

Isolation and Characterization of the Mycorrhiza Helper Bacteria from the Fairy Rings of *Floccularia Luteovirens*

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Abstract

Floccularia luteovirens is an edible ectomycorrhizal fungus which forms fairy rings (FRs) distributed on the Qinghai-Tibet Plateau. Our previous study found four isolates of potential mycorrhiza helper bacteria (MHB), but they were only isolated from a single FR and their mycorrhizal promoting ability was not tested. Thus, it was necessary to collect samples from a larger area and measure the mycorrhizal promoting ability of the potential candidates. Of 184 bacterial isolates from five fairy rings located in Qinghai-Tibet Plateau belonging to 12 species, and 7 of them (58.3%) significantly promoting the growth of *F. luteovirens*. We also tested the symbiosis-promoting ability of the isolates, and the results showed that only four of them stimulated the formation of mycorrhizal symbiosis. This is the first report of the construction of mycorrhizal symbiosis between *F. luteovirens* and its host plant in greenhouse conditions. Our results showed that the strain A89 (*Achromobacter marplatensis*) could produce antifungal properties but nonetheless stimulated the fungal growth. This may due to the nutrient condition of medium and the genotype specificity of MHBs. The results suggest that the promotion of significant growth is not a good predictor for mycorrhiza formation promotion ability of MHB.

Key Points:

We found ten strains promoted hyphal growth, some was the first time been reported.

We constructed *F. luteovirens* and its host plant in the laboratory.

We found four strains MHB of *F. luteovirens*.

Introduction

The rhizosphere is an important habitat for microorganisms and plants and is different from bulk soil (Raaijmakers et al. 2009). The plants supply carbon resources to the root-associated bacteria and fungus through photosynthesis processes and get available nutrients back from them (Caravaca et al. 2002). Meanwhile, microorganisms also interact together physically and metabolically in competition for nutrients and space (Garbaye 1991; Nazir et al. 2010). The Ectomycorrhiza (ECM) is the symbiotic entity, consisting of a fungus and the roots of various plant species. It plays an important role in material recycling in forest and grassland ecosystems because this kind of fungus can enhance water and mineral absorption by its host plants (Nehls et al. 1998). As a part of the rhizosphere, the ECM fungus without any doubt is positively and negatively affected by other microbes. For instance, some bacteria can reduce the growth of fungal hyphae and the colonization rate of ECM fungi to the host plant root (Varese et al. 1996). The other bacteria isolated from rhizosphere or mycorrhizosphere can promote the formation of the ectomycorrhizal and are called mycorrhiza helper bacteria (MHB) (Freyklett et al. 2007). The MHB is good bio-fertilizers for the cultivation of mushrooms and enhances its productivity (Oh and Lim 2018). According to the studies in the past decade, the MHB has been classified into many bacterial groups, such as Proteobacteria, Firmicutes and Actinomycetes which have been isolated from various

environments (for example, from mycorrhizas and fruit bodies of ECM fungi) (Freyklett et al. 2007). Mechanisms by which MHB promote the formation of symbiosis are as follows: 1. Stimulation of the hyphal extension of ECM fungi, thereby increasing the probability of root-fungus contacts. For example, growth factors produced by MHB can enhance spore germination and hyphal growth of ECM fungi and consequently enhance the formation of mycorrhizal symbiosis (Garbaye 1994). The hyphal formation can be changed by MHB that facilitate the colonization of ECM: the MHB *Pseudomonas fluorescens* BBc6R8 enhanced the hyphal branching angle and the hyphal branching density and then the hyphal extension was stimulated significantly (Deveau et al. 2007). The MHB also can protect the plant and ECM fungi from root pathogens: Selim et al. (2005) isolated a strain of *Paenibacillus* from the rhizosphere of *sorghum bicolor* and found that it was antagonistic towards soil-borne fungal pathogens (Selim et al. 2005). Another important mechanism is to alleviate soil-mediated stress: Vivas et al. (2003) showed that, in conditions of heavy metal pollution, spore germination and fungal growth were positively influenced by MHB (Vivas et al. 2003). Some studies found that there is a significant correlation between the influence of MBH on fungal hyphal growth and mycorrhizal formation (Garbaye 1991). Nevertheless, in contrast, Bowen and Theodorou observed that the fungus-bacterium reactions *in vitro* cannot predict the mycorrhiza formation ability of the same fungus-bacterium combination (Bowen and Theodorou 1979). Dunstan et al. (1998) also found that, although a significant growth promotion had been observed in eight fungus-bacterium combinations *in vitro*, only the combinations of *Laccaria laccata-pseudomonad* Elf29 showed a significant enhancement of mycorrhizal formation (Dunstan et al. 1998).

Floccularia luteovirens, mostly distributed in the altitude of about 3 000 ~ 4 800 m in Qinghai Province, is a valuable chinese well-known mushroom for the thick sporocarp, the smooth and delicious taste, the special fragrance and the rich nutrition, there are actual medicinal properties as well (Kun. 1997; Zhou et al. 2008). However, it can not be cultured by artificial method (Xingbao H *et al.* 2015). The antecedent researchers showed *Floccularia luteovirens* is a kind of ECM fungus co-exist which is associated with *Kobresia humilis* (Xing et al. 2014). It plays essential roles in the Plateau terrestrial ecosystem. It can also form fairy rings comprised of its fruit bodies. Vegetative production located near the fruiting bodies is stimulated (Xing et al. 2017). Our previous study compared the microbial communities located in different zones of its fairy rings and found a distinct bacterial community under the fairy rings. The diversity of edaphon under the fairy rings is significantly lower than outside and inside, and there was no other ECM. Four potential mycorrhiza helper bacteria strains were isolated (Xing et al. 2018). However, this study only investigated one single soil sample collected from the fairy rings of *F. luteovirens* and only tested hyphal growth promoting ability. Thus it was necessary to collect samples from a larger area and measure the mycorrhizal promoting ability of ECM-plant symbiotic system of the potential candidate MHBs.

Materials And Methods

The soil samples under the five fairy rings of *F. luteovire* were collected from Yeniugou, Qilian, Qinghai province, China, in August 2018 (Table 1). The occurrence of sporocarp of *F. luteovire* was observed 1 ~ 2 years before sampling. The distances among fairy rings were 0.45 to 23.3 km. After the natural drying

and sieving, quickly keep 4 °C. The bright and large mushrooms were also collected. *Trifolium subterraneum* L. was bought from the Yanji Town Shuyang County seed Business Department in China.

Table 1
Sampling information of five fairy rings in Qinghai-tibet plateau

Fairy ring	Location	Latitude	Longitude	Elevation (m)
Fairy ring1	Qinghai province, China	N 38°27'49"	E 99°32'43"	3353
Fairy ring2	Qinghai province, China	N 38°37'07"	E 99°20'12"	3477
Fairy ring3	Qinghai province, China	N 38°31'18"	E 99°29'15"	3360
Fairy ring4	Qinghai province, China	N 38°27'31"	E 99°32'49"	3389
Fairy ring5	Qinghai province, China	N 38°31'55"	E 99°29'58"	3407

For bacterial isolation, all the soil samples (10 g for each sample) were diluted into 990 mL sterilized distilled water and 10^{-2} and 10^{-3} serial dilutions were prepared and were spread onto Petri dishes containing TSA (Tryptic soy agar; Difco, USA) and R2A (Reasoner's 2A agar medium; Difco, USA). After the DNA extraction, the concentration of DNA was recorded after the measurement of Ultraviolet-Visible Spectrophotometer (UV-759) under 340 nm and the analysis of Nano Drop microfluid. The 16S ribosomal RNA primer 27F and 1492R primers were used to identify all the bacterial isolates (Weisburg et al. 1991). PCR was performed in a 15 μ L solutions containing genomic DNA, 200 mM 10 \times PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 200 nM of each primer, and 1 U of Taq DNA polymerase (Takara, Dalian, China). The following PCR program was used: initial denaturation for 5 min at 95 °C, 35 cycles of 30 s each at 95°C, 57 °C for 90 s, 40 s at 72 °C, and a final extension for 10 min at 72 °C. After purification using the PCR product purification kit (Qiagen), following the recommended protocol, the PCR products were sequenced on an ABI 3730 automated sequencer (Sangon Biotech (Shanghai) Co., Ltd.). The sequences were then compared to available bacterial sequences in GenBank using the BLAST program in the National Center for Biotechnology Information (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). The mOTUs were identified based on the UPGMA tree with *p*-distance, strains with less than 1% dissimilarity were identified as one molecular operational taxonomic unit (mOTU). A phylogenetic dendrogram was then generated based on the 16S rRNA gene sequences using the neighbor-joining method in MEGA X software package (Kumar et al. 2007). All the isolates have been submitted to GenBank under accession numbers MK453311-MK453322.

We selected the collected *F. luteovire* mushroom after cap opening soon, without disease and growing well, scrubbed 2 ~ 3 times with alcohol cotton balls and rinsed off with distilled water. Then, a piece of tissue from the connection of cap and stem of the sporocarp were cultured on optimized Potato Dextrose Agar (PDA) medium (0.25 kg not sprout and healthy potatoes per 1000 mL, the filtrate mixed with $CaCl_2$ 0.125 g, $MgSO_4$ 0.5 g, maltose 1.5 g, D-glucose 7.5 g, $K_2HPO_4 \cdot 3H_2O$ 0.5 g, thiamine hydrochloride) 0.0001 g, $(NH_4)_2HPO_4$ 0.25 g, KNO_3 0.5 g, agar 10 g, the PH adjusted to 6.0 ~ 6.5) at 25 °C,

the growth condition, germination days and full package days were recorded. Then, we selected the growing well hypha and inoculated on the center of 9-cm-diameter Petri dish containing 15 ml PDA medium, inversed the plate with the surface facing down after 2 ~ 3 days. After growing enough, cutted a plug of the fungus block which growing uniform during the edge of the a colony to 4 mm × 4 mm and transformed into the center of the PDA-plate.

We selected the brilliant identified 20 bacteria to test the hyphal growth enhancement ability. The processes were as follows: 1) A plug of the growing uniform *F. luteovirens* strain was cutted out from the edge of a purified colony and transferred into the center of a new PDA-plate with the surface facing down and then cultured at 25 °C for 10 days (three dishes per fungus-bacterium treatment and one dish with fungus only). 2) Bacterial isolates were grown to late exponential phase at 25 °C for 24–36 h and washed with sterile physiological saline (9 g/L⁻¹ NaCl). Fifteen micro-liter of bacterial suspension (OD_{600 nm} 0.7 in sterile physiological saline) were inoculated into to each plug. 3) Petri dishes were stored at 25 °C for 20 days and irrigated with 10 µL of sterile glucose (10 mM) in physiological saline (9 g/L⁻¹ NaCl) every 7 days. 4) After that, diameters of *F. luteovirens* isolates on each plug were measured five times with Toup view software and averaged, and potential hyphal growth promotion ability of each isolate was compared using Student's t-tests (Dunstan et al. 1998).

Due to the low seed germination rate of *kobresia sp.*, we used *Trifolium repens* as the plant material to test the symbiosis promotion ability of each potential MHB. The seeds were surface-sterilized using 8% NaClO for 10 min. The potting medium comprised sand, soil, and vermiculite (1:2:1, v/v/v) and was autoclaved under 121 °C for 20 min twice. Forty seeds were planted into each plot (five plots for each treatment) and the culture conditions were as follows: 12 h light and 12 h dark at 25 °C and irrigated every three days. After germination, 10 mL of *F. luteovirens* liquid inoculum into each pot; the control received 5 mL of diluted PDA liquid medium, after that 10 ml of selected bacterial (with the hyphal growth ability) suspension was also injected into the pot. Sixty days after inoculation, the roots were washed carefully and cut into 1 cm long pieces and the roots were visualized using 5% ink diluted in vinegar as described by Vierheilig *et al.* (2010). The inoculum rate was estimated as the percentage of total root tips which was colonized by *F. luteovirens*.

Results

Due to the different composition of the two mediums, the time for the growth of a single scattered colony was also different. The growth rate (2 days) of bacteria cultured in R2A medium was significantly faster than that (4 days) cultured in TSA medium. In total, 184 bacteria were isolated from the soil of five *F. luteovirens* fairy rings on TSA and R2A media. During the identification of the isolates we found that the relationship of the concentration of bacterial DNA with the PCR strip condition and the sequencing results (Table 2). The concentration of DNA should be over 30 ng/µL, the result of PCR and sequencing may be ideal. From 184 bacterial, 20 strains was identified. Most of the bacteria were only can cultured at TSA or R2A medium, only *Bacillus cereus*, *Bacillaceae bacterium* can be cultured at both (Table 3). Then, isolates were clustered into 20 mOTUs using partial sequence analysis based on 16S rDNA sequences.

Then, phylogenetic tree shows 12 species belonging to three phyla, four classes, seven orders, 10 families, and 10 genera (Fig. 1). Proteobacteria (66.16%) is the most dominant phylum, followed by Firmicutes (31.45%). At the genus level, *Bacillus* (28.65%) is the most dominant one followed by *Achromobacter* (27.53%) (Fig. 2a). At the species level, we isolated 47 *Achromobacter spanius* strains and 40 *Bacillus cereus* strains which were the dominant species in our study. Moreover, different mediums performed differently in the isolation of fairy ring soil bacteria. Eight strains (*Bacillus*, *Acinetobacter*, *Stenotrophomonas*, *Microbacterium*, *Brevibacterium*, *Pseudomonas*, *Cupriavidus*, *Leuconostoc*) were found from 97 TSA isolates, and by contrast, only four strains (*Bacillus*, *Pseudomonas*, *Achromobacter*, *paenarthrobacter*) were unique from 87 R2A isolates (Fig. 2b). The brilliant identified 20 bacteria (the bold) were to conduct the following study (Table 3).

Table 2

The relationship of the concentration of bacterial DNA with the PCR strip condition and the sequencing results.

Bacterial concentration (ng/uL)	Sample size	PCR strip condition	The sequencing results
0 ~ 10	21	Not good	None
10 ~ 20	25	Good	Most of ideal
20 ~ 30	13	Good	Some of not ideal
30 ~ 605	224	Good	Ideal

Table 3

Identification of bacteria from the soil of five *F. luteovirens* fairy rings on TSA and R2A media

Bacteria number	Identification of bacteria	Bacteria number
	<i>Arthrobacter spanius</i>	1-201-30
	<i>Acinetobacter rhizosphaerae</i>	1-101-502-1902-2002-2102-2202-2302-2502-2602-2802-3502-3602-6502-8102-8202-84
	<i>Achromobacter sp.</i>	A10A90A120A130A180A190A230A240A260A280A290A300A370A380A390A400A410A420A480A490A510A520A530A540A550A560A580A590A600A610A630A640A650A660A670A690A750A760A770A780A790A820A830A840A870A880A910A810A89
	<i>Bacillus cereus</i>	A1401-3402-24
1-31	<i>Bacillus sp.</i>	
	<i>Bacillus thuringiensis</i>	A7002-730A430A7101-301-1701-1901-2001-2201-2501-2701-2901-3801-3901-4001-4101-4201-4301-4401-4502-202-602-702-1302-1402-1502-1802-3402-3702-3802-302-4002-4102-4302-4602-4702-6102-6602-6802-7002-8002-8702-4502-76
	<i>Bacillaceae bacterium</i>	A210A570A8502-85
1-21	<i>Brevibacterium frigoritolerans</i>	
2-94	<i>Cupriavidus respiraculi</i>	
2-58	<i>Leuconostoc sp.</i>	
	<i>Microbacterium sp.</i>	1-401-2601-2801-3502-74
1-6	<i>Microbacterium oxydans</i>	
A6	<i>Pseudomonas fluorescens</i>	
	<i>Pseudomonas koreensis</i>	A160A270A340A350A470A720A740A50A100A110A150A250A320A80
A17	<i>Pseudomonas moraviensis</i>	
	<i>Pseudomonas reinekei</i>	2-4902-50
	<i>Pseudomonas sp.</i>	A20A30A40A80A220A620A90

Bacteria number	Identification of bacteria	Bacteria number
	<i>Stenotrophomonas rhizophila</i>	1-10, 2-29, 2-62, 2-69, 2-90
	<i>Stenotrophomonas sp.</i>	1-7, 1-8, 1-11, 1-12, 1-14, 1-15, 1-16, 1-18, 2-10
1-33	<i>Uncultured bacterium</i>	

The *F. luteovirens* mycelium started germinating at the 10th day, overgrew with the test-tube about 22 ~ 24th day at 25 °C. Then, the white and sparse hypha could be used to conduct the following study for its brilliant quality. For bacteria-fungus co-culture experiments, 10 strains fairy ring bacteria significantly promoted the growth of *F. luteovirens* on PDA medium, accounted for half of culturable strains. These bacteria were identified as *Achromobacter marplatensis* (A89), *Leuconostoc pseudomesenteroides* (2-58), *Acinetobacter rhizosphaerae* (1-5), *Microbacterium oxydans* (1-28), *Stenotrophomonas rhizophila* (2-62), *Pseudomonas fluorescens*(A6), *Cupriavidus respiraculi* (2-94), *Stenotrophomonas sp.* (1-12), *Bacillus cereus* (A14), *Bacillus thuringiensis* (2-85). Meanwhile, we found 6 strains *Bacillus thuringiensis* (A70), *Pseudomonas reinekei* (2-49), *Arthrobacter spanius* (1-2), *Uncultured bacterium* (1-33), *Pseudomonas koreensis* (A11), *Pseudomonas sp.* (A4) fairy ring bacteria did not have any effect on the growth of *F. luteovirens* on PDA medium, however, 4 strains fairy ring bacteria *Microbacterium oxydans* (1-6), *Bacillus sp.* (1-31), *Pseudomonas moraviensis* (A17), *Brevibacterium frigoritolerans* (1-21) was surprised to have the inhibiting effect (Table 4). Although there is still 3 strains of the bacteria had the inhabiting effect on *F. luteovirens* mycelium, but the multiple of hypha detrimental bacteria was only 2.99% of all the multiple of bacteria, most of the the bacteria was hypha helper bacteria (64.18%) (Fig. 3a).

Table 4
Initial screening of MHB independent sample t test results

Bacteria number	The first comparison results	The second comparison results	The third comparison results	Final comparison results
A89	∅	∅	∅	∅
A70	0	0	0	0
1-6	-	-	0	-
2-58	∅	∅	∅	∅
2-49	0	0	0	0
1-2	0	0	0	0
1-5	∅	∅	∅	∅
1-28	∅	∅	∅	∅
1-31	-	-	0	-
1-33	0	0	0	0
2-62	∅	∅	∅	∅
A6	∅	∅	0	∅
2-94	∅	∅	-	∅
A11	0	0	0	0
1-12	∅	∅	∅	∅
A4	0	0	0	0
A17	-	-	-	-
A14	∅	∅	∅	∅
1-21	-	-	-	-
2-85	∅	∅	0	∅

We constructed the mycorrhizal symbiosis between *T. epens* and *F. luteovirens* and then tested the symbiosis promoting ability of 12 potential MHBs (ten strains from the present study and two strains from our previous study). From the Staining and microscopic examination results, the discolored mycorrhiza root structure can be clearly seen, the red arrow pointed to the hypha penetrated into the root, the distinct septum and protuberant structure of the hypha was seen clearly. The blue arrow pointed to the spores of the *F. luteovirens*, and the blue arrow pointed to the Hartig-net of the ectomycorrhiza, hence the mycorrhizal symbiosis between *T. epens* and *F. luteovirens* was successfully constructed (Fig. 4).

Ultimately, we got 4 strains MHB *Achromobacter marplatensis* (A89), *Stenotrophomonas rhizophila* (2–62), *Bacillus cereus* (A14), *Pseudomonas fluorescens* (A6) to stimulate the formation of mycorrhizal symbiosis between *T. epens* and *F. luteovirens*, the infection rate increased respectively 8.2%–9.0%–22.1%–37.4%, the highest infection effect can reach 90.3%. However, we also got four strains bacteriac *Leuconostoc pseudomesenteroides* (2–58), *Acinetobacter rhizosphaerae* (1–5), *Cupriavidus respiraculi* (2–94), *Bacillus thuringiensis* (2–85) inhibited the inoculum rate of *F. luteovirens* to the roots (Fig. 3b).

Discussion

In the present study, we isolated 184 bacterial strains from the fairy ring soil of *F. luteovirens*. These bacteria belong to 20 strains, a very limited number compared with our previous study on microbial community of fairy ring soil based on high throughput sequencing (Xing et al. 2018) and with other studies on bacterial communities of the fairy ring (Caesar-Tonthat et al. 2013; Kim et al. 2014). Based on current bacterial culture methods, less than 1% of bacteria are known to be culturable (Amann et al. 1995), that is because of unknown species lack of necessary information about suitable culture methods. In other studies, where attempts were made to isolate bacteria from fairy ring soil, the number of isolates from soil were also very limited: Kim and Whang 2007 showed that only 5–8% of total bacteria were cultured from fairy rings of *Tricholoma matsutake* and that Acidobacteria, which form a large part of the total bacteria community, were not isolated (Kim and Whang 2007). Many subsequent studies on the same topic also failed to isolate large portions of bacterial taxa (such as Acidobacteria) (Jiang et al. 2015; Ohara and Hamada 1967). Despite the drawbacks of the culture-dependent method, we isolated the species *Pseudomonas*, *Paenarthrobacter*, and *Bacillus* which is the dominant species in its fairy ring soil, and these bacteria are the ideal materials for conducting the following test. Even so, future studies based on culturomics are needed.

For the 20 species isolated, we tested their hyphal growth promoting potential firstly and the results showed that seven strains promoted the hyphal growth rate of *F. luteovirens*, among them the genera *Pseudomonas*, *Bacillus*, *Arthrobacter*, *Microbacterium* were previously reported to contain the MHBs (Freyklett et al. 2007). However, as far as we know for other isolates such as *Leuconostoc* (2–58), *Acinetobacter* (1–5), *Stenotrophomonas* (2–62), *Cupriavidus* (2–94), *Stenotrophomonas* (1–12), no studies have shown their hyphal growth promoting ability before. We also found that the isolates *Achromobacter marplatensis* (A89) had antifungal properties described in a former study (Wolf et al. 2002), but exhibited fungal growth promoting ability in the present study.

These interesting new findings could be explained as follows: 1). the mode of interaction between bacteria and ECM fungi may vary under different nutrient conditions (Oh et al. 2018) found that bacteria isolated from the root of *T. matsutake* showed different promoting potential under different nutrient conditions (glucose-rich medium and glucose-poor medium). In the nutrition-poor medium, *Pseudomonas fluorescens* promoted the growth of *Laccaria bicolor* but inhibited it in nutrient-rich medium (Oh and Lim 2018). Furthermore, *Paenibacillus* only showed promotion ability on glucose-poor medium due to their nitrogen-fixing ability (the provision of nitrogen for ECM on glucose-poor medium (Freyklett et al. 2007).

In the present study, all the bacteria have only been tested on PDA medium, which is a nutrition-rich medium. Thus, to investigate the promoting ability of all the isolates comprehensively, testing on nutrition-poor medium must be done in future experiments. 2) Regarding the genotype specificity of MHBs, many studies have shown that different genotypes of ECM fungi may exhibit differently when co-cultured with bacteria (Mediavilla et al. 2016). In our study, we used the single strain *F. luteovirens*, thus results may vary when multiple strains are used. Moreover, Keller et al. (2006) concluded that *Streptomyces sp.* AcH 505 could accelerate the growth of *Suillus bovinus* but inhibits the growth of *Hebeloma cylindrosporum*. This is due to the fact that the antibiotic produced by AcH 505 can inhibit the growth of *H. cylindrosporum* but *A. muscaria* can tolerate such antibiotic (Keller et al. 2006). In addition, Oh et al. (2018) found that anti-fungal activity was more frequently observed in bacteria grown in the glucose-poor medium than in glucose-rich medium (Oh and Lim 2018). Under low nutrient conditions, the bacteria have to compete with other co-existing microbes, this is why the strain *Achromobacter marplatensis* (A89) did not show anti-fungal activity in PDA medium.

Finally, we only identified four strains of MHBs from 10 candidates, all of which showed hyphal growth acceleration ability. Our results confirmed the hypothesis by Dunstan that significant growth promotion is not a reliable predictor of mycorrhiza formation promotion ability (Dunstan et al. 1998). However, as far as we know, this is the first study that constructs mycorrhizal symbiosis between *F. luteovirens* and its host plant. Furthermore, due to the genotype specificity of MHBs and their varied performance in low nutrient conditions, further testing with different genotypes of ECM fungi in nutrient-low conditions is suggested.

Declarations

Declarations

Ethics approval and consent to participate

This article does not contain any studies with animals performed by any of the authors.

Consent for publication

All authors approve the manuscript for publication. I would like to declare on behalf of my co-authors that the work described was original research that has not been published previously, and not under consideration for publication elsewhere, in whole or in part. All the authors listed have approved the manuscript that is enclosed.

Availability of data and material

Materials described in the manuscript, including all relevant raw data, can be freely available to any scientist wishing to use them for non-commercial purposes, without breaching [participant confidentiality](#).

Competing interests

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

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Authors' contributions

Rui Xing, Ming-hang Xu contributed equally to this work. Rui Xing, Ming-hang Xu conceived and designed research, conducted experiments. Fa-qi Zhang, Qing-bo Gao contributed new reagents or analytical tools. Rui Xing, Ming-hang Xu analyzed data. Rui Xing, Ming-hang Xu wrote the manuscript. All authors read and approved the manuscript.

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Figures

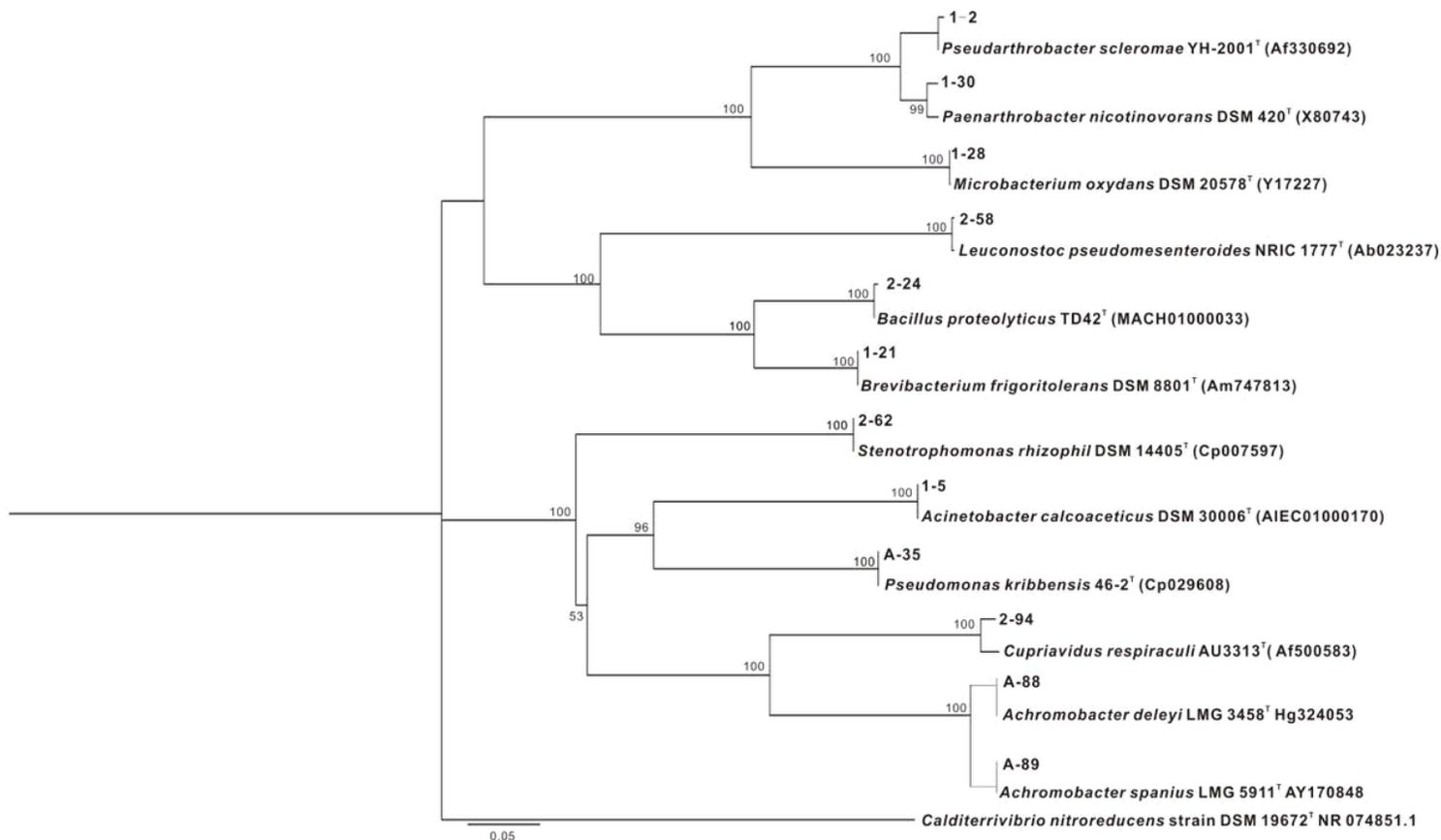


Figure 1

Phylogenetic analysis based on 16S rDNA sequence. Distances and clustering using the neighbor-joining method were performed with the software MEGA X. Bootstrap values based on 1 000 replications are listed as percentages at the branching points

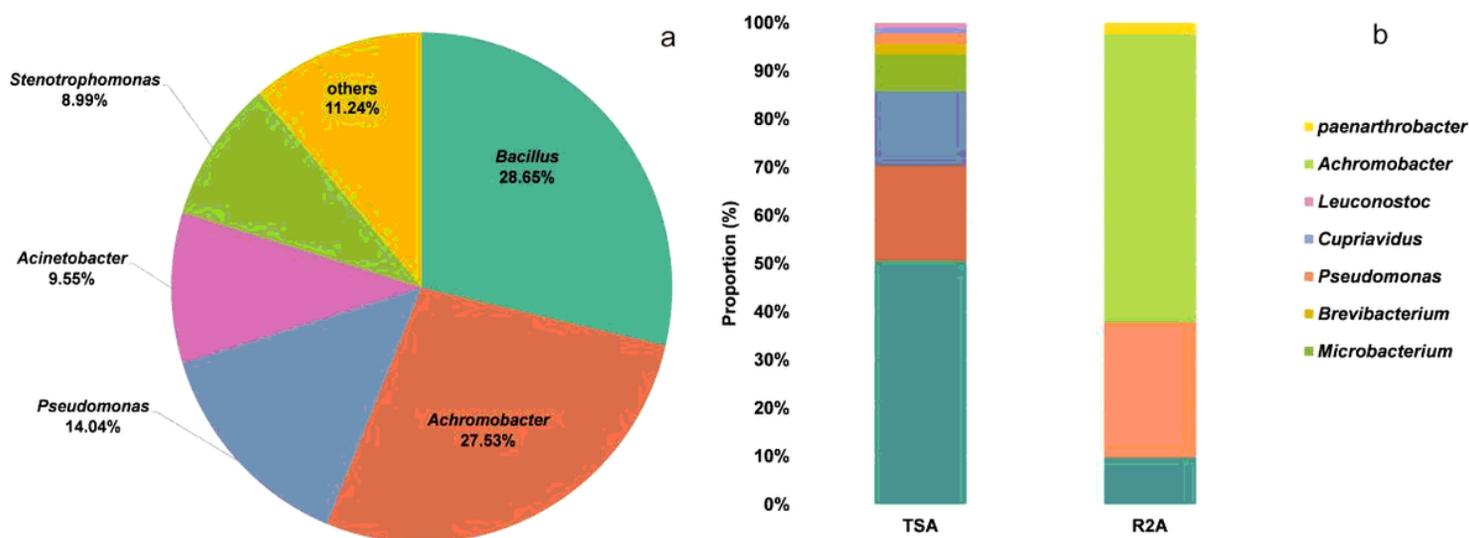


Figure 2

The bacteria isolated from the fairy ring soil of *F. luteovirens* at the genus level (a). The bacteria isolated from different media (TSA and R2A) at the genus level.

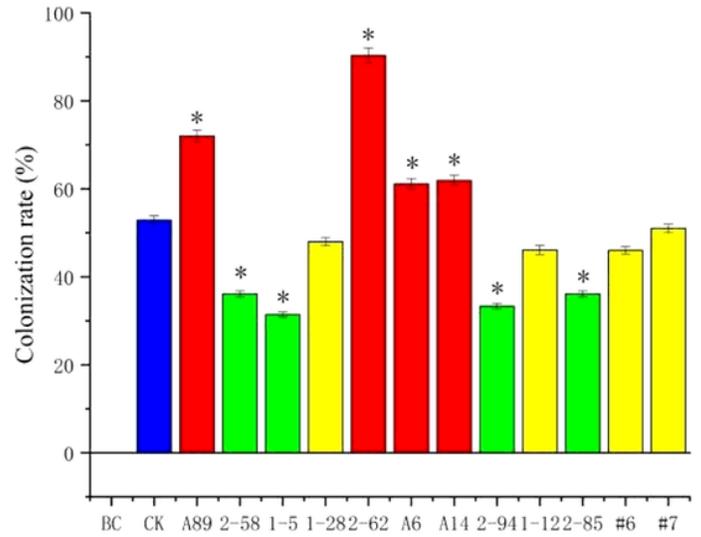
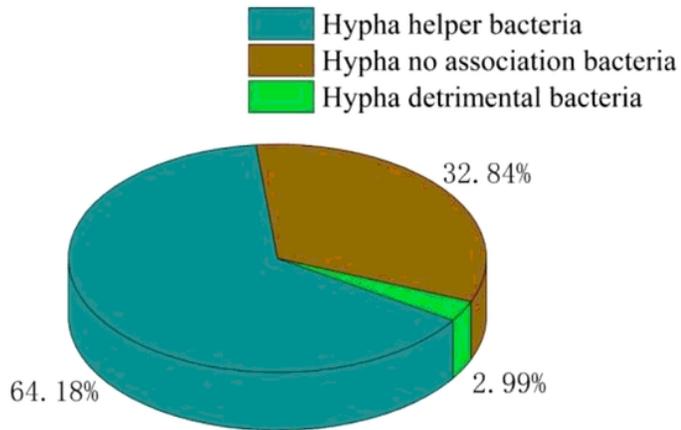


Figure 3

The influence ratio of all three isolates on mycelia (a). Average colonization rate of *F. luteovirens* to *Trifolium repens* when co-cultured with bacteria isolated from the fairy ring soil (b).

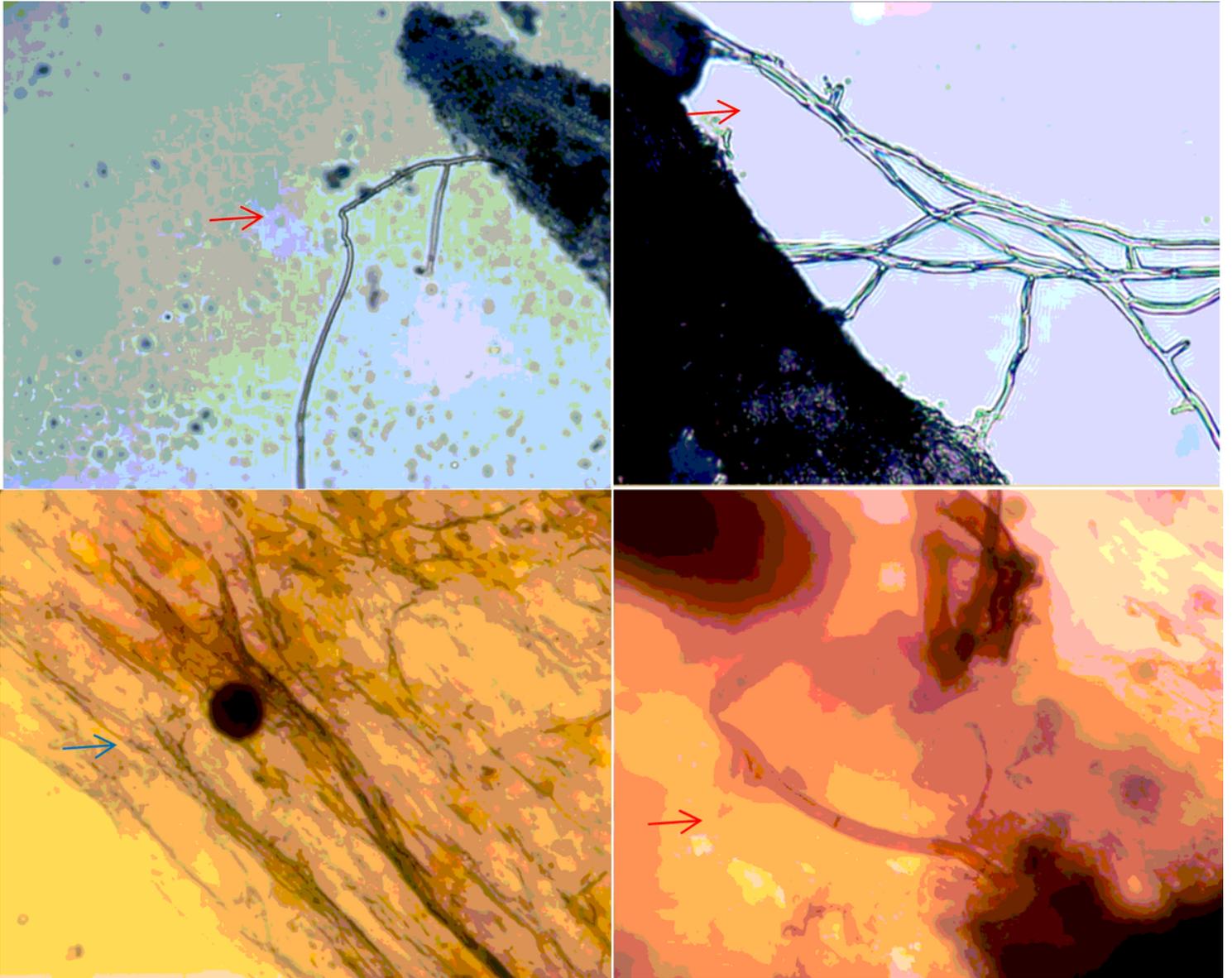


Figure 4

Results of mycorrhizal staining and microscopic examination