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Short Communication

Effect of *Pseudomonas* sp. P7014 on the growth of edible mushroom *Pleurotus eryngii* in bottle culture for commercial production

Min Keun Kim [c], Renukaradhya K. Math [a], Kye Man Cho [a], Ki Jae Shin [a], Jong Ok Kim [a], Jae San Ryu [c], Young Han Lee [c], Han Dae Yun [a,b,*]

[a] *Division of Applied Life Science, Gyeongsang National University, Chinju 660-701, Republic of Korea*
[b] *Research Institute of Agriculture and Life Science, Gyeongsang National University, Chinju 660-701, Republic of Korea*
[c] *Division of Plant Environmental Research, Gyeongsangnam-do Agricultural Research and Extension Service, Chinju 660-360, Republic of Korea*

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Abstract

Addition of bacterial culture strain P7014 and its supernatant to the mushroom growing media resulted in mushroom mycelia run faster. Mycelial growth rate of *Pleurotus eryngii* was increased up to 1.6 fold and primordial formation was induced one day earlier. Moreover, it was supposed that addition of bacteria had beneficial applications for commercial mushroom production, which appreciably reduced total number of days for cultivation of about 5 ± 2 days compared with uninoculated, which took 55 ± 2 days.

Keywords: *Pleurotus eryngii*; Commercial production; Growth promoting; *Pseudomonas* sp. P7014

1. Introduction

Among the edible mushrooms, the king oyster mushroom (*Pleurotus eryngii*) is one of the most popular mushrooms in Asia, Europe and North America (Estrada and Royse, 2007). The increasing popularity of *P. eryngii* among consumers is due to its flavor, texture and shelf life.

Proper composting followed by pasteurization and conditioning treatments, a wide range of plant wastes such as sawdust, wheat straw (Bernardo and Edgardo, 2007), and weed plants can also be used as substrates for *P. eryngii* production (Nirmalendu and Mina, 2007). The casing step is required for the induction of fruiting-body formation, induction being due to the presence of saprophytic bacteria such as pseudomonad in the casing medium (Rainey, 1991). Ntougias et al. (2004) reported that Gram-negative bacteria are existed in spent mushroom compost (SMC), or even in the mushroom cultivation substrate which was analyzed just after pasteurization and cooling. Modern commercial mass cultivation method includes autoclaved compost in polypropylene bottle to prevent the contamination due to casing. The mushroom mycelium cultured in liquid is inoculated aseptically without casing. The biological properties of composts appear to be very important for induction of fruiting body formation.

The present study mainly focuses on the isolation of mushroom growth promoting bacterium and studies its effect on the early induction of fruiting body for commercial production. Experiments were conducted to examine the effects of bacterial culture and supernatant of the isolate on growth, early fruiting body initiation. The final aim of this study is to explore application in commercial field.

* Corresponding author. Address: Division of Applied Life Science, Gyeongsang National University, Chinju 660-701, Republic of Korea. Tel.: +82 55 751 5469; fax: +82 55 757 0178.
E-mail address: hdyun@nongae.gsnu.ac.kr (H.D. Yun).

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2. Methods

2.1. Media, culture conditions and Isolation of growth promoting bacteria

Bacteria from the mixture of three samples extract were cultured on nutrient broth/agar. To observe the effects of selected bacterial on growth of axenic cultures of mycelia and fruiting body, *P. eryngii* was cultured on potato dextrose agar. *P. eryngii* used in this study was provided by the Laboratory of Mushroom, Gyeongnam Agricultural Research and Extension Services. Several pieces of cultivated medium and casing soil samples on which previously *Pluerotus* sp. were grown, was taken and suspended in 9 ml of sterile distilled water, and then the suspensions were plated out on nutrient agar medium by a decimal dilution and incubated at 28 °C for 24 h.

2.2. Screening and plate assay for novel bacteria promoting mycelia growth of P. eryngii

To screen for effective bacterium among eight all bacteria were cultivated with mycelia and the cultures were maintained at 25 °C, after 12 days of growth period the plates were examined to select the highly significant growth promoting bacterium. The effect of most effective isolate on mycelial growth was examined by co-culturing the bacterium with *P. eryngii* on PDA. Control plates were not inoculated with bacteria. The hyphal growth was measured in the presence and absence of bacteria. Measurements were confined to the colony margin and the same lines were measured over consecutive time intervals. The mycelial growth rate was determined by the formulae πr^2 ($\pi = 3.14$), radius value was taken from both inoculated (90 mm) and uninoculated (50 mm) plates, the obtained values were divided, which gives the total diameter of the mycelia in a Petri plate.

2.3. Effect of supernatant on the mycelia of mushroom

Experiment was performed to access the growth promotion effect of isolated strain P7014 and its supernatant on *P. eryngii* as previously described by Estrada and Royse (2007). Substrate preparation and humidity maintenance in bottle was performed according as described by Ko et al. (2005). Spawn was taken from the previous spent culture, was added 10 g per bottle. Simultaneously the isolate P7014 was grown for 24 h in nutrient broth and 10 ml aliquots of the bacterial supernatant was added along with the spawn into the bottles in sterile condition. Control was inoculated with spawn without bacterium. Substrate residues binding to the stipes were removed before weighing. Mushrooms were weighed individually to determine average mushroom weight per bottle.

2.4. Identification of isolated bacterium

Isolated growth promoting bacterium was characterized and analyzed by 16 S rDNA accordingly as previously described by Cho et al. (2006). Nucleotide sequence data were submitted to the GenBank nucleotide sequence databases and were assigned the accession number (EF154396).

3. Results and discussion

A total of 1215 numbers of bacteria (colonies) were isolated, these were initially subjected for preliminary test to verify for growth promotion effect on the mycelium of *P. eryngii*, described in Section 2. Preliminary screening revealed eight possible growth promoting candidates. Among them one which promoted most effective mycelia growth was obtained form casing soil sample.

The growth promotion effect by the bacteria was determined by measuring the length of the mycelia towards the bacteria and finally designated the isolate as P7014. Co-culturing of the isolated P7014 on PDA plates with *P. eryngii* for 10 days resulted in the increase of mycelial length compared to control (uninoculated). The mycelial length of the co-cultured plate (90 mm) was comparatively double then that of control plate (50 mm). The growth time and the length of the mycelia of co-cultured plate were comparatively higher than that of control plate. The growth rate was obtained about 3.2 from the calculation, which indicates there was more than 3 times increase in growth rate compared to uninoculated.

To characterize bacterium, partial 16S rDNA sequence of merely the most effective growth promoting bacterium was obtained, where as sequence of others seven bacteria was not obtained since they were comparatively less effective to promote growth, and compared with that of other related *Pseudomonas* from GenBank. The level of 16S rDNA similarity between strain P7014 and the *Pseudomonas* species ranges from 94.5% to 99.2%. The highest 16S rDNA sequence similarity (99.2%) is observed between strain P7014 and *Pseudomonas koreensis* Ps 9–14^T (Fig. 1).

After two days of spawning the growth was relatively similar with that of uninoculated. However, after 10 days, it was observed that the mycelial growth was comparatively 1.4 times higher than that of uninoculated. After 22 ± 2 days of supernatant addition the mycelia were completely run in the medium where as uninoculated bottles took 28 ± 2 days. In case of supernatant added bottles primordia were observed early on 6 ± 1 days where as uninoculated took 7 ± 1 days, after scratching period. Harvesting of the well grown single fruiting body was done on the 50 ± 1 days. In contrast, uninoculated obtained 55 ± 2 days. The cumulative change of fruiting body weight among inoculated and uninoculated bottles can be easily explained by the graph (Fig. 2), which shows that just after passing of scratching period (inoculated 30; uninoculated

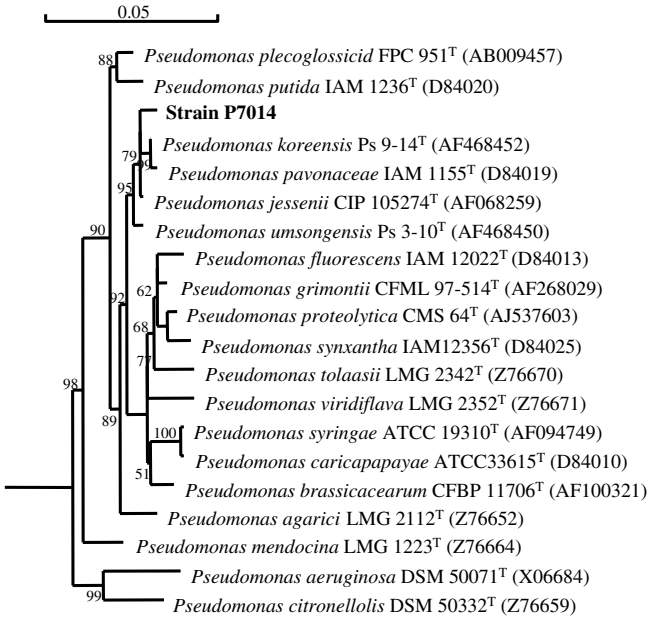

Fig. 1. Phylogenetic relationships of the strain P7014 and other closely related *Pseudomonas* species based on 16S rDNA gene sequences. Numbers above each node are confidence levels (%) generated from 1000 bootstrap trees.

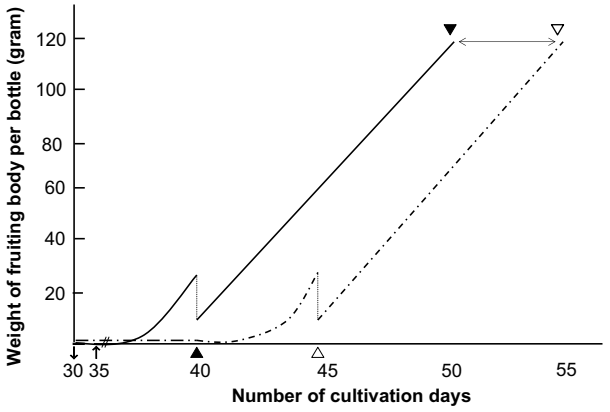

Fig. 2. Cumulative change of fruiting body weight with time course after scratching. −, inoculated with *Pseudomonas* sp. P7014; -.-, uninoculated; ↓, inoculated scratching at 30th ± 2 day; ↑, uninoculated scratching at 35th ± 2 day; ▲, start point of single fruiting body stage (inoculated); ▽, start point of single fruiting body stage (uninoculated); ▼, harvesting time (inoculated); ▽, harvesting time (uninoculated); ↔, 5 ± 2 days gap between inoculated and uninoculated bottles. Data are means of 16 replicates.

35 days) when the primordia starts forming, the weight of fruiting body per bottle randomly increased.

The culture and its supernatant of the isolate *Pseudomonas* sp. P7014 showed effective growth promotion. Addition of supernatant with *P. eryngii* made spawn run faster (5 ± 2 days earlier) compared to uninoculated bottle, which took 28 ± 2 days and formation of primordia were one day earlier. These results provide us evidence of effectiveness of strain P7014 and its importance in mushroom cultivation. Experiment with other seven growth promoting bacteria was ruled out; since they were comparatively less effective on plate assay.

There is possibility that autoclaving (121 °C for 90 min) compost method without casing will lower growth, stimulation of mycelium and induction of fruiting body. The complete time period required to isolate single fruiting body usually takes about 55 ± 2 days.

In addition, supernatant of isolated strain P7014 was extracted by inoculating single colony of P7014 was subcultured into 10 ml nutrient broth (NB) and incubated at 30 °C for 24 h. The resulting culture was used to inoculate a 2-l flask containing 500-ml NB. The flask was incubated at 30 °C for 24 h in a rotary shaker with agitation at 195 rpm. Cells in the flask were separated by centrifugation at 12,000*g* for 20 min. The resulting cell free supernatant was add (20 ml/per bottle) to the mushroom growth media which in turn promoted the growth of mycelium, which provides evidence that there were some growth promoting factors. This research provides us to develop new tool for identifying the specific compounds that are released by certain pseudomonads to trigger mycelium and basidiome formation. This may bring us more easy way of apply and cultivate mushroom.

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