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ORIGINAL ARTICLE

Effect of fairy ring bacteria on the growth of *Tricholoma matsutake* in vitro culture

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Abstract

Tricholoma matsutake (pine mushroom) (Basidiomycota, Agaricales) is a valuable edible fungal species that cannot be cultivated artificially. As an ectomycorrhizal fungus, *T. matsutake* interacts with trees belonging to the Pinaceae and Fagaceae, and forms fairy rings around host trees that are arc-shaped areas with dense hyphae of *T. matsutake* in the soil. Because the fairy rings maintain their dense hyphae for several years and form fruiting bodies, the characteristics of the fairy ring may be important in understanding the ecology of *T. matsutake*. Recent studies have shown that diverse bacteria co-exist in the fairy ring, and suggest that the fairy ring bacteria may influence on the growth of *T. matsutake*. However, the effect of the fairy ring bacteria on the growth of *T. matsutake* is largely unknown. In this study, we isolated fairy ring bacteria and investigated their effect on the growth of *T. matsutake* in co-culture experiments. In addition, the relationship between bacterial effects and nutrient conditions was tested using different media with varying glucose concentrations. A total of 237 bacteria (28 species) were isolated from fairy rings of four different *T. matsutake* producing areas: Proteobacteria (17 species), Firmicutes (7 species), and Actinobacteria (4 species). Burkholderiaceae (*Burkholderia* and *Paraburkholderia*) was most abundant in the fairy ring bacteria communities. Most bacteria showed a negative effect on the growth of *T. matsutake* when it grew on glucose rich medium (20 g/L). In glucose deficient medium (2 g/L), however, some bacteria promoted the growth of *T. matsutake*. In addition, the mode of interaction between bacteria and *T. matsutake* is different, depending on the glucose concentration.

Keywords Pine mushroom · Growth promoting bacteria · *Paenibacillus* · *Staphylococcus* · Glucose

Introduction

In many forest soils, bacteria readily co-exist with fungi and some of these receive nutrients from fungal hyphae by degrading them (Leveau and Preston 2008) or from a flow of plant photosynthetic products (Warmink et al. 2009). Some forest soil bacteria, mycorrhiza helper bacteria (MHB) in particular, promote the development of ectomycorrhizal fungi (Frey-Klett et al. 2007). MHB can facilitate mycorrhiza formation, promote growth and even serve as biocontrol agents to inhibit pathogens and competitors. These kinds of bacteria can be used as bio-fertilizer to facilitate cultivation of mushroom forming fungus and enhance its productivity (Kim et al. 2008; Young et al. 2013; Zarenejad et al. 2012). However, bacteria who co-exist with an ectomycorrhizal fungus do not always mean that they have a consistently positive interaction with the ectomycorrhizal fungus (Varese et al. 1996). In addition, the mode of the relationship (positive vs. negative) may be not fixed, but is changeable depending on soil factors such as carbon availability (Brulé et al. 2001; Deveau et al. 2016; Duponnois 1992; Garbaye 1994). For example, *Pseudomonas fluorescens* BBc6R8, a MHB of the ectomycorrhizal fungus *Laccaria bicolor*, showed an effect on the growth of *L. bicolor* depending on the nutritional condition of the growing medium (Brulé et al. 2001). If MHB are used as bio-fertilizer, therefore, effects of MHB need to be tested in various nutritional conditions for identifying conditions that its effects maintain.

Tricholoma matsutake (Pine mushroom) is an ectomycorrhizal fungus associated with tree species in the Pinaceae and Fagaceae (Matsushita et al. 2005). The unique

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taste and pine-like aroma of fruiting bodies of *T. matsutake* make it one of the most precious gastronomic mushrooms in Asia (Wang et al. 1997). Despite many attempts, artificial cultivation of the fruiting bodies has not yet been achieved (Yamada et al. 2006). Fruiting bodies of *T. matsutake* form in an arc-shaped area around host trees called "fairy ring" (Wang et al. 1997). A high density of *T. matsutake* hyphae, which aggregate with soil within the fairy ring (Ogawa 1975), is maintained for several years, expanding at a rate of 10–15 cm per year (Yamaguchi et al. 2016). It is thought that better understanding of the maintenance of dense hyphae of *T. matsutake* within fairy ring will ultimately solve the mystery on fruiting body formation of *T. matsutake* which, to date, can only happen in nature.

Bacterial diversity associated with fairy rings of *T. matsutake* has been relatively well-studied, while their effect on the growth of *T. matsutake* has not. For example, culture-dependent studies have shown that several species of bacteria live within fairy rings of *T. matsutake* (Jiang et al. 2015; Kataoka et al. 2012; Kim and Whang 2007) while their abundance may be low (Ohara and Hamada 1967). Moreover, culture-independent methods (e.g., DGGE or next generation sequencing) illuminated distinctive bacterial communities in fairy rings of *T. matsutake* compared to it in adjacent bulk soil (Kim et al. 2014; Oh et al. 2016; Vaario et al. 2011), which suggests that *T. matsutake* may also have helper bacteria for growth and maintenance of hyphae in the soil.

The objectives of this study were to isolate and identify fairy ring bacteria and to investigate their influence on the growth of *T. matsutake* hyphae. Fairy ring bacteria were co-cultured with *T. matsutake* isolate to determine their effects. In addition, we tested whether their relationship is fixed or changeable depend on the nutrient condition by using media varying in glucose concentration.

Materials and methods

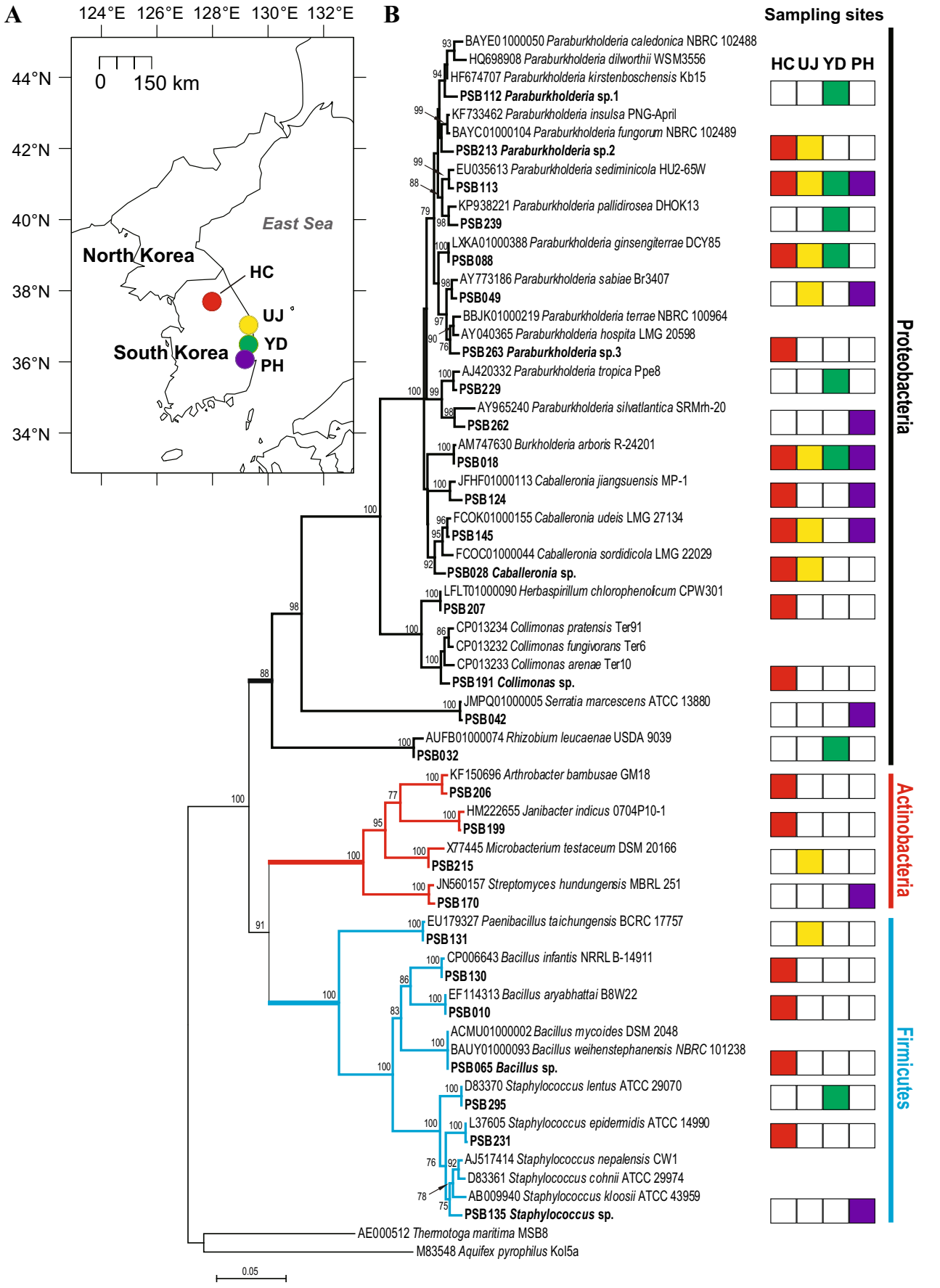
Sampling and isolation of bacteria

In September and October, 2013, soil samples were collected from within three fairy rings (Fig. S1) at each of four sampling sites with permission: Hongcheon (N 37° 41′ 35″, E 127° 58′ 51″), Uljin (N 37° 02′ 09″, E 129° 17′ 62″), Yeongdeok (N 36° 29′ 20″, E 129° 17′ 54″), and Pohang (N36° 06′ 21″, E129° 07′ 24″) (Fig. 1a). The Hongcheon site is a research forest of the National Institute of Forest Science (Seoul, South Korea), and the other locations are sites where annual productivities of the fruiting body of *T. matsutake* are high. All sampling sites are mixed forests (c.a. 30–40 years old) dominated by *Pinus densiflora* co-existing partially with *Pinus koraiensis*, *Quercus* spp., and *Rhododendron* spp. as understory vegetation. For bacterial isolation, 5 g of soil were diluted in 495 mL of sterilized distilled water and serial dilutions ranging from 1/100 to 1/1000 were prepared, and 100 μl of dilution were spread onto Petri dishes containing Tryptic soy agar (TSA; Difco, USA) and Reasoner's 2A agar medium (Difco, USA). After incubating at 30 °C for 2–7 days, each bacterial isolate was subcultured onto TSA. Bacterial strains were then stored in 20% glycerol at −80 °C until the co-culture experiments.

Fig. 1 **a** Map of sampling sites and **b** phylogenetic tree for identification of bacteria isolated from fairy rings of *T. matsutake* based on 16S rRNA region. Phylogeny was constructed by neighbor-joining method with Kimura-2-parameter and 1000 bootstrap replicates. High bootstrap values (≥ 75%) are presented at the nodes. Sequences of type strains were used as references retrieved from ezBiocloud. Species presence on each site is indicated by a colored box (red: Hongcheon-HC; yellow: Uljin-UJ; green: Yeongdeok-YD; purple: Phohang-PH)

Molecular experiment and identification

Identification of bacterial isolates was conducted using the 16S ribosomal RNA region sequence amplified using 27F and 1492R primers (Weisburg et al. 1991). For colony PCR, single colony (c.a. 1 μl) of bacteria was diluted in 100 μl of sterilized distilled water. The PCR mixture was made using a Maxime PCR PreMix kit (iNtRON Biotechnology, Korea) containing 2 μl of diluted bacteria colony, 1 μl of each primer, and 16 μl of distilled water. PCR amplification was performed using a C1000™ thermal cycler (Bio-Rad, USA) under the following conditions: 95 °C for 10 min, and 35 cycles of 95 °C for 40 s, 55 °C for 40 s, and 72 °C for 60 s, and then incubation at 72 °C for 5 min. The PCR products were checked by gel electrophoresis on a 1% agarose gel. After purification using Expin™ PCR Purification Kit (GeneAll Biotechnology, Korea), PCR products were sequenced at Macrogen (Seoul, Korea). All strains were sequenced using 27F and 1100R primers (c.a. 1 kb) to group molecular operational taxonomic units (mOTUs). Sequences were proofread using MEGA5 (Tamura et al. 2011) and aligned using MAFFT (Katoh and Standley 2013). mOTUs were constructed based on UPGMA tree with p-distance. Clades showing less than 1% of dissimilarity were selected as a mOTU. Then, one to three sequences that were frequent genotypes in mOTU were selected as representative of mOTUs and we sequenced the remaining partial region to species using 1100F and 1492R primers. Initial identification was conducted using BLAST on the EzBioCloud, where curated type sequences are deposited (Yoon et al. 2017). Retrieved top match sequences were used for phylogenetic analysis. Multiple alignment was conducted using MAFFT and alignment was reviewed and edited with MEGA5. A phylogenetic tree based on the neighbor-joining method was constructed using MEGA5 with Kimura-2-parameter model and 1000 bootstrap replications. Final identification was assigned based on the phylogenetic tree. If a


A
124°E 126°E 128°E 130°E 132°E
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Sampling sites
HC UJ YD PH
BAYE01000050 Paraburkholderia caledonica NBRC 102488
HQ698908 Paraburkholderia dilworthii WSM3556
HF674707 Paraburkholderia kirstenboschensis Kb15
PSB112 Paraburkholderia sp.1
KF733462 Paraburkholderia insulsa PNG-April
BAYC01000104 Paraburkholderia fungorum NBRC 102489
PSB213 Paraburkholderia sp.2
EU035613 Paraburkholderia sediminicola HU2-65W
PSB113
KP938221 Paraburkholderia pallidirosea DHOK13
PSB239
LXKA01000388 Paraburkholderia ginsengiterrae DCY85
PSB088
AY773186 Paraburkholderia sabiae Br3407
PSB049
BBJK01000219 Paraburkholderia terrae NBRC 100964
AY040365 Paraburkholderia hospita LMG 20598
PSB263 Paraburkholderia sp.3
AJ420332 Paraburkholderia tropica Ppe8
PSB229
AY965240 Paraburkholderia silvatlantica SRMrh-20
PSB262
AM747630 Burkholderia arboris R-24201
PSB018
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PSB124
FCOK01000155 Caballeronia udeis LMG 27134
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FCOC01000044 Caballeronia sordidicola LMG 22029
PSB028 Caballeronia sp.
LFLT01000090 Herbaspirillum chlorophenolicum CPW301
PSB207
CP013234 Collimonas pratensis Ter91
CP013232 Collimonas fungivorans Ter6
CP013233 Collimonas arenae Ter10
PSB191 Collimonas sp.
JMPQ01000005 Serratia marcescens ATCC 13880
PSB042
AUFB01000074 Rhizobium leucaenae USDA 9039
PSB032
KF150696 Arthrobacter bambusae GM18
PSB206
HM222655 Janibacter indicus 0704P10-1
PSB199
X77445 Microbacterium testaceum DSM 20166
PSB215
JN560157 Streptomyces hundungensis MBRL 251
PSB170
EU179327 Paenibacillus taichungensis BCRC 17757
PSB131
CP006643 Bacillus infantis NRRL B-14911
PSB130
EF114313 Bacillus aryabhattai B8W22
PSB010
ACMU01000002 Bacillus mycoides DSM 2048
BAUY01000093 Bacillus weihenstephanensis NBRC 101238
PSB065 Bacillus sp.
D83370 Staphylococcus lentus ATCC 29070
PSB295
L37605 Staphylococcus epidermidis ATCC 14990
PSB231
AJ517414 Staphylococcus nepalensis CW1
D83361 Staphylococcus cohnii ATCC 29974
AB009940 Staphylococcus kloosii ATCC 43959
PSB135 Staphylococcus sp.
AE000512 Thermotoga maritima MSB8
M83548 Aquifex pyrophilus Kol5a
0.05
Proteobacteria
Actinobacteria
Firmicutes

sequence was not clustered monophyletically with one species, we assigned it at the level of genus. Based on final identification, relative abundance was calculated by the number of isolate for each species divided by the total number of isolates. All sequences we generated by Sanger sequencing were deposited in GenBank under accession numbers MF948887-MF948914 and MG572505–MG572713.

Co-culture of bacteria and *T. matsutake* isolate

The bacteria that were successfully isolated from within the fairy rings at our four sampling sites were co-cultured with a *T. matsutake* isolate to identify their effect on mycelial growth of *T. matsutake*. Representative isolates selected from each bacterial species were cultured on TSA, and *T. matsutake* strain (KMRB20121004–05) provided from Korea Mushroom Resource Bank (Seoul, South Korea) was cultured in potato dextrose broth (Difco, USA) for 6 months at 25 °C. Before the experiments, *T. matsutake* isolate was washed and homogenized in 30 mL of sterilized distilled water using a HG-15A homogenizer (DAIHAN, South Korea). Co-culture plates were prepared by first placing 20 μl of homogenized *T. matsutake* isolate at the center of 60 mm Petri dishes containing "Tricholoma matsutake" media (TMM) (glucose 20 g/L, yeast extract 1.5 g/L, soytone 1.5 g/L, and agar 20 g/L) (Kim et al. 2005). Each bacterial isolate was then inoculated onto plates by streaking with a sterile 1-μl inoculating loop (SPL Life Science, Korea) along a 30 mm line situated 15 mm away from the center of each Petri dish. To investigate if carbon availability affected how mycelial growth of *T. matsutake* responded to fairy ring bacteria in culture, we conducted another, similar set of co-culture experiments where glucose content of TMM was 2 g/L, i.e., ten times lower than in normal TMM. We refer to the reduced glucose media as low TMM (lTMM) and the normal media as high TMM (hTMM). In previous study, total of carbon (TOC) in the fairy ring of *T. matsutake* was 1.7–1.9% (Huh et al. 1998; Kim et al. 2014). Given that sugar contents are 4–7% of TOC in soil (Gunina and Kuzyakov 2015; Jolivet et al. 2006), lTMM that was glucose poor condition (0.2% glucose contents) is similar to forest soil where the fairy rings of *T. matsutake* exist. In contrast, hTMM was unlikely happened condition in nature, but, if we find growth promoting bacteria in this condition, these bacteria can be used as bio-fertilizer because the growth of *T. matsutake* can be maximized in glucose rich condition. For each bacterial species at each of the two glucose concentrations, the experiment was conducted in quadruplicate. After incubation of co-culture plates at 25 °C for 2 months, the diameter of *T. matsutake* isolate on each plate was measured twice and averaged. Differences in growth between treated and control (*T. matsutake* grown alone) plates were calculated by percentage changed with the formula as follows:

$$\text{Growth difference}\ (\%) = \frac{\text{Average growth of control} - \text{Average growth of treated}}{\text{Average growth of control}} \times 100$$

If growth difference was lower than zero, we noted "% decrease" after an absolute value of growth difference. Statistical significances on the growth difference were tested using pairwise Wilcoxon rank sum tests adjusted by the false discovery rate of Benjamini and Hochberg (1995).

Results

Identification of fairy ring bacteria associated with *T. matsutake*

A total of 237 bacterial isolates were obtained from soil within fairy rings of *T. matsutake* at four sample sites. Based on the partial 16S rDNA sequences, bacterial isolates were initially clustered into 28 mOTUs using partial sequence analysis and then identified them to species level using full sequence analysis (Fig. 1b). Representative sequences showed high similarity with reference type sequences (98–100%). Twenty of mOTUs were assigned to species but eight of mOTUs were assigned to the genus level due to high similarity with multiple reference species. The phylogenetic tree shows 28 species belonging to 3 phyla, 4 classes, 7 orders, 11 families, and 14 genera. Among the phyla, Proteobacteria had the highest number of species (17 species), followed by Firmicutes (7 species), and Actinobacteria (4 species). At the genus level, *Paraburkholderia* was the most dominant (69.6%), followed by *Burkholderia* (16.0%), *Staphylococcus* (5.1%), and *Caballeronia* (3.4%) (Fig. 2a). At the species level, *Paraburkholderia sediminicola* was most dominant (55.3%) found in all locations, followed by *Burkholderia arboris* (16.0%) and *Paraburkholderia ginsengiterrae* (5.9%) (Fig. 2b). A total of 8–16 species was isolated from each locations, and major species (total relative abundance > 5%; three species) comprised more than 70%. With the exception of two species, *Paraburkholderia sediminicola* and *Burkholderia arboris*, all other bacterial species that we isolated from within the fairy rings of *T. matsutake* showed geographically distinct distributions.

Radial growth of *T. matsutake* in co-culture experiments

For the co-culture experiments using the high glucose medium (hTMM) (Fig. S2A), most of the fairy ring bacteria we tested

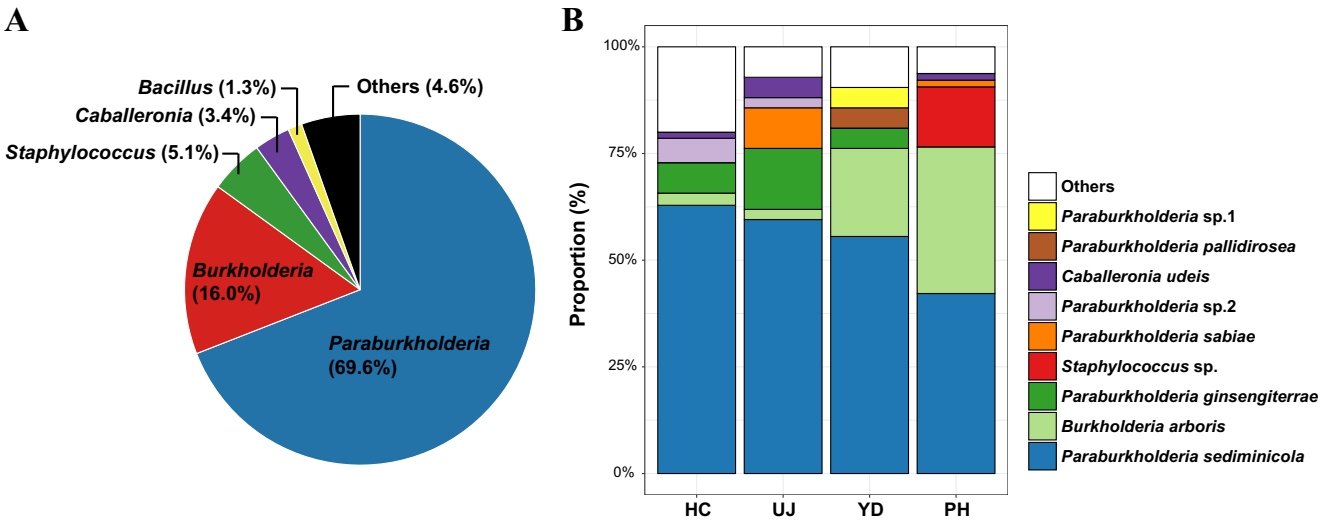

Fig. 2 Graphic showing diversity of bacteria isolated from soil within fairy rings of *T. matsutake* at four sample sites. **a** Composition of major genera of bacteria (> 1%). **b** Composition of major species of bacteria (> 1%). HC, Hongcheon; UJ, Uljin; YD, Yeongdeok; PH, Phohang

significantly suppressed the growth of *T. matsutake* (Growth differences 33–100% decrease) (Fig. 3). Eight of the bacterial species (*Burkholderia arboris*, four *Paraburkholderia* species, *Rhizobium leucaenae*, *Serratia marcescens*, and two *Staphylococcus* species) showed a significantly strong inhibitory effect with no growth of *T. matsutake* on these plates, while six species of bacteria did not show significant effect (neutral effect) on the growth of *T. matsutake* compared to it in control dishes. For the co-culture experiments with the low glucose medium (lTMM) (Fig. S2B), only three of the 28 fairy ring bacteria made *T. matsutake* grow and, of those, two (*Paenibacillus taichungensis* and *Staphylococcus* sp.) significantly promoted the growth of *T. matsutake* (Growth differences: 346–404% increase) (Fig. 3).

Discussion

The frequent interactions between fungi and other microbes in forest soils emphasizes that the biotic environment is critical to understand fungal ecology and physiology in nature. In this study, we isolated and identified bacteria occurring in soil within fairy rings of *T. matsutake* at four different sites in South Korea. We also co-cultured 28 fairy ring bacteria individually with a *T. matsutake* isolate at both low and high concentrations of available carbon to determine whether the fairy ring bacteria influence on the growth of *T. matsutake* positively or negatively. Given the importance of *T. matsutake* and its difficulty of cultivation, we believe that investigation of *T. matsutake* growth promoting bacteria is crucial step to understanding the ecology of *T. matsutake* in nature.

Because fairy rings are important components on the life history of *T. matsutake*, microorganisms associated with these fairy rings have been extensively studied. Previous studies showed that bacterial communities in the fairy ring were different from it in adjacent bulk soil, which suggests that some bacteria may be favored by *T. matsutake* and/or they might be able to resist its antibiotic activity (Kataoka et al. 2012; Oh et al. 2016; Vaario et al. 2011). Despite these encouraging results with the isolation of soil bacteria, less than 1% of them are known to be culturable (Amann et al. 1995). For fairy rings of *T. matsutake*, the direct viable bacteria count method using carboxyffuorescein diacetate (CFDA) showed that culture isolation captured only 5–8% of bacteria actually present within the fairy ring; a large proportion of the Acidobacteria was missing (Kim and Whang 2007). Similar to previous studies (Jiang et al. 2015; Kim and Whang 2007; Ohara and Hamada 1967), we did not discover large portion of bacterial taxa including Acidobacteria that was the second most abundant phylum in fairy rings of *T. matsutake* (Kim et al. 2014), primarily due to the innate limitations of the culture-dependent method we used. Given that the *Bacillus*, *Burkholderia*, and *Paenibacillus* species we isolated were abundant in fairy rings of *T. matsutake* (Jiang et al. 2015; Kataoka et al. 2012; Kim et al. 2014; Oh et al. 2016), identifying the effect of these bacteria on the growth of *T. matsutake* is meaningful attempts to catch a glimpse of microbial interaction between the fairy ring bacteria and *T. matsutake*, although we isolated just a portion of the diversity of fairy ring bacteria.

Burkholderiaceae, which included the genera *Burkholderia*, *Caballeronia*, and *Paraburkholderia*, was the most dominant family that we isolated from fairy rings of *T. matsutake* (Fig. 2a); these three genera together previously belonged to *Burkholderia*. Recent studies of multi gene phylogeny separated *Paraburkholderia* and *Caballeronia* from the *Burkholderia* clade (Dobritsa and Samadpour 2016; Sawana et al. 2014). Strains from the *Burkholderia* genus, which is known as a fungiphile (Warmink et al. 2009) of MHB (Poole et al. 2001),

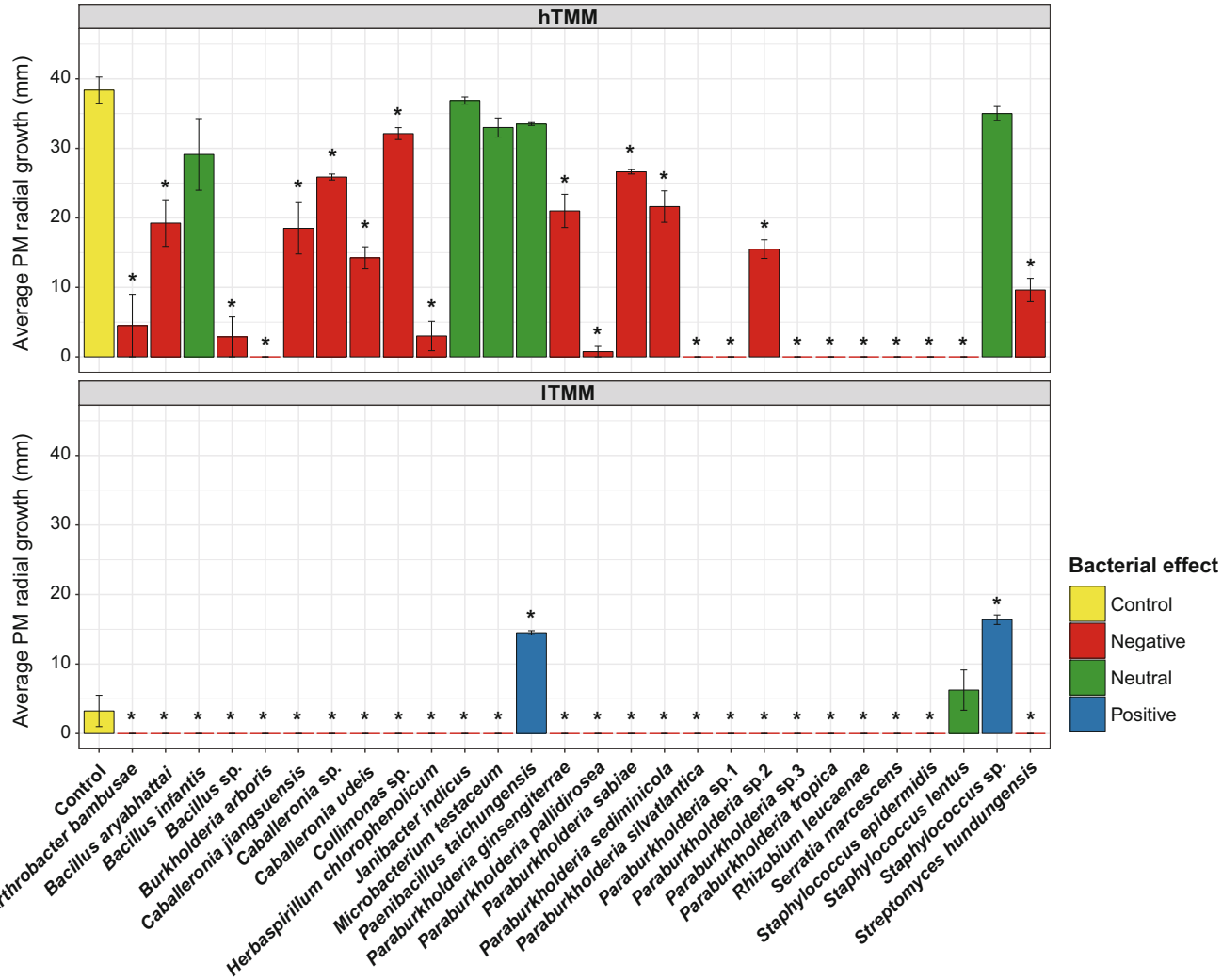

Fig. 3 Average radial growth (mm) of *T. matsutake* isolate co-cultured with the fairy ring bacteria in a high glucose medium (hTMM) and a low glucose medium (lTMM). Growth of *T. matsutake* on treated plates was compared to growth on control plates using pairwise Wilcoxon rank sum tests. An asterisk indicates a significant difference ($P < 0.05$; adjusted by the false discovery rate of Benjamini and Hochberg)

are frequently detected in the fungi-associated environments (Stopnisek et al. 2016) including *T. matsutake*-associated environment (Jiang et al. 2015; Kataoka et al. 2012; Oh et al. 2016). Similar to Burkholderiaceae case, minor genera we isolated (e.g. *Paenibacillus*, *Serratia*, *Staphylococcus*, and *Streptomyces*) have also frequently been detected from fungi-associated environments (Citterio et al. 1995; Frey-Klett et al. 2007; Li et al. 2016; Warmink et al. 2009), which suggests that the fairy ring bacteria may have conserved traits favoring hyphal dominant habitats in the genus level.

Many bacteria living in fungi-associated environments have been revealed as MHB, showing helping mycorrhiza formation, growth promotion, and the fruiting body maturations (Frey-Klett et al. 2007; Warmink et al. 2009). For example, *Pseudomonas fluorescens* BBc6R8 and *Streptomyces* sp. AcH 505 can induce gene expression associated with the hyphal growth of ectomycorrhizal fungi *Laccaria bicolor* and *Amanita muscaria*, respectively, as MHB effects (Schrey et al. 2005; Deveau et al. 2007). Despite the high abundance of *Paraburkholderia* species we isolated in our study, they did not have a positive effect on the growth of *T. matsutake* in hTMM experiment (Fig. 3). Instead, most of the bacteria significantly suppressed the hyphal growth of *T. matsutake*, with eight species showing strong inhibition, which indicates that the fairy ring bacteria cannot be used as bio-fertilizer in artificial medium with glucose rich condition.

In contrast to the result from our hTMM experiment, in the lTMM condition where similar to forest soil, some fairy ring bacteria showed significant growth promotion of *T. matsutake* hyphae (Fig. 3). While the majority of bacteria negatively affected the growth of *T. matsutake* in the lTMM, *Paenibacillus taichungensis* and *Staphylococcus* sp. stimulated growth of *T. matsutake* at a rate of 346–404% higher than that of *T. matsutake* grown alone (Fig. 3). These bacteria did not showed significant effect (neutral effect) in the hTMM experiment. Our results corroborate previous studies where

different bacterial effects occurred, depending on nutrient availability (Brulé et al. 2001; Duponnois 1992; Garbaye 1994). For example, *Pseudomonas fluorescens* strain BBc6R8 showed a negative effect on the fungus, *Laccaria bicolor*, in a nutrient rich condition, while it promoted growth in a nutrient limited condition (water agar) (Brulé et al. 2001). Also, we could infer that the two fairy ring bacteria we showed to promote the growth of *T. matsutake* in a carbon-limited environment may act as helper bacteria in forest soil which is generally oligotrophic condition. Nevertheless, most fairy ring bacteria showed strong inhibition on the growth of *T. matsutake* in lTMM, which suggests that these bacteria may exist in the fairy ring to exploit nutrients by degrading hyphae (Leveau and Preston 2008) without positive effect on the growth of *T. matsutake*. According to our results (Fig. 3), therefore, *T. matsutake* may experience harsh antagonism from bacteria in forest soil, while some bacteria help the growth of *T. matsutake*.

Negative or positive interaction between bacteria and fungi are well known and can be found in recently reviews (Frey-Klett et al. 2011; Scherlach et al. 2013). Negative effect of several bacteria on the growth of *T. matsutake* agree with previous reports of the species or genera that have antifungal activity (Kai et al. 2009; Zarei et al. 2011; Nimaichand et al. 2013; Tenorio-Salgado et al. 2013). Considering that bacteria living in forest soil secrete antibiotic or antifungal compound for competing resources (e.g., nutrition or space) to other microbes (de Boer et al. 2005; Raaijmakers et al. 2009), severe antifungal activity in lTMM may be due to bacterial response on nutrient limitation. However, there are several other mechanisms that can be involved in antagonistic relationship between bacteria and fungi such as pH alteration in a growth medium (Frey-Klett et al. 2011). Mechanism of growth promoting effect of the fairy ring bacteria is uncertain while some potential mechanisms can be explainable. (1) Bacteria may secrete nutrients that can be used for the growth of *T. matsutake* when nutrient condition is limited. For example, bacteria secrete different volatile compound (VOC) depend on nutrient condition (Blom et al. 2011; Garbeva et al. 2014), and some VOC can be used as energy source by other microbes (Prenafeta-Boldu et al. 2001; Schmidt et al. 2016). (2) Other explanation is that growth promoting compound may target same gene which is targeted by glucose, thus it is not effective in glucose rich condition because glucose already activates the gene. This is similar case with mycelial growth promoting compound (auxofuran) secreted by *Streptomyces* sp. AcH 505, MHB of *Amanita muscaria* (Riedlinger et al. 2006). Auxofuran have same target with glucose that activates acetoacyl-CoA synthetase gene (*Aacs*) which is associated with ergosterol synthesis, an important component of cell structure (Schrey et al. 2005). (3) Bacteria may degrade self-inhibitory compound secreted from fungi, which is revealed from the growth promoting effect of *Pseudomonas putida* on the growth of *Agaricus bisporus* (Chen et al. 2013). However, it is necessary to investigate which mechanism is involved in the interaction between the fairy ring bacteria and *T. matsutake*. One thing we need to mention is that bacterial interaction may have genotype specificity. Several studies have showed that different genotype pairs may have opposite direction of relationship (Duponnois and Garbaye 1990; Varese et al. 1996; Labbe et al. 2014). In this study, we only used single strain of *T. matsutake*, thus further study based on our results is need to use multiple strains for generalizing interaction between bacteria and *T. matsutake*.

In conclusion, we isolated and identified several bacteria from fairy rings of *T. matsutake*, and we investigated whether or not some of the fairy ring bacteria had an effect on the growth of *T. matsutake* in culture. *Paraburkholderia* was the most dominant genus successfully isolated from fairy rings of *T. matsutake*, and it was found at all four geographical locations we sampled. Bacterial effects on the growth of *T. matsutake* were affected by glucose concentrations; bacteria mostly suppressed growth in artificially high glucose condition, while some promoted the growth of *T. matsutake* in the low glucose condition. The role of bacteria in the growth of *T. matsutake* is changed depending on nutrition conditions, which suggests that *T. matsutake* growth promoting bacteria may be used as bio-fertilizer in forest soil for increasing productivity of *T. matsutake*, although they cannot be used in nutrient-rich medium. We believe that our results provide information required for understanding *T. matsutake* ecology associated with bacterial interaction in the fairy ring. Mechanisms by which bacteria promote the growth of *T. matsutake* need to be elucidated at the genetic or metabolite level.

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