PGPB-induced physical and chemical changes that enhance plant tolerance to abiotic stresses. The genera *Azospirillum*, *Azotobacter*, *Bacillus*, *Burkholderia*, *Pseudomonas*, *Streptomyces*, and *Rhizobia* are well-known PGPB that have been commercialized for agricultural management (Glick, 2012).

Microbial inoculation can influence the composition and structure of microbial communities in the rhizosphere and endosphere, as observed when *Bacillus velezensis* NJNU-Z9 was inoculated into pepper, enhancing bacterial diversity in the rhizosphere (Trabelsi and Mhamdi, 2013; Zhang et al., 2019b). Treatments with bio-organic fertilizers, which are the mixtures of organic matter and PGPB, alter microbial communities in the rhizosphere, thereby suppressing plant diseases and improving the soil chemical properties including organic carbon, nitrogen, and potassium (Liu et al., 2018; Tao et al., 2020; Wang et al., 2015; Xue et al., 2015; Zhang et al., 2019a). Three different *Bacillus* species (*B. cereus*, *B. subtilis*, and *B. amyloliquefaciens*) modulate bacterial diversity, evenness, and composition of the bacterial community in the root endosphere (Gadhave et al., 2018).

Previous studies have revealed that *Bacillus mesonae* strain H20-5, isolated from soil-cultivated lettuce, enhanced tolerance to salinity stress in tomato plants (Sawant et al., 2019; Yoo et al., 2019b). The plants treated with H20-5 have exhibited significantly decreased electrolyte leakage and accumulated proline, abscisic acid (ABA), and antioxidant enzyme activities compared to untreated plants (Yoo et al., 2019b). Strain H20-5 has also upregulated *9-cise-poxycarotenoid dioxygenase 1* (*NCED1*) and *abscisic acid-response element-binding protein 1* (*AREB1*), which are involved in ABA biosynthesis and signaling, respectively (Yoo et al., 2019b). Field tests revealed that strain H20-5 improves fruit productivity and quality of tomato, strawberry, and cucumber (Yoo et al., 2019a). Whole genome analysis revealed that H20-5 possesses putative functional genes involved in biofilm formation and in the biosynthesis of auxin, proline, and spermidine (Sawant et al., 2019).

This study identified the effects of administering *Bacillus mesonae* strain H20-5 on bacterial communities in the bulk and rhizosphere soils of tomato (*Solanum lycopersicum* L.) plants grown under different soil salinity levels. To analyze bacterial communities, we conducted Illumina MiSeq sequencing of 16S rRNA genes and examined bacterial species richness and diversity, bacterial community structure, enriched taxa, and microbial network topology using bioinformatics tools.

Materials and Methods

Plant growth conditions and treatment of bacterial inoculant. The soil used in this study was collected from an experimental vegetable field at the National Institute of Agricultural Sciences (35°49'33.08"N, 127°2'38.82"E). The soil was air dried in the shade for a week and passed through a 2-mm sieve. The soil EC value was 0.4 dS/m. To establish soils with different salinity levels, we adjusted the soil EC values to 2, 4, and 6 dS/m by supplementing diluted −1,000 kPa salinity solutions (Polonenko et al., 1986) at ratios of 2.4:7.6, 5.5:4.5, and 8.6:1.4 (−1,000 kPa salinity solution:water), respectively. Tomato (*Solanum lycopersicum* "Juiken") seeds were germinated on wet filter papers for 3 days and sown in plastic pots (5 cm in diameter) filled with 50 g of dried soil. Diluted saline solutions (10 ml) were added. To maintain the initial soil moisture levels, the pots were watered daily according to their respective weights.

Bacillus mesonae strain H20-5 (Yoo et al., 2019b) was cultured in R2A broth at 28°C and 150 rpm for 2-3 days. After centrifugation at 8,000 rpm for 10 min, the supernatants were removed, and the cells were resuspended using 10 mM $MgSO_4$ such that OD_{600} = 0.25 (>10^8 cfu/ml). A week after sowing the tomato seeds in saline soils, 5 ml of the *B. mesonae* H20-5 suspension was added. $MgSO_4$ (10

mM) was used as the untreated control.

Soil pH and EC values were measured using a pH meter (CyberScan pH1500, EUTECH, Singapore) and an EC meter (D-54, Horiba, Kyoto, Japan), respectively, after shaking the soil:water (1:5 w/v) mixture for 30 min at 200 rpm.

Fresh weight measurement and rhizosphere sample preparation. After two weeks of bacterial inoculum treatment, we measured the fresh weight of tomato seedlings and collected bulk and rhizosphere soil samples. Loose soil without tomato roots was carefully collected as the bulk soil sample. Subsequently, the tomato roots were vigorously shaken by hand to remove adherent soil particles. The roots with firmly attached soil were placed into 50-ml tubes containing 0.85% NaCl and shaken vigorously using a shaker (CUTE MIXER CM-1000, EYELA, Tokyo, Japan) for 30 min. The roots, which contained soil and saline water, were removed from the tubes and were centrifuged at 8,000 rpm for 15 min. The supernatants were removed, and the remaining soil samples were used as the rhizosphere samples.

DNA extraction and quantitative PCR analysis. Bulk and rhizosphere soil samples (0.5 g each) were used in triplicates for DNA extraction. The Power Soil DNA Isolation Kit (MoBio, Carlsbad, CA, USA) was used for extraction according to the manufacturer's instructions, and the samples were subsequently pooled. Prior to DNA extraction, soil samples derived from individual tomato plants were prepared separately and pooled together to minimize variation. The extracted DNA was quantified using the Qubit dsDNA BR Assay Kit (Invitrogen, Carlsbad, CA, USA). The abundance of total bacteria and strain H20-5 was quantified using real-time PCR (Bio-Rad CFX96 System, Hercules, CA, USA) using the primer pair 16S 338F (5′-ACTCCTACGGGAGGCAG-3′)/518R (5′-WTTACCGCGGCTGCTGG-3′) and InsK1F (5′-ACTTGCCCACGGTA TGAACG-3′)/InsK1R (5′-GATCTAGTAAAGGTCGCTGGTATTG-3′), respectively.

Bacterial 16S rRNA gene amplicon sequencing. To generate bacterial libraries, universal 16S rRNA gene primers (799F: 5′-AACMGGATTAGATACCCKG-3′ and 1193R: 5′-ACGTCATCCCCACCTTCC-3′) were used for PCR amplification. These target-specific primers were attached to the Nextera consensus and adaptor sequences with the forward (5′-TCGRCGGCAGCGTC-AGATGTGTATAAGAGACAG-target sequence-3′) and reverse (5′-GTCTCGTGGGCTCGG-AGATGTGTATAAGAGACAG-target sequence-3′) primers for the first round of PCR amplification. The PCR conditions were as follows: initial denaturation at 94°C for 3 min, 25 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, and final extension at 72°C for 5 min. Following initial amplification of the bacterial target sequences, the library size was verified via agarose gel electrophoresis, and the PCR products were cleaned using Agencourt AMPure XP (Beckman Coulter, Inc., Miami, FL, USA). A second round of PCR amplification was conducted with primers containing Illumina dual indices and sequencing adapters, namely, S502F (5′-AATGATACGGCGACCACCGAGATCTACAC-55555555-TCGTCGGCAGCGTC-3′) and N701R (5′-CAAGCAGAAGACGGCATACGAGAT-77777777-AGTCTCGTGGGCTCGG-3′), under the same conditions as those used for the first round of PCR. The PCR products were cleaned using Agencourt AMPure XP (Beckman Coulter, Inc.) and quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen). Purified amplicon libraries were pooled at equimolar concentrations and sequenced with an Illumina MiSeq system using the MiSeq Reagent Kit v3 (Illumina Inc., San Diego, CA, USA). Bacterial DNA samples were sequenced at the National Instrumentation Center for Environmental Management (NICEM; Republic of Korea). Sequence data are available in the GenBank SRA database under the BioProject accession number PRJNA674044.

Sequence data processing. The sequences obtained from the MiSeq platform were processed using the UPARSE pipeline (ver. 9.1.13_i86linux64, http://www.drive5.com/usearch) (Edgar, 2013). The paired-end reads were merged when the number and ratio of mismatches in the overlap region were <10 bp and 10%, respectively. Low-quality reads that were above the expected error threshold (>1) and short reads (<300 bp) were removed. To minimize the impact of sequencing artifacts, singletons were removed from the datasets. Chimeric sequences were removed using the UCHIME *de novo* algorithm. The remaining high-quality sequences were clustered into operational taxonomic units (OTUs) with 97% identity using the UPARSE algorithm. Representative sequences of bacterial and archaeal OTUs were classified using the naïve Bayesian classifier (Wang et al., 2007) based on the Ribosomal Database Project (RDP) database (Cole et al., 2014) with a 60% confidence threshold. OTUs affiliated with chloroplasts and non-bacterial cells were subsequently removed from the bacterial OTU table. To assess alpha-diversity indices, sequence reads of bulk soil and rhizosphere samples were rarefied to 14,000 reads, and six indices including coverage, number

of OTUs, Chao1, ACE, Shannon, and Inverse Simpson were subsequently calculated using Mothur (version 1.29.1, http://www.mothur.org) (Schloss et al., 2009).

Data analyses. Statistical analyses in this study were performed using R 3.3.1 (R Development Core Team, 2014). The OTU abundances in the dataset were normalized using Hellinger transformation (Legendre and Gallagher, 2001) using the "*decostand"* function of the *vegan* package (Oksanen et al., 2013) in R. Subsequently, non-metric multidimensional scaling (NMDS) analysis was performed based on the Bray-Curtis dissimilarity matrix using the "*metaMDS*" function of the *vegan* package. The differences in community structure between rhizo-compartments, soil EC levels, and H20-5 treatment were tested by ANOSIM (Clarke, 1993) using the "*anosim*" function and permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) using the "*adonis*" function. These calculations were based on the Bray-Curtis dissimilarity matrix, which was calculated using the "*vegdist*" function within the *vegan* package. To identify the OTUs that were specifically abundant due to H20-5 treatment, indicator species analysis was conducted using the "*multipatt*" function with the "*r.g*" option in the *indispecies* package (De Cáceres and Legendre, 2009), and OTUs with high indicator values (>0.5) and high abundances (>0.1% relative abundance) were screened from the results.

Network analysis was conducted using the Molecular Ecological Network Analyses pipeline based on random matrix theory (RMT) at the University of Oklahoma's Institute for Environmental Genomics web server (http://ieg2.ou.edu/MENA/). The details of the process are provided by Shi et al. (2016). The input datasets were separated using H20-5 treatment and non-treatment. For network construction, the following options were used: OTUs detected in all nine samples were used, 0.01 was filled in the blanks with paired valid values, logarithm values were obtained, Pearson correlation coefficient was used for correlation analysis, and calculation was made by decreasing the cut-off from the top using only Poisson regression. A similarity threshold was selected automatically using the RMT-based approach to define the adjacency matrix. The topological descriptors were measured using R package *QuACN* (Mueller et al., 2011). The modularity of the network was calculated using the greedy modularity optimization method. Subsequently, the within-module connectivity (Zi) and among-module connectivity (Pi) of each node were examined, and topological roles were classified based on the values of Zi (2.5) and Pi (0.62).

Results

Strain H20-5 promotes tomato growth under salinity stress conditions. To establish soils with different salinity levels, we sowed germinated tomato seeds in air-dried soils and drenched the soils in saline solutions of different concentrations. We confirmed that the soils maintained the EC levels at 2, 4, and 6 ds/m, respectively, and soil pH were between 6.0-6.5 (Supplementary Fig. 1). We treated the inoculant of strain H20-5 7 days after sowing germinated tomato seeds. The fresh weight of the tomato plants was measured 14 days after H20-5 treatment. Consistent with previous results (Yoo et al., 2019a), we confirmed that tomato growth was higher in the H20-5 treatment group than in the controls at different soil salinity levels (Supplementary Fig. 2).

To examine the sustained abundance of strain H20-5 in soils after H20-5 treatment, the bulk and rhizosphere soils were separately collected at 0, 2, 4, 7, and 14 days after treatment, and the copy numbers of strain H20-5 were measured via real-time quantitative reverse transcription PCR with H20-5 specific primers. On the day of the H20-5 treatment, the copy number of strain H20-5 was high in the bulk soil when compared to the control, at EC values of 2, 4, and 6 ds/m (Supplementary Fig. 3). However, the abundance of H20-5 rapidly decreased and became insignificant at 14 days after treatment. The abundance of total bacteria quantified by the 16S rRNA gene was not significantly different between the H20-5 treatment and control (Supplementary Fig. 3).

Effect of strain H20-5 on bacterial richness and diversity. To investigate the effects on bacterial community due to the strain H20-5 treatment, bulk and rhizosphere soil samples at 14 days after H20-5 treatment were used for Illumina MiSeq sequencing using the amplicon library of the V5-7 region of the 16S rRNA gene. From a total of 36 samples (3 salinity levels × 2 treatments × 2 compartments × 3 replicates), 2,011,070 high-quality sequences were generated and clustered into 2,688 OTUs. For alpha-diversity analysis, the number of sequences was rarefied to 14,000 reads, which is the minimum number of reads among samples. The sequence depth was saturated at more than 97% coverage.

In the bulk soils, bacterial richness and diversity, represented by the Chao1 richness estimate and Shannon's diversity index, were significantly reduced as soil EC levels increased, regardless of H20-5 treatment (Fig. 1A and C). In the rhizosphere soils, Chao1 values were not signifi-

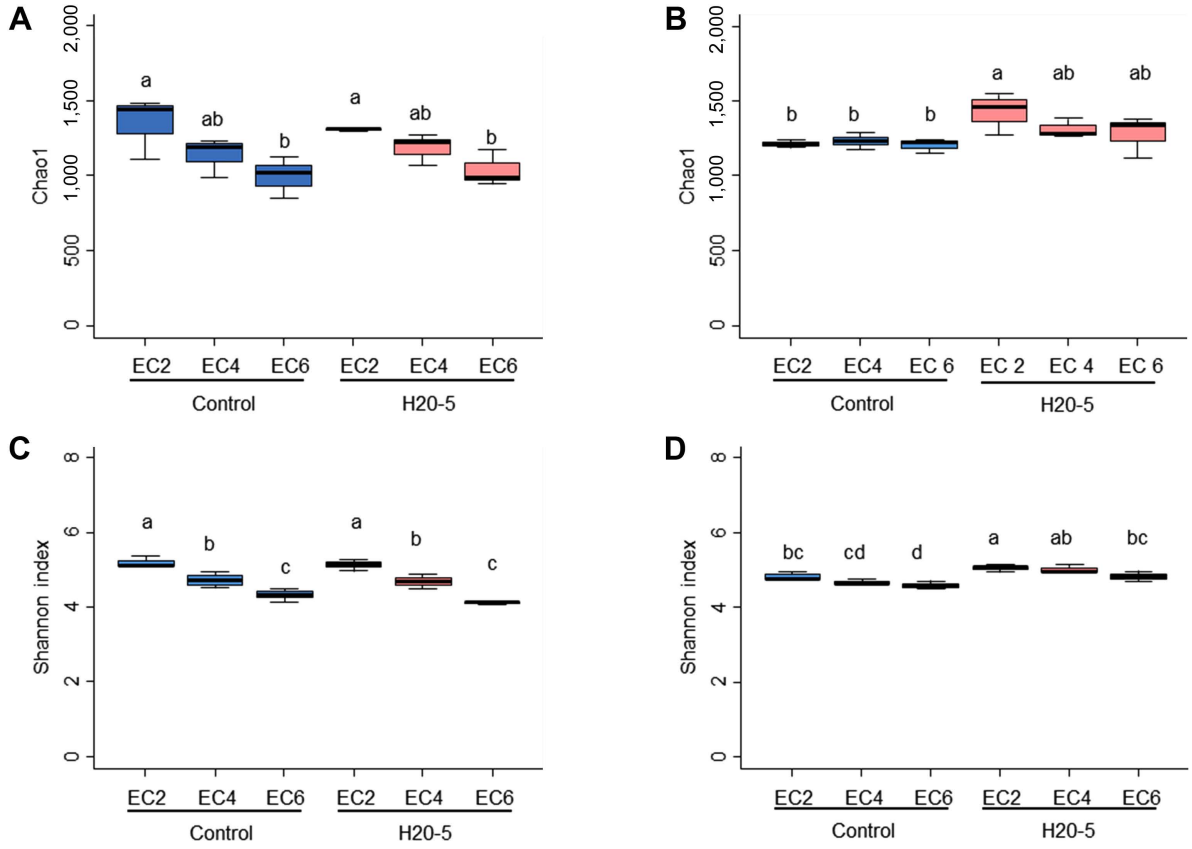

Fig. 1. Box plots showing the bacterial richness and diversity in bulk (A, C) and rhizosphere (B, D) soils 14 days after the *Bacillus mesonae* H20-5 treatment. Different letters indicate a significance by the least significant difference test at $P < 0.05$.

cantly affected when soil EC levels were increased, and the EC value of 2 ds/m of soil treated with H20-5 was higher than that in the control (Fig. 1B). Although the patterns of the Shannon index changes were slightly decreased by increasing soil EC levels, overall values of Shannon index were higher in the H20-5 treated rhizosphere soil than in the control (Fig. 1D).

Effect of strain H20-5 on bacterial community assemblages. The bacterial community assemblages represented by NMDS biplots based on the Bray-Curtis dissimilarity were differentiated by rhizo-compartments (bulk and rhi-

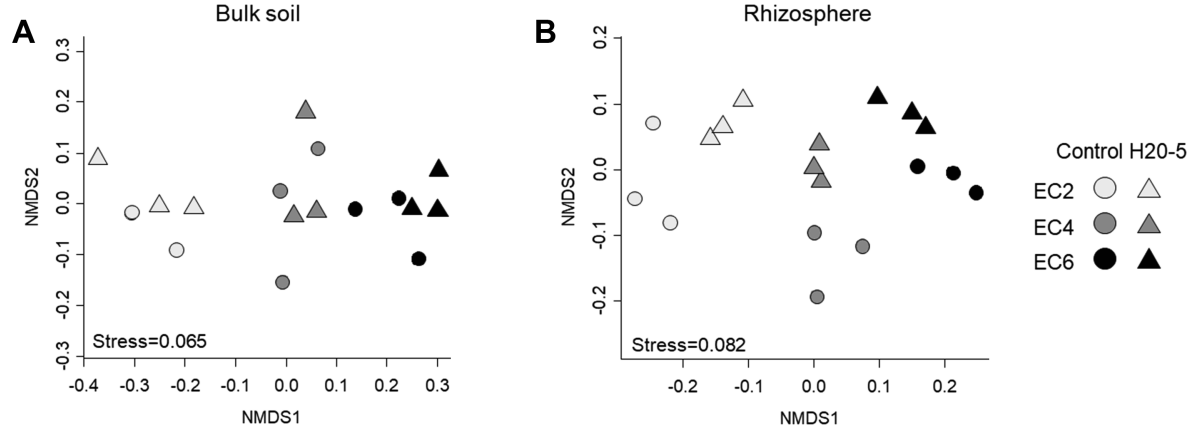

Fig. 2. Non-metric multidimensional scaling (NMDS) ordinations of bacterial community composition in the bulk (A) and rhizosphere (B) soils with and without *Bacillus mesonae* H20-5 treatment under different soil salinity levels.

zosphere soil**s**) along the y-axis (Supplementary Fig. 4). The bacterial community structures were clearly separated by EC levels along the x-axis in both the bulk and rhizosphere soils. The ordination results were further supported by a non-parametric analysis of similarities (ANOSIM) and PERMANOVA based on the Bray-Curtis dissimilarity (Supplementary Table 1). ANOSIM ($R = 0.6278$, $P < 0.001$) and PERMANOVA ($R^2 = 0.3073$, $P < 0.001$) revealed a significant separation of the bacterial communities by soil EC levels. The ANOSIM ($R = 0.4294$, $P < 0.001$) and PERMANOVA ($R^2 = 0.1523$, $P < 0.001$) results also demonstrated that the bacterial communities were significantly different in each rhizo-compartment.

To compare the shift in bacterial community upon H20-5 treatment in the bulk and rhizosphere soils, NMDS biplots were analyzed separately by rhizo-compartments (Fig. 2). Bacterial communities in the bulk and rhizosphere soils were clustered according to different soil EC levels. Intriguingly, the composition of bacterial communities in the H20-5 treated rhizosphere was similar regardless of the EC values, compared to the control. This suggests that strain H20-5 affects bacterial communities and is more stable toward the changes in salinity levels.

Effect of strain H20-5 on bacterial taxonomic distribution. Because the bacterial communities in the rhizosphere soils were more affected by H20-5 than those in the bulk soils, we focused on the composition of bacterial communities in the rhizosphere samples. The most dominant phylum in the rhizosphere soils was *Proteobacteria*, followed by *Actinobacteria, Acidobacteria*, and *Bacteroidetes* (Fig. 3). The relative abundances of *Alphaproteobacteria*, *Acidobacteria*, and *Gemmatimonadetes* decreased with increasing soil EC levels in the rhizosphere soils of the control, whereas those of *Gammaproteobacteria* and *Firmicutes* gradually increased. The decreasing patterns of *Alphaproteobacteria*, *Acidobacteria*, and *Gemmatimonadetes* were diminished in the H20-5 treated rhizosphere soils.

A species indicator analysis was performed to identify OTUs enriched upon H20-5 treatments. Dominant OTUs (>0.1% average relative abundance) with high indicator values (>0.5) are listed in Supplementary Table 2. Only three OTUs exhibited higher relative abundances in the non-treated rhizosphere soils than in the H20-5 treatments. The relative abundances of 19 OTUs were higher in the H20-5 treated rhizosphere soils than in the control. Among the indicator OTUs, the relative abundance of OTU62 was increased upon H20-5 treatment, independent of the soil EC levels (Fig. 4). The relative abundances of OTU164, OTU131, OTU79, OTU119, and OTU436 were similar at an EC of 2 ds/m and gradually increased with soil EC levels in the rhizosphere upon H20-5 treatment. The relative abundance patterns of OTU81, OTU70, OTU90, OTU66, and OTU12 in the H20-5 treated rhizosphere soils were relatively stable over the different soil EC levels, whereas those in non-treated rhizosphere soils decreased with

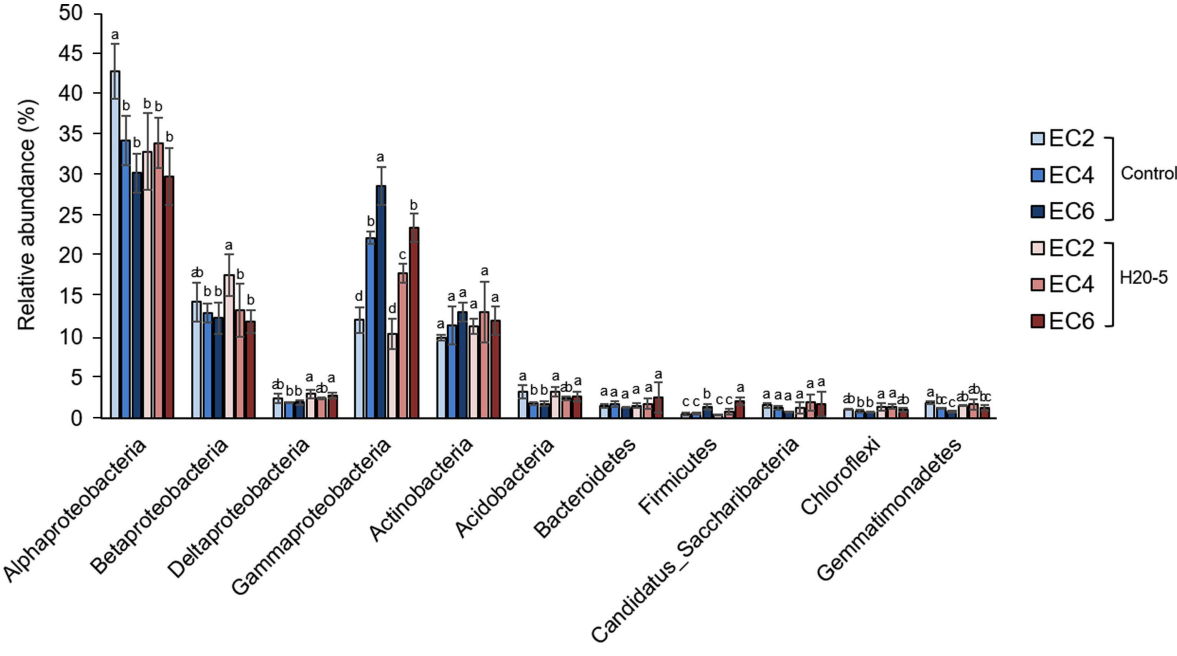

Fig. 3. Effect of *Bacillus mesonae* H20-5 on bacterial taxonomic distribution at phylum level in rhizospheres with different soil salinity levels. Error bars indicate standard error. Different letters indicate a significance by the least significant difference test at $P < 0.05$.

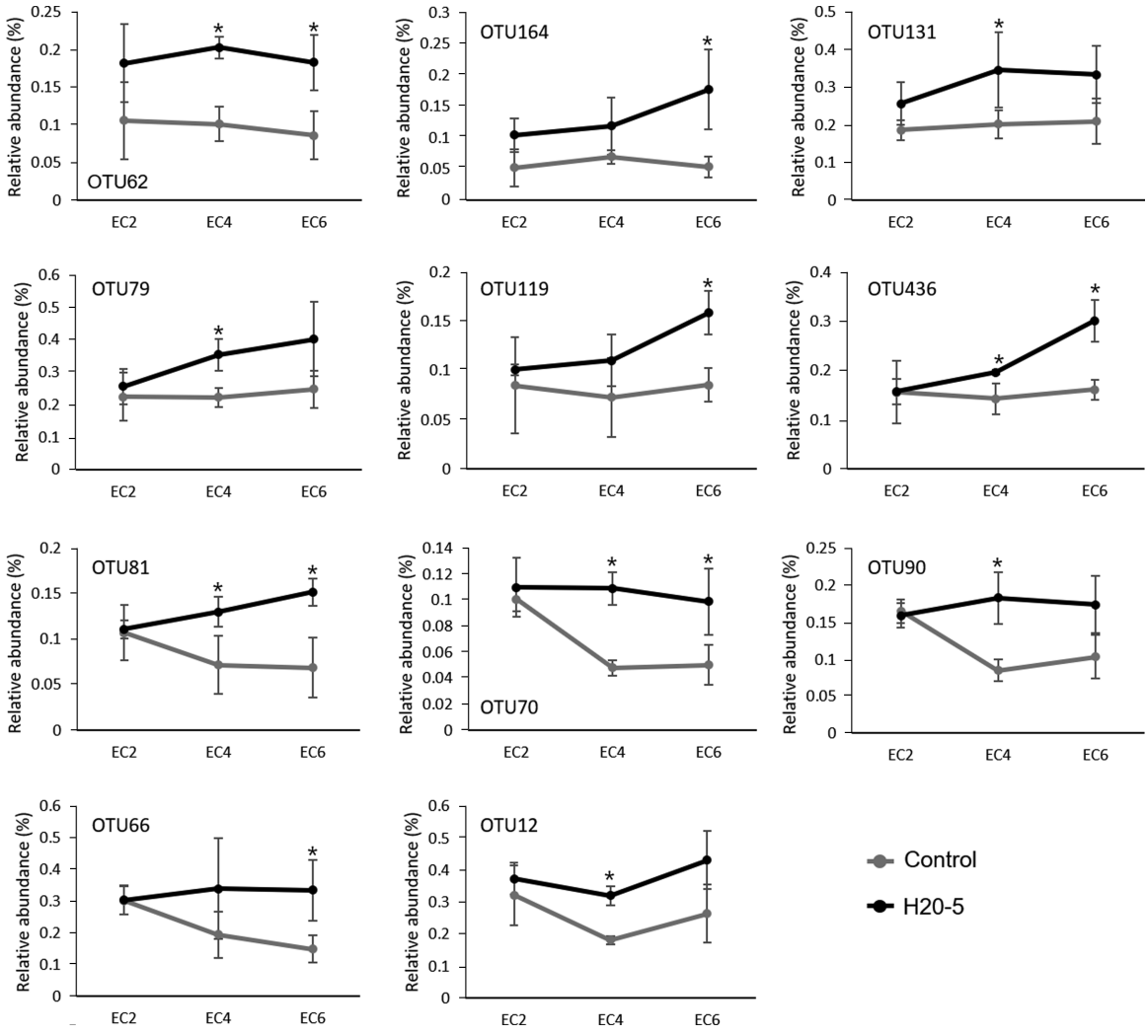

Fig. 4. Relative abundance of bacterial operational taxonomic units (OTUs) at different soil salinity levels. The asterisks indicate significant differences between the treatment and control determined by Student's t-test (*$P < 0.05$). Error bars indicate standard error.

increasing soil EC levels. Among these OTUs enriched by H20-5 treatment, eight OTUs were affiliated with *Actinobacteria* and four were *Alphaproteobacteria*. In the phylum *Actinobacteria*, several putative genera including *Kineosporia*, *Virgisporangium*, *Actinoplanes*, *Gaiella*, *Blastococcus*, and *Solirubrobacter* were enriched by H20-5 treatment. In the phylum *Alphaproteobacteria*, the genera *Pseudolabrys*, *Bradyrhizobium*, and *Hyphomicrobium* were enriched.

Effect of strain H20-5 on bacterial network. To understand the effect of strain H20-5 on bacterial interactions in the rhizosphere with different soil EC levels, co-occurrence network analysis was performed using an RMT-based network analysis. To compare the network structure, the input data were categorized as H20-5 treatment and control, and core OTUs that were present in all nine samples (three EC levels × three replicates) were used for network construction (Supplementary Fig. 5). The total number of the core OTUs was 702 and 431 in the H20-5 treated rhizosphere and control, respectively. The network connectivity in all compartments was well fitted by the power law, with an R^2 value > 0.7, indicating scale-free properties. The numbers of nodes and modules, which were associated with the microbial co-occurrence network, were higher in the H20-5 treated rhizosphere soils. Although the average degree (avgK), which is the average number of connectors per node, was lower than that of the control (Table 1), the values of topological descriptors representing structural complexity of the network were higher in the H20-5 treated rhizosphere soils than in the control (Supplementary Table 3). This finding suggests that microbial communities in the

Table 1. Topological properties of the networks of bacterial communities in the rhizosphere soils with and without *B. mesonae* H20-5 treatment

Network index	Control	H20-5
No. of core OTUs	431	702
Total nodes	307	492
Total links	1346	1839
R² of power-law	0.75	0.856
Average degree (avgK)	8.769	7.476
Average path distance (GD)	3.949	4.316
Average clustering coefficient (avgCC)	0.278	0.185
Modularity	0.402	0.487
No. of modules	32	44
No. of module hubs	4	6
No. of connectors	11	22

OUT, operational taxonomic units.

H20-5 treated rhizosphere soils formed complex networks than those in the control soil.

The treatment with strain H20-5 also affected the topological roles of the taxa in the network. The topological roles of the taxa in the network were classified into following four categories based on the values of within-module connectivity (Zi) and among-module connectivity (Pi): peripherals, few interactions with other nodes ($Zi < 2.5$ and $Pi < 0.62$); connectors, many links with other modules ($Zi < 2.5$ and $Pi > 0.62$); module hubs, many interactions within the module ($Zi > 2.5$ and $Pi < 0.62$); and network hubs, many interactions within and among modules ($Zi > 2.5$ and $Pi > 0.62$) (Supplementary Fig. 6). We identified six module hubs and 22 connectors in the bacterial network of H20-5 treated rhizosphere soils; however, the bacterial network of the control included four module hubs and 11 connectors (Supplementary Fig. 6). The higher numbers of module hubs and connectors in the bacterial network of H20-5 treatments suggested that more members in the soils were associated with the bacterial interactions induced by H20-5 treatment, although the average connectivity was reduced.

Discussion

Previous studies have revealed that *Bacillus mesonae* strain H20-5 promotes tomato growth under different salinity conditions, with soil EC levels of 2, 4, and 6 ds/m. Although the copy numbers of H20-5 measured via qRT-PCR were rapidly decreased in the bulk and rhizosphere soils, a shift in the bacterial community was observed upon H20-5 treatment.

Bacterial richness and diversity, represented by the Chao1 and Shannon index values, were significantly reduced with increasing EC levels in the bulk and rhizosphere soils; however, they were not decreased in the H20-5 treated rhizosphere soils when compared to the control. The number of the core OTUs, which were detected in all the rhizosphere soil samples at different salinity levels, was also higher in the H20-5 treated rhizosphere soils. This is consistent with previous reports that bacterial species diversity in the rhizosphere and root endosphere was increased upon treatment with PGPRs belonging to the genus *Bacillus* (Gadhave et al., 2018; Zhang et al., 2019b). Considering that higher bacterial diversity was positively correlated with plant growth promotion (Garbeva et al., 2004; Liu et al., 2018), the bacterial community harboring more diverse bacterial members in the H20-5 treated rhizosphere enhanced plant growth in tomato.

NMDS analyses revealed that soil EC, which represents salinity, and rhizo-compartments, differentiated as bulk and rhizosphere soils, were the major factors shaping the microbial community structure. Soil EC is a critical factor not only for plant growth, but also for microbial community assembly (Casamayor et al., 2002; Kim et al., 2016; Min et al., 2016). Microbial community analysis with diverse soils across continents that have variations in edaphic factors, such as pH, EC, organic matter, and nitrogen, revealed that microbial community structure was highly correlated with pH and EC (Lee et al., 2019). Because the soil pH was unchanged in this study, a gradual shift was observed in the bacterial community according to soil EC levels. The diversity and composition of the microbial community in the rhizosphere were distinguished from those in bulk soil. The rhizosphere is a nutrient-rich region due to root exudation and rhizodeposition. The microorganisms that attract these compounds reshape their communities in the rhizosphere (Bulgarelli et al., 2013). A microbial community study of field soil cultivated with tomato plants also revealed that the microbial community structures were clearly differentiated by rhizo-compartments, regardless of soil type (Lee et al., 2019).

We observed that the effect of H20-5 treatment on the diversity and structure of the bacterial community was greater in the rhizosphere soil than in the bulk soil. Considering that the density of H20-5 in the bulk and rhizosphere soils was extremely low, strain H20-5 might have indirectly affected the bacterial communities through the tomato plants. The priming activity of the tomato plants exposed to H20-5 conferred tolerance to salinity stress, promoting the growth, quantity, and quality of the fruits (Yoo et al., 2019a, 2019b). The tomato plants IST upon H20-5 treat-

ment, which changed its physiology. The tomato plants treated with H20-5 exhibited increased levels of Ca^{2+}, proline, and ABA and enhanced antioxidant enzyme activities, including catalase, superoxide dismutase, and glutathione peroxidase activities compared to the controls (Yoo et al., 2019b). These physiological changes could lead to differences in the composition of root exudates between tomato plants with and without H20-5 treatment, which affects the bacterial community. There were variations in the bacterial communities associated with different *Arabidopsis* mutants related to biosynthesis and signaling pathways of plant hormones, including ABA and jasmonic acid (Carvalhais et al., 2013; Lebeis et al., 2015; Liu et al., 2017). The microbial communities in the rhizosphere differ according to crop species and plant development, owing to the varying compositions of root exudates (Chaparro et al., 2014; Edwards et al., 2015, 2018). Greater diversity and richness of bacterial communities in the H20-5 treated rhizosphere soil indicated that the H20-5 treated tomato plants recruited more bacterial members around the soil.

The relative abundances of *Alphaproteobacteria*, *Acidobacteria,* and *Gemmatimonadetes* were gradually decreased depending on soil EC levels in the non-treated rhizosphere soils, and they were relatively stable in the H20-5 treated rhizosphere soils. NMDS analysis revealed that the variation in the structures of bacterial communities with increasing soil EC was relatively less in the H20-5 treated rhizosphere soils compared to the control. These results suggest that the H20-5 treated bacterial communities were relatively stable compared to the control. Microbial communities rapidly respond to environmental changes; however, the bacterial communities in the H20-5 treated rhizosphere soils minimized the environmental effect by conferring tolerance to salinity stress on tomato plants.

We identified enriched OTUs belonging to *Steroidobacter*, *Kineosporia*, *Virgisporangium*, *Nitrospira*, *Pseudolabrys*, *Gaiella*, *Bradyrhizobium*, *Hyphomicrobium*, *Blastococcus*, and *Solirubrobacte* upon H20-5 treatment under salinity stress conditions. The relative abundance of *Steroidobacter*, *Gaiella*, and *Pseudolabrys* was significantly altered by increasing salinity in the rhizosphere soil of Jerusalem artichoke (Yang et al., 2016). *Bradyrhizobium* is well-known for nodulation and nitrogen fixation in leguminous plants, and it promotes the growth of non-leguminous plants (Antoun et al., 1998). *Bacillus* spp. improved plant growth by recruiting other bacterial members from the soil. For example, *Bacillus amyloliquefaciens* enhanced soybean growth and nodulation by recruiting the natural symbiont *Bradyrhizobium japonicum* (Masciarelli et al., 2014).

Bacterial co-occurrence networks in the rhizosphere with different soil EC levels indicated that the structural complexity of the network quantified by topological descriptors was higher in the H20-5 treatment group than in the control despite the lower average degree. A relatively large number of nodes with low degree in the H20-5 treatment group networks would result in an overall low average degree. Despite this low value, more bacterial members were associated in the bacterial network of the H20-5 treated rhizosphere soils than in the control. The number of modules, which are the groups of nodes that are highly connected to each other, and keystone taxa representing module hubs and connectors, which play potential roles in the network structure, were relatively higher in the H20-5 treated rhizosphere soils than in the control. H20-5 likely has an impact on the dispersion of ecological functional processes into various modules. Efficient communication among different members of microbial communities can effect rapid responses to environmental changes (Deng et al., 2012). Modularity is an important concept in ecology for system stability and resilience (Olesen et al., 2007). Overall, the bacterial community affected by strain H20-5 might be relatively stable to salinity changes.

In summary, *B. mesonae* H20-5 IST of tomato plants under salinity stress, which affected the structure and composition of the soil bacterial community. The bacterial species richness and diversity in the rhizosphere were increased upon H20-5 treatment. There were no abrupt changes in the composition of bacterial communities at different salinity levels in the H20-5 treated rhizosphere soils compared to control. More bacterial species and more modules of the bacterial network were identified in the H20-5 treated rhizosphere soil compared to the control. These findings demonstrate the effects of introducing PGPB on bacterial communities under different salinity conditions. The results suggest that not only plant physiology, but also the diversity and structure of the microbial community, should be considered to improve sustainable crop productivity under changing environmental conditions.

Conflicts of Interest

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

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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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