



Growth promotion of *Pleurotus ostreatus* by *Pseudomonas fluorescens* on different culture media

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ABSTRACT

The edible mushroom *Pleurotus ostreatus* is widely grown globally. The use of biological agents to promote mushroom growth and yield has always been a concern for scientists and mushroom growers. This study employed co-cultivation of *Pleurotus ostreatus* and *Pseudomonas fluorescens* on various culture media to investigate the impact of *Pseudomonas fluorescens* on the growth of *Pleurotus ostreatus*. The results showed that *Pseudomonas fluorescens* promoted the growth of the edible fungus *Pleurotus ostreatus*. They increased the mycelium extension rate on PDA, paddy rice, and rubber sawdust media. The addition of *P. fluorescens* to the mushroom-growing media resulted in faster mycelial growth, with a rate of up to 5 mm/day, and a thicker mycelium density compared to the control (*Pleurotus ostreatus* mycelium not incubated with *P. fluorescens*). Moreover, under the same culture conditions, *Pseudomonas fluorescens* stimulated primordium formation 5 days earlier and reduced the total number of days for cultivation compared with no co-culture. The research results suggest that adding beneficial bacteria to mushroom growth will help mycelium grow more vigorously and shorten propagation time and total culture time, providing a basis for further research into their potential as a mushroom growth promoter in commercial cultivation. The results indicate that *P. fluorescens* promoted mycelial growth and accelerated primordium formation of *P. ostreatus*, suggesting its potential as a biological growth-promoting agent. Further studies are needed to evaluate its effects on fruiting body yield and its practical application in commercial cultivation.

Key words: Edible mushroom, growth substrate, vigorous, growth, mycelium, fruiting body.

INTRODUCTION

Pleurotus spp. are commonly known as oyster mushrooms. They belong to the class Agaricomycetes, the order Agaricales, and the family Pleurotaceae (Seethapathy *et al.*, 16). The global of *P. ostreatus* has grown considerably over the past several decades. Its rising popularity can be attributed to its simple cultivation, as well as its significant nutritional and medicinal benefits. *P. ostreatus* is rich in proteins, carbohydrates, lipids, vitamins, minerals, amino acids, and dietary fiber, while containing relatively low levels of calories, fat, cholesterol, and sodium (Raman *et al.*, 14). In addition to its nutritional value, *P. ostreatus* contains various bioactive compounds, including polysaccharides, peptides, oligosaccharides, fatty acids, glycoproteins, terpenoids, lectins, and nucleosides, which are associated with antioxidant, antidiabetic, immunomodulatory, anti-inflammatory, antihypertensive, antitumor, and antibacterial activities (Devi *et al.*, 6; Tiupova *et al.*, 18). Furthermore, its ability to grow on diverse lignocellulosic materials, such as sawdust, cornstalks, paddy straw, bagasse, cotton waste, and banana leaves, makes *P. ostreatus*

an important crop for the valorization of agricultural and industrial residues (Raman *et al.*, 13).

The cultivation and productivity of edible mushrooms are influenced by numerous microorganisms associated with the cultivation substrate and growing environment. Some bacteria are pathogenic to cultivated mushrooms and can negatively affect yield and quality (Saiyad & Srivastava, 15). In contrast, other bacteria can establish beneficial interactions with mushroom mycelia and promote mycelial growth, morphological development, enzyme production, gene expression, and fruiting body formation (Park *et al.*, 12).

Recent research indicates that certain bacteria have the potential to enhance mushroom production (Kaur *et al.*, 7; Kumari & Naraian, 9). Various species within the *Pseudomonas* genus play key roles in mushroom growing (Park *et al.*, 12). Previous studies have shown that plant growth-promoting bacteria, particularly fluorescent *Pseudomonas*, can enhance the growth of oyster mushrooms. However, their effects vary depending on the bacterial strain, mushroom species, and cultivation substrate. Therefore, indigenous bacterial strains continue to be studied to identify strains that possess optimal

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characteristics suited to local cultivation conditions. Promotion of mushroom growth by biological agents instead of chemicals is a matter of great concern in the mushroom-growing industry. In this study, a native *P. fluorescens* strain was evaluated for its ability to promote *Pleurotus ostreatus* growth under three cultivation conditions: agar, grain, and sawdust media. The findings provide preliminary evidence for the potential use of native *P. fluorescens* as a biological growth-promoting agent for *P. ostreatus* and provide a basis for further investigation of the underlying bacterium–mushroom interaction.

MATERIALS AND METHODS

Bacterial strain *P. fluorescens* (PF) and fungal strain *P. ostreatus* (P003) were obtained from the Institute of Life Sciences, Vietnam Academy of Science and Technology (VAST), Ho Chi Minh City, Vietnam. *P. fluorescens* (PF) was originally isolated from the fruiting bodies of the ectomycorrhizal mushroom *Cantharellus cibarius* (Loan *et al.*, 11). Strains were maintained on potato dextrose agar (PDA) at 4 °C. They were activated on the same medium at 25 °C for 10 days in a petri dish to use for experiments.

PDA was prepared using 200 g potato extract, 20 g dextrose, 15 g agar, and 1,000 mL distilled water (pH 6.0). The medium and Petri dishes (80 mm diameter) were sterilized at 121 °C for 15 min. A 10-mm-diameter mycelial plug of *Pleurotus ostreatus* P003 was placed in the center of each PDA plate. P003 alone served as the control, whereas P003 co-cultured with *Pseudomonas fluorescens* PF was used as the treatment. Each treatment included three Petri dishes in each experimental run, and the experiment was independently repeated three times. The plates were randomly arranged in the incubator and maintained at 25 °C, 75% relative humidity (RH), and in darkness. Mycelial diameter was recorded daily for 8 days, and colony morphology was observed.

Paddy rice was washed, soaked for 12 h, boiled for 20 min, and drained. Approximately 150 g of prepared grain was transferred into each 150-mL glass bottle and sterilized at 121 °C for 20 min using a Hirayama autoclave (HV-110, Hirayama, Japan).

After cooling to room temperature, the grain was inoculated with *P. ostreatus* P003 alone (control) or P003 spawn associated with *P. fluorescens* PF. Three bottles were used per treatment in each experimental run, and the experiment was independently repeated three times. The bottles were randomly arranged and incubated at 25 °C in darkness. Mycelial growth was assessed after 5, 7, and 9 days.

The substrate consisted of rubber sawdust, rice bran, and agricultural lime at a ratio of 100:10:1 (dry weight basis), with the moisture content adjusted to approximately 65%. Approximately 500 g of substrate was placed in each polypropylene bag (15 × 25 cm) and sterilized at 121 °C for 30 min. After cooling to room temperature, each bag was inoculated with 2 g of spawn. The control treatment received *P. ostreatus* P003 spawn, whereas the treatment received P003 spawn associated with *P. fluorescens* PF. Ten bags were used per treatment in each experimental run, and the experiment was independently repeated three times. The bags were randomly arranged and incubated vertically at 25 °C in darkness. Mycelial growth was measured every 6 days for 20 days, and colony morphology was recorded.

All statistical analyses were carried out using Statistical Package for Social Sciences (SPSS) version 16. One-way analysis of variance (ANOVA) and Tukey test were applied for comparisons; the results were considered significant at $p < 0.05$. Each value is expressed as mean $\pm$ standard deviation. All the experiments were conducted in triplicate.

RESULTS AND DISCUSSION

To evaluate the effect of *P. fluorescens* on the growth of *P. ostreatus* on agar medium, we co-cultured them on PDA medium, monitored the mycelium expansion, and the mycelial density daily. The results are shown in Table 1 and Figures 1.

The results show that the PF strain promoted P003 mycelium growth. The mycelium is thicker and grows faster, especially when the P003 mycelium is present in the presence of PF, the mycelium grows much stronger than in the area without this bacterium. After 8 days of culture on PDA media, the colony diameter of P003 + PF was 75.6 mm, while the diameter of P003 mycelium was 70.0 mm. By daily

Table 1: *P. ostreatus* mycelial growth and density on PDA medium incubated with *P. fluorescens*.

Treatment	Mycelial growth (mm)				Mycelial density
	5 days	6 days	7 days	8 days	
P003 + PF	43.8 $\pm$ 0.84	53.4 $\pm$ 0.89	64.4 $\pm$ 0.55	75.6 $\pm$ 0.89	++
Control (P003)	39.6 $\pm$ 0.55	48.2 $\pm$ 0.45	58.6 $\pm$ 0.55	70.0 $\pm$ 0.71	+

P003+PF: *P. ostreatus* mycelium was incubated with *P. fluorescens*; Control: *P. ostreatus* mycelium culture only; ++: thick; +: thin



Fig. 1. The mycelial morphology of *P. ostreatus* on PDA medium inoculated with *P. fluorescens* after 7 days.

monitoring, we observed that the mycelial expansion rate increased up to 5 mm/day when treated with the PF strain.

Bacteria play an important role in the life cycle of fungi, as they can either promote or inhibit fungal growth (Suarez *et al.*, 17). Bacteria can negatively affect fungi by partially or completely inhibiting colonization of the substrate by the mycelium, decomposing the fruiting body, affecting fruiting body development, and reducing yield (Carrasco and Preston, 3). In addition, some reports have shown that certain bacteria can stimulate fruiting body formation, mycelial growth, and the biological control of fungal pathogens (Braat *et al.*, 2). The use of growth-promoting bacteria in the cultivation of edible mushrooms can increase yields and support the production of healthy foods. Therefore, researchers are particularly interested in this group of bacteria. In this study, the effects of *P. fluorescens* on *P. ostreatus* growth were investigated on PDA agar, grain, and sawdust media. We confirmed that the growth rate of *P. ostreatus* mycelium increased on all media used.

Normally, during fungal culture, when infected by another fungus or bacterium, a septum forms between them and inhibits the growth of the cultured mushroom. However, the presence of PF bacteria in the PDA culture medium not only did not inhibit the growth of P003 but also promoted the growth of this species. Within the scope of this study, we only investigated the effect of PF on the growth of P003 mycelium. Besides promoting mycelial growth and shortening cultivation time compared with the control, *P. fluorescens* also stimulated *P. ostreatus* primordial formation on PGA and grain medium (Fig. 2). These results are quite similar to those of previous reports studying the effects of *Pseudomonas* spp. on the growth of edible mushrooms such as *A. bisporus*, *P. eryngii*, and *P. ostreatus* (Cho *et al.*, 4; Kim *et al.*, 8; Park *et al.*, 11; Rainey, 13).

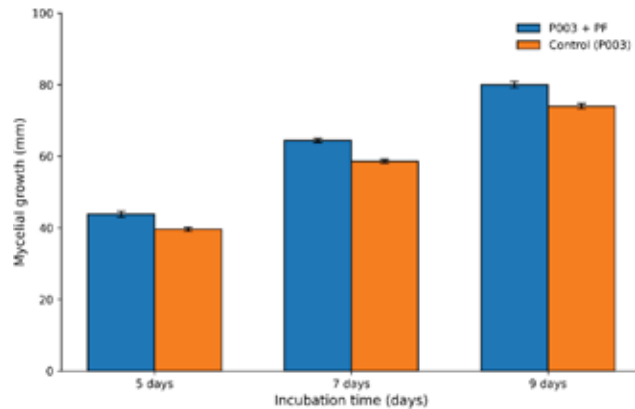


Fig. 2. P003+PF and control (P003) mycelium growth in agar medium.

Different species of the genus *Pseudomonas* affect fungal growth differently. Rainey (12) showed that *P. putida* increased the mycelial extension rate and mushroom yield by up to 26.6% compared with the uninoculated control. In contrast, *P. tolaasii* and *P. aeruginosa* significantly inhibited the growth and mycelial degradation of *A. bisporus* and significantly reduced mushroom yield (-72.5%). When studying the effect of growth-promoting bacteria on the growth of button mushroom mycelium and the control of pathogenic factors in vitro, Asgharzadeh *et al.* (1) found that all *Pseudomonas* strains used in the study were capable of promoting mycelial growth. Many species of the genus *Pseudomonas* play an important role in mushroom cultivation, of which *P. putida* is considered the most important.

P. fluorescens, a symbiotic bacterium, belongs to the Rhizobacteria group. This is an important group of bacteria that plays a major role in promoting plant growth, biocontrol, enhancing resistance, reducing the severity of various diseases, and improving soil (Danish *et al.*, 5; Li *et al.*, 10). Yang *et al.* (20) reported that *P. fluorescens* has several growth-promoting properties, such as IAA and siderophore production and the decomposition of organic phosphorus, inorganic phosphorus, and insoluble potassium. Application of *P. fluorescens* to soil increased the diversity of soil microbial communities, reduced soil pH, changed the structure of soil microbial communities, and increased enzyme activity and nutrient content in the soil. *P. fluorescens* also increased the photosynthesis rate, increased the number of lateral roots, and improved root structure, thereby increasing nutrient content and biomass, and ultimately promoting plant growth.

Paddy rice was used as media to indicate the effect of *P. fluorescens* on the mycelial growth of *P. ostreatus*. The results showed that the presence of

P. fluorescens in culture medium increased mycelial growth of *P. ostreatus*. Mycelium extensions were faster than those of the control treatments. On the 9th day of incubation, the mycelium length medium was 81 mm, while the control was 74.3 mm. For this experiment, in the presence of bacteria, the time for the mycelium of *Pleurotus pulmonarius* cover the whole bottle was 9 days, while the control was 11 days. Besides promoting mycelial growth and shortening cultivation time compared to the control, *P. fluorescens* also stimulates *P. ostreatus* primordial formation in PGA and grain medium (Fig. 3). These results are quite similar to those of previous reports studying the effects of *Pseudomonas* spp. on the growth of edible mushrooms such as *A. bisporus*, *P. eryngii*, and *P. ostreatus* (Cho *et al.*, 4; Kim *et al.*, 8; Park *et al.*, 11; Rainey 13). Different species of the genus *Pseudomonas* affect fungal growth differently. Rainey (12) showed that *P. putida* increased the mycelial extension rate and mushroom yield by up to 26.6% compared with the uninoculated control. In contrast, *P. tolaasii* and *P. aeruginosa* significantly inhibited the growth and mycelial degradation of *A. bisporus* and significantly reduced mushroom yield (-72.5%). When studying the effect of growth-promoting bacteria on the growth of button mushroom mycelium and the control of pathogenic factors in vitro, Asgharzadeh *et al.* (1) found that all *Pseudomonas* strains used in the study were capable of promoting mycelial growth. Many species of the genus *Pseudomonas* play an important role in mushroom cultivation, of which *P. putida* is considered the most important.



Fig. 3. *P. fluorescens* promoted *P. ostreatus* primordial formation in PGA (left) and grain (right) medium.

P. fluorescens, a symbiotic bacterium, belongs to the Rhizobacteria group. This is an important group of bacteria that plays a major role in promoting plant growth, biocontrol, enhancing resistance, reducing the severity of various diseases, and improving soil (Danish *et al.*, 5; Li *et al.*, 10). Yang *et al.* (20) reported that *P. fluorescens* has several growth-promoting properties, such as IAA and siderophore production and the decomposition of organic phosphorus, inorganic phosphorus, and insoluble potassium. Application of *P. fluorescens* to soil increased the diversity of soil microbial communities, reduced soil pH, changed the structure of soil microbial communities, and increased enzyme activity and nutrient content in the soil. *P. fluorescens* also increased the photosynthesis rate, increased the number of lateral roots, and improved root structure, thereby increasing nutrient content and biomass, and ultimately promoting plant growth.

On sawdust medium, we investigated the effect of PF on the growth of P003 based on the parameters of mycelium length and time of primordial formation. The results are shown in Table 2 and Figure 4. The results showed that on sawdust medium, the mycelium length of P003 + PF was higher than that of the control. After 20 days of culture, P003 + Pf mycelium length was 81 mm, and that of P003 was 75.33 mm. In addition, the presence of *P. fluorescens* stimulated primordial formation 5 days earlier.

Park *et al.* (12) confirmed that even within the same *Pseudomonas* species, the degree of impact on fungal growth varies. Orban *et al.* (19) demonstrated that the growth-promoting effect was mainly caused by volatile organic compounds, while



Fig. 4. *P. ostreatus* mycelium and body fruit in a rubber sawdust medium inoculated with *P. fluorescens* P003 (control); P003+PF (*P. ostreatus* co-cultivation with *P. fluorescens*).

Table 2: *P. ostreatus* growth on rubber sawdust medium incubated with *P. Fluorescens*.

Treatment	Mycelial growth (mm)			Primordial formation (days)
	8 days	14 days	20 days	
P003 + PF	17.67 ± 0.58	46.33 ± 1.53	81.00 ± 1.73	47
Control (P003)	12.3 ± 1.00	41.00 ± 1.00	75.33 ± 0.58	52

Values represented as mean ± standard deviation (SD) of triplicate analysis

the inhibitory effect was most likely related to non-volatile compounds. Volatile organic compounds from strain M48F of *P. peoriae* enhanced the growth of *P. ostreatus* and *P. eryngii* compared with the control without bacteria. Furthermore, the effects of bacteria on mycelial growth are highly species-dependent and may affect different fungal species. Bacteria and fungi influence their environment in a species-dependent manner to create habitats that are mutually beneficial. Bacteria that promote fungal growth may inhibit the growth of other organisms; therefore, further research on their ecological relationships, as well as research to identify natural compounds that promote fungal growth, is needed in the future.

In conclusion, *P. fluorescens* promoted mycelial growth and primordial formation of *P. ostreatus* and shortened the time required for mycelial colonization. These findings suggest that *P. fluorescens* may support the vegetative growth and early developmental stages of *P. ostreatus*. Further studies evaluating fruiting body development and yield parameters are required to determine its potential application in mushroom production.

AUTHOR'S CONTRIBUTION

Writing original draft, methodology, and data curation (PNH); Writing review & editing, methodology, data curation (PVH); Writing original draft, data curation (TKD); Writing original draft, data curation (TTNH); Writing original draft, formal analysis (NTTH).

DECLARATION

The authors declare no conflict of interest.

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