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RESEARCH ARTICLE

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Growth Characteristics of *Pseudomonas putida* and *Pleurotus ostreatus* After Co-Cultivation

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ABSTRACT

This study was conducted to investigate the growth characteristics and morphology of *Pleurotus ostreatus* mycelium during co-cultivation with *Pseudomonas putida*. For selection of the most effective *Pseudomonas* species in coculture, Three strains of *Pseudomonas putida* and one strain *of Pseudomonas fluorescens* were co-cultured with heuktari, one of the varieties of *P. ostreatus*, on NPDA agar medium to observe the growth pattern of mycelium. It was found that the microorganism affecting mycelium growth during co-cultivation was *P. putida* KACC 10275, and co-cultivation of these two species resulted in enhanced mycelium growth on both NPDA and sawdust agar media. Furthermore, while primordium formation and fruit body development did not occur on plates inoculated only with heuktari, fruit bodies were observed only on plates where heuktari and *P. putida* were co-cultured, as confirmed by cultivation in the growth chamber for the same duration.

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Pleurotus ostreatus; *Pseudomonas*; co-cultivation; sawdust medium

1. Introduction

Fungi, through interactions with other microorganisms including plants, engage in material exchange and form diverse microbial communities, playing crucial roles in ecology and survival [1]. Within microbial communities, the interaction between fungi and bacteria not only involves competition for protection, dispersal, and nutrient acquisition but also significantly influences each other's growth and development, thus playing a key role in ecosystem functioning [2]. Specifically, bacteria have been found to exert positive or negative effects on the fungal life cycle, influencing mycelial growth and fruit body primordium formation [3,4]. Among them, bacteria such as *Pseudomonas putida* and *Pseudomonas fluorescens* are known to aid in mushroom mycelium growth and primordium formation [5–7].

There are some research findings on fruit body formation and promotion of mycelial growth in *P. ostreatus* due to contact with *P. putida* and *P. fluorescens* [8,9]. However, most previous studies have been limited to *P. putida*, *P. fluorescens*, and mushrooms, particularly those belonging to the Basidiomycota phylum (such as *Pleurotus ostreatus*, *Pleurotus eryngii*, *Lentinula edodes*), remain largely insufficient [5,10–12]. Additionally, even in Agaricus bisporus, not all Pseudomonas putidastrains have been effective in promoting mycelial growth [13]. Therefore, research is needed to identify which strains are effective in promoting mycelial colonization in Pleurotus ostreatus.

Edible mushrooms, comprising approximately 2,000 species worldwide, hold high nutritional value [14]. Commonly cultivated globally are species such as *Agaricus bisporus*, *Lentinula edodes*, and *Pleurotus ostreatus* [15]. Among them, *P. ostreatus* is known as a representative white-rot fungus capable of growing on agricultural lignocellulosic waste [16] and is one of the most widely cultivated and consumed mushrooms worldwide [17]. P. ostreatus exhibits variations in morphology depending on external factors such as nutritional substrates and environmental conditions (temperature, humidity, light intensity, and pH) during growth [18]. Therefore, in this study, we aimed to investigate the morphological changes of *P. ostreatus* mycelium through co-cultivation with *P. putida* on sawdust agar medium, aiming to provide foundational data for Bacterial-Fungal Interactions (BFI) in *P. ostreatus* cultivation.

2. Materials and methods

2.1. Microbial strains

For investigating the growth characteristics of *P. ostreatus* mycelium under beneficial microorganism treatment, the

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strain "Heuktari (KMCC00463)" of *P. ostreatus* variety was chosen. The heuktari strain was obtained from the Microbial Research Institute of the Gyeonggi-do Agricultural Research and Extension Services, cultured at 25°C on PDA, and subcultured every 9 days for use as an inoculum.

The three strains of *P. putida* (KACC 10275, KACC 10267, KACC 10192) and one strain of *P. fluorescens* (KACC 10278) used in the experiment were obtained from the Korean Agricultural Culture Collection (KACC) at the National Institute of Agricultural Sciences (Table 1).

2.2. Comparison of mycelial growth characteristics for beneficial microorganism selection

2.2.1. Mycelial growth characteristics on NPDA agar medium

We utilized NPDA (Nutrient Broth Agar + Potato Dextrose Agar) medium, a 1:1 mixture of NA (Nutrient Broth Agar) commonly used for bacterial culture and PDA (Potato Dextrose Agar) frequently used for fungal culture.

2.2.1.1. Cultivation and growth patterns of 4 strains of Pseudomonas mycelium. The four strains of *Pseudomonas* were inoculated as spots onto NPDA plates after incubating in NB (Nutrient Broth) medium at 25 °C for 48 h. Subsequently, the plates were incubated at 25 °C for 24 h in a dark chamber. *P. ostreatus* mycelium was inoculated onto the plates using a 5 × 5 cm cork borer, and on the 6th day, the length of the co-cultivated mycelium with the four strains of *Pseudomonas* from the center was measured using Vernier calipers (CD-15PSX, Kawasaki, Japan).

2.2.1.2. Petri dish cultivation. *P. putida* KACC 10275 was cultured in NB medium at 25 °C for 48 h, and the fungal culture was diluted using a spread plate method on NPDA medium to measure CFU (Colony Forming Units), resulting in 6.4 × 10^-4 CFU/mL with an OD value of 0.22 at 600 nm. *P. putida* was cultured in a Petri dish (90 × 15 mm) in a cross shape at 25 °C for 24 h under dark conditions. *P. ostreatus* mycelium was inoculated onto the NPDA plate in a cross shape at the center using a 5 × 5 mm cork borer, then incubated at 25 °C for 6 days under dark conditions before measuring growth using Vernier calipers.

2.2.1.3. Partition petri dish cultivation. Using a partition petri dish (90 × 15 mm, SPL Life Sciences, Korea), *P. putida* (KACC 10275) was inoculated with 100 µL onto NPDA medium and cultured at 25 °C for 24 h. Then, heuktari was inoculated onto the non-inoculated section, and mycelium length was measured using Vernier calipers for up to 6 days.

2.2.1.4. Comparison of mycelial growth on sawdust agar medium. The medium was composed of a ratio of 5 parts poplar, 3 parts barley, and 2 parts beet pulp by volume, adjusted to 65 ± 2% moisture and pH 7.0 ± 0.3. Each test tube (30 × 200 mm) was filled with 80 ± 2 g of the medium and sterilized at 121 °C for 90 min using a high-pressure sterilizer (HV-110, Hirayama, Japan). After cooling for 12 h, *P. putida* was diluted to 6.4 × 10^-4 CFU/mL and 1 mL was inoculated into the center. The tubes were incubated at 25 °C for 24 h, then "heuktari" PDA inoculum was cut into 1/8 and inoculated. Growth was measured at 10-day intervals up to 30 days using Vernier calipers in a 25 °C incubator.

2.3. Statistical analysis

The statistical analysis of the experimental results was conducted to test the significance among the mean values using Duncan's multiple range test. T-tests and analysis of variance (ANOVA) were performed using the R (version 4.3.2) program to test statistical significance at a 5% level of significance.

3. Results

3.1. Selection of beneficial microorganisms

3.1.1. Comparison of Pseudomonas species

Regarding the growth data of *P. putida* (3 strains) and *P. fluorescens* (1 strain) cultured as spots on the 6th day, it was observed that the growth for KACC10275 was 4.3 cm, for KACC10278 was 3.8 cm, for KACC10192 was 3.67 cm, and for KACC10267 was 3.35 cm (Figures 1 and 2).

To observe the growth pattern of mycelium according to beneficial microorganisms, *P. putida* was treated on NPDA agar medium, followed by inoculation of heuktari for co-cultivation. The results are shown in Figures 3 and 4. When co-cultivated with *P. ostreatus*

Table 1. Oyster mushroom cultivation: a list of experimented effective microorganisms.

KACC No.	*Pseudomonas*	Source	Growth temperature	Characteristics
10275	*P. putida*	Soil	25 °C	Oxidation of phenol and aromatic ring compounds
10267	*P. putida*	Soil by camphor enrichment	26 °C	6-oxocineole utilization. Diketocamphane monooxygenases production.
10192	*P. putida*	–	26 °C	Oxidation of L-tryptophan *via* aromatic pathway
10278	*P. fluorescens*	–	25 °C	Degradation of naphthalene and phenol Species

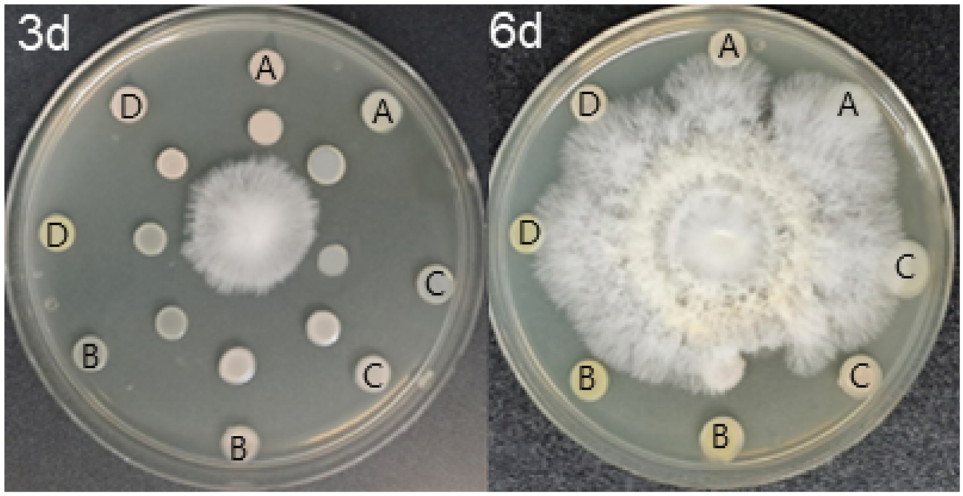

Figure 1. Morphology of *Pseudomonas ostreatus* mycelium on NPDA medium inoculated with 3 strains of *P. putida* and 1 strain of *P. fluorescens* on the 3rd and 6th days. A, KACC10275; B, KACC10267; C, KACC192; D, KACC10278.

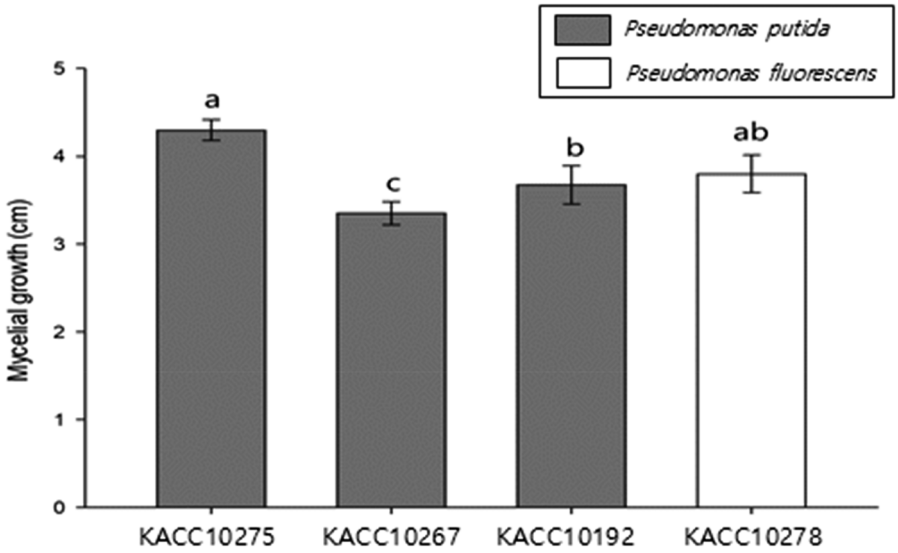

Figure 2. Mycelial growth of oyster mushrooms in NPDA medium inoculated with *Pseudomonas* on the 6th day. Different letters are significantly different by Duncan's multiple range test ($p < 0.05$).

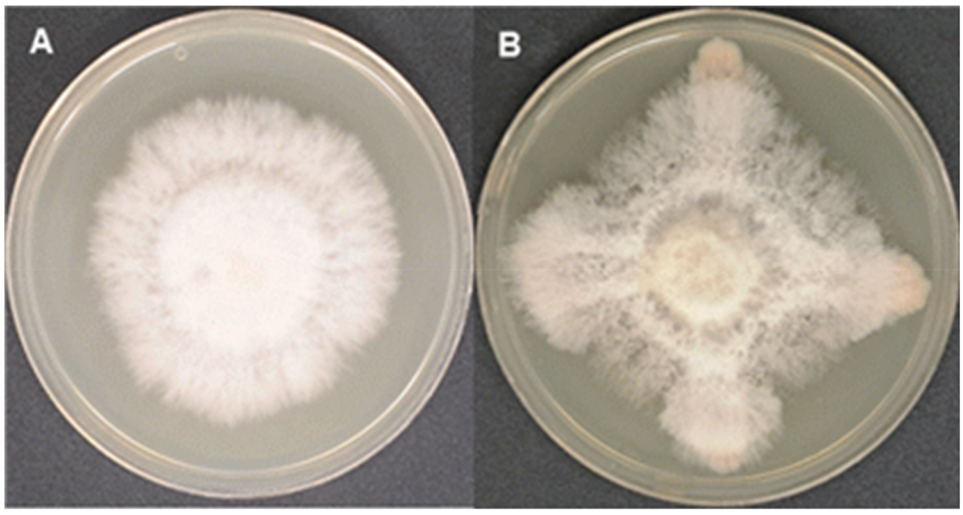

Figure 3. Morphology of mycelium for *Pseudomonas ostreatus* in NPDA petri dish with PPI in 6 days.

mycelium using the strain KACC10275 of *Pseudomonas* spp., the diameter of *P. ostreatus* mycelium after 6 days of cultivation was measured. The PPI (*Pseudomonas putida* inoculation) treatment showed a diameter of 6.58 cm, which was higher than the control at 5.76 cm.

The results of cultivating heuktari and *P. putida* in a Partition petri dish (Figures 5 and 6) showed similar outcomes to those observed in conventional Petri dishes. The mycelium of heuktari grown in the partition area opposite to where *P. putida* was inoculated exhibited superior growth compared to the untreated control (Figure 7).

3.1.2. Comparison of fruiting body growth on sawdust agar medium

To observe the growth of oyster mushroom fruiting bodies on sawdust agar medium, the growth on days 10, 20, and 30 in test tubes was examined. The control measurements were 2.38, 6.54, and 7.93 cm, respectively. In contrast, for the PPI treatment, the measurements were 3.38, 7.48, and 9.3 cm, respectively. It was observed that in all instances, the PPI treatment showed a tendency to grow more than 1 cm longer than the control (Table 2 and Figure 8).

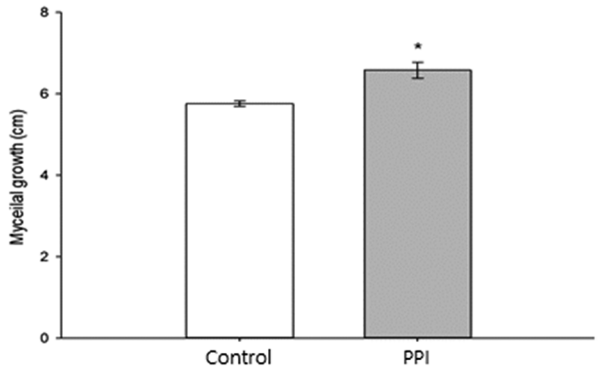

Figure 4. Control and *Pseudomonas putida* cross culture on NPDA agar after 6 days. T-test; *, Significant at the 0.05 probability levels, respectively.

4. Discussion

The genus *Pseudomonas* includes more than 300 validly recognized species and is characterized by rich genetic diversity [19]. Although members of the genus *Pseudomonas* share specific morphological, metabolic, and genomic traits, the diversity of niches and lifestyles adopted by the family members is vast. Introduction Representatives of the genus Pseudomonas became favored workhorses in biotechnology in recent years [20, 21], due to their versatile metabolism and high tolerance toward organic compounds [22–24].

One species of the group, *Pseudomonas putida*, thrives as a colonizer of plant roots and frequently inhabits soils polluted with various types of chemical waste [25]. *P. putida*, identified as a common mycorrhizal helper bacteria (MHB) [26], thereby enhancing the mycelial expansion rate of *A. bisporus* [27]. demonstrated that among 23 microbial inoculants evaluated, *P. putida* exhibited the most pronounced effect, enhancing *A. bisporus* yield by 14.4% [28]. In this study, we also confirmed results consistent with the aforementioned findings, observing an enhancement in the mycelial expansion rate of *A. bisporus*. *P. putida* is generally a soil-derived bacterium, with mechanisms elucidated primarily in compost-based substrates used for the cultivation of A. bisporus. In *P. ostreatus*, some studies have also reported the promotion of fruiting body formation and mycelial growth through interaction with *P. putida* and *P. fluorescens* [8, 9], but the principles of their interactions remain unclear. Furthermore, not all strains of *P. putida* are effective in promoting the growth of *A.*

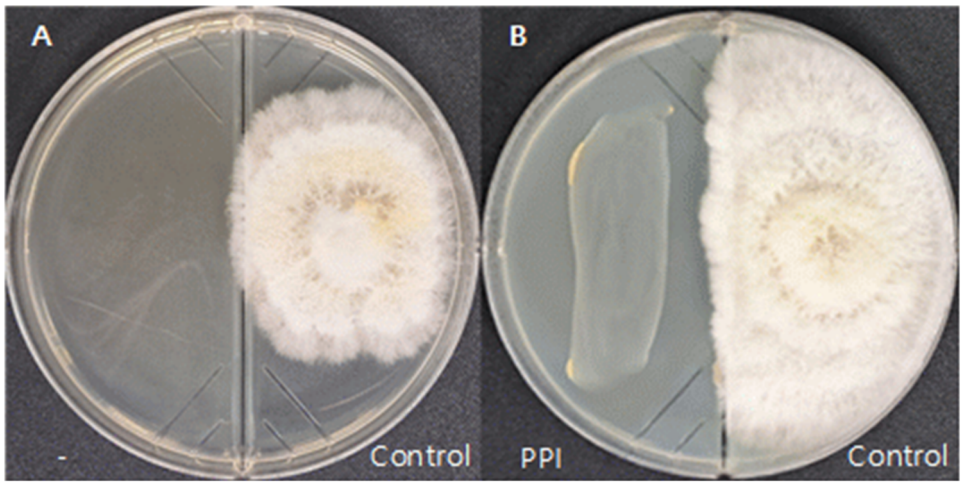

Figure 5. Morphology of mycelium for *Pseudomonas ostreatus* in NPDA partition petri dish with PPI in 6 days. A, control; B, PPI (*P. putida* inculation).

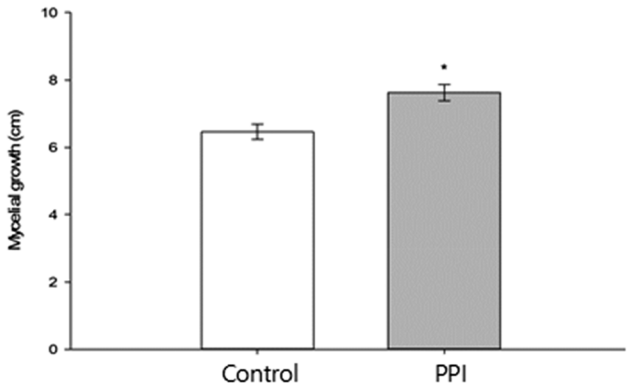

Figure 6. Control and PPI day 6 mycelium cultured in partition petri dish medium. T-test; *, Significant at the 0.05 probability levels, respectively.

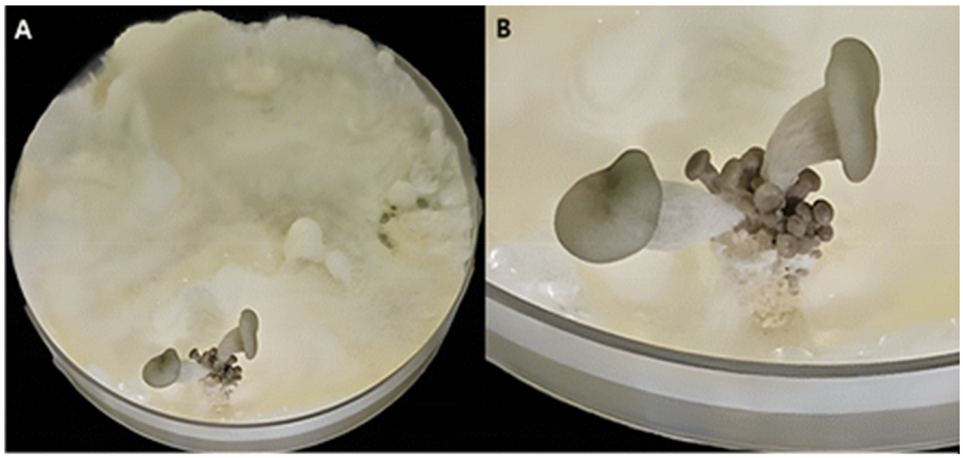

Figure 7. Morphology fruit body for oyster mushroom inducted by *Pseudomonas putida* inoculation.

Table 2. *Pseudomonas ostreatus* mycelium in substrate with *P. putida*.

	Mycelium growth (cm)		
Treatment	10days	20days	30days
Control	2.38	6.54	7.93
PPI	3.38*	7.48*	9.30*

Substrate (Sawdust: Beet pulp: Cotton seed meal = 5: 3: 2).
T-test; *, Significant at the 0.05 probability levels, respectively.

bisporus [13], and it is assumed that a similar phenomenon may occur in *P. ostreatus*. Therefore, for reliability and stability, we did not perform direct screening of strains. Instead, we selected three strains of *P. putida* and one strain of *P. fluorescens* with well-characterized properties that were obtained from the Agricultural Microbiology Department at the National Institute of Agricultural Sciences (KACC), which maintains environmental conditions similar to the cultivation temperature of *P. ostreatus*.

Furthermore, upon comparing four *Pseudomonas* species, we found that *P. putida* obtained from soil demonstrated the most significant improvement in the mycelial expansion rate of *A. bisporus*. Through methods used in Zhao et al. [29], Jasim et al. [30], and Shu et al. [31], we confirmed that even within the same *Pseudomonas* specie, the degree of impact on A. bisporusvaries.

When co-cultivated, c strains attach to *A. bisporus* mycelium, and during this process, ACC deaminase produced by the strains degrades ACC, a precursor of ethylene produced by *A. bisporus* mycelium. This mechanism results in a reduction in ethylene production by the mushroom mycelium, leading to accelerated mycelial growth [32]. Additionally, the metabolism of *P. putida* could accelerate the growth rate of the mycelium [8]. This led to faster mycelial growth on PPI in co-cultivation with *P. putida*. The presence of saprophytic bacteria such as *Pseudomonas putida* which colonize the casing layer normally used in commercial cultivation of *A. bisporus* stimulates perimordia formation [33–35]. Additionally, we confirmed that the mycelial expansion rate of *A. bisporus* increased not only on PDA medium but also on actual sawdust substrate. Furthermore, when inducing primordia formation on PDA medium, we observed that *A. bisporus* primordia formation occurred only on the PPI plates within the same time period.

Figure 8. Growth of *Pseudomonas ostreatus* mycelium in sawdust medium with *P. putida*.
A, 10 days; B, 20 days; C, 30 days.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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