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PLANT GROWTH PROMOTING RHIZOBACTERIA AFFECT BUTTON MUSHROOM YIELD AND QUALITY

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ABSTRACT. *Microorganisms in mushroom casing play a crucial role in initiation and development of primordia and uniform distribution of sporophores, higher mushroom yield, and early cropping. We investigated the effects of plant growth promoting rhizobacteria (PGPR) on yield and quality of button mushroom (Agaricus bisporus Sing) by inoculating in casing and substrate. In the first experiment the casing was inoculated with suspensions of the PGPR Bradyrhizobium japonicum, Rhizobium leguminosarum, and Pseudomonas putida and compared to a non-inoculation control. In the second experiment the best PGPR from the first experiment was inoculated in the substrate, casing and both substrate and casing. In the first experiment, the highest mushroom nitrogen and protein contents were 17.5 % and 1.73 % of the control, respectively with P. putida inoculation. In the second experiment, yield, weight of mushroom per tray and biological efficiency were higher 12.1% and 20.64 %, 12.1% and 20.64 %, and 13.43% and 21.08% of the control, respectively when substrate or both substrate and casing were inoculated with P. putida.*

KEYWORDS: *Agaricus bisporus, Bradyrhizobium japonicum, Pseudomonas putida, Rhizobium leguminosarum*

INTRODUCTION

Although the role of the casing is not precisely defined, it must have particular physico-chemical and microbiological properties which determine its function (Cai et al. 2009). Cultivated mushroom does not fruit in axenic condition, consequently, the presence of bacteria in casing is key to mushroom sporophore initiation (Wood 1976). Microorganisms in casing play a crucial role in initiation and development of primordia; uniform distribution of sporophores; higher mushroom yield, and early completion of cropping. There is a succession of microbial populations in the stages of mushroom cropping which is influenced by casing type affecting mushroom yield (Garland 1997, Ebadi et al. 2012).

Hume and Hayes (1972) reported that the population of *Pseudomonas* influenced mushroom sporophore initiation. The bacterial genera *Bacillus* sp., *Alcaligenes* sp., *Rhizobium japonicum*, *Azotobacter vinelandii*, *Rhodopseudomonas palustris*, and the yeast *Lipomyces starkeyi* have been reported to stimulate mushroom sporophore formation (Park and Agnihotri 1969, Curto and Favelli 1972, Han 1999, Fermor et al. 2000).

In spite of previous research on microbial effect on mushroom production little information is available on different microbial community effect in the casing on mushroom sporophore formation. The aim of this work was to investigate if plant growth promoting rhizobacteria (PGPR) affected yield and button mushroom (*Agaricus bisporus* Sing) quality when the bacteria incubated in casing or substrate.

MATERIAL AND METHODS

Isolates of PGPR *Bradyrhizobium japonicum*, *Rhizobium Leguminosarum*, and *Pseudomonas putida*, obtained from the Soil and Water Research Institute, Karaj, Iran, I.R., were used. Bacteria were grown on Sperber nutrient agar (Sperber 1958). For preparation of inocula, strains were cultured in liquid LB medium and incubated at 25°C overnight. Bacterial suspensions of 9.8×10^7 CFU·mL^{-1} in bottles containing retentive solution (90.6 mg NaCl, 3.85 g Na_2HPO_4, 2.1 g KH_2PO_4·L^{-1}) were transported to the mushroom farm at room temperature. The casing was

sterilized before use (121ºC, 2 kg/cm^2, 1 hr, 3 times), to avoid possible microbial contaminants. In the first experiment bacterial suspensions (300 mL) were inoculated onto casing. Non-inoculated casing was the control. In the second experiment suspensions of *P. putida* which lead to higher nitrogen and protein content in mushroom at the first experiment were inoculated onto substrate, casing and both substrate and casing.

Starting material for the production of mushroom compost, consisting of a mix of straw, chicken manure, gypsum and water, was obtained from a grower in the Karaj of Iran. Composting of substrates was with the method of Yang (1986). The total outdoor composting process (Phase I) took 21 days, including pre-wetting. The second treatment (Phase II) was processed indoors for 7 days. Nitrogen content following Phase II was approximately 2.15%. Twenty kg of wet compost, equal to 6.30 kg of dry compost, was placed in a 60×40×20 cm tray with 200 g of spawn of *A. bispora*. Running, to increase mycleia, was in a growing room under controlled conditions of 85±5% relative humidity and compost temperature of 23±2°C. After 20 days of running, a 4 cm layer of pasteurized top soil was placed over the compost, the casing procedure. Ten days after casing, room temperature was lowered to 16±1°C, and 95±4% relative humidity.

Harvesting began 16 days after casing and lasted 40 days. All mushrooms were picked before the veil was broken. Fresh weight and number of harvested mushrooms were recorded daily. For each treatment, and flush, 3-6 representative fruit bodies were dried at 100°C to constant weight to determine dry matter content. Protein content of fruit bodies was calculated from the nitrogen content (N × 6.25) as determined by the micro-Kjeldahl method (Guebel et al., 1991).

RESULTS AND DISCUSSION

Pseudomonas putida inoculation in casing and substrate increased button mushroom yield and quality. Casing inoculation with bacterial strains affected mushroom nitrogen and protein contents during the first experiment without affecting yield and yield components. The highest nitrogen and protein content in mushroom were from trays covered with casing inoculated with *P. putida* (Table 1). In the first experiment, the highest mushroom nitrogen and protein contents were 17.5 % and 1.73 %

Table 1. Effect of plant growth promoting bacteria on button mushroom

Plant promoting bacteria	No. mushrooms per tray	Mushroom weight per tray (g)	Mushroom yield (g per m^2 compost)	Biological efficiency (kg per 100 kg dry weight of substrate)	Nitrogen (%)	Protein (%)	Potassium (%)
Bradyrhizobium japonicum	317.33	6105.42	18316.26	90.29	5.05	31.56	4.99
Rhizobium leguminosarum	313.67	6028.38	18085.14	89.26	4.78	29.85	4.74
Pseudomonas putida	308.67	5932.08	177796.24	87.85	5.30	33.10	4.96
Control	281.00	5412.06	16236.18	79.59	4.51	28.21	4.38
LSD (P<0.05)	46.06	1076.6	3229.8	12.17	0.54	3.35	0.77

of the control, respectively with *P. putida* inoculation. In the second experiment inoculation method affected yield, weight of mushroom per tray and biological efficiency (Kg fresh weight of mushroom per Kg dry weight of substrate). These characteristics were higher than control when substrate or both substrate and casing were inoculated with *P. putida* (Table 2). In the second experiment, yield, weight of mushroom per tray and biological efficiency were higher 12.1% and 20.64 %, 12.1% and 20.64 %, and 13.43% and 21.08% of the control, respectively when substrate or both substrate and casing were inoculated with *P. putida*. Number of mushroom was significantly higher than control when substarte, casing or both substrate and casing inoculated with *P. putida*. Total nitrogen and protein content of mushroom similar first experiment were significantly higher than control when casing inoculated with *P. putida.*

Presence of bacteria in casing is thought to be key to mushroom sporophore initiation (Wood 1976) and lead to higher number of mushroom in second experiment. *Pseudomonas putida* in casing plays a role in initiation and development of primordia; more uniform distribution of sporophores; and higher yield of mushroom (Hayes 1974, Park and Agnihotri 1969, Curto and Favelli 1972, Han 1999, Fermor et al., 2000). It appears *P. putida* can be used to increase production of button mushroom if used in substrate or both substrate and casing. It appears also that *P. putida* can be used as substrate bacteria to benefit some aspects of button mushroom cultivation. Additional study needs to be undertaken to determine effects of mixing *P. putida* and other strains of *Pseudomonas*, or other species of bacteria on button mushroom production. It remains to be determined whether specific substrates, or mushroom strains, are provided adequate benefit by bacterial strain. It should be determined whether concentrations of the bacteria are optimum for use in incubation.

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Table 2. Effect of *Pseudomonas putida* inoculation onto substrate, casing, and both substrate and casing on button mushroom

Incubation condition	No. of mushroom per plot	Mushroom weight per plot (g)	Mushroom yield (g per m^2 compost)	Biologica efficiency (kg per 100 kg dry weight of substrate)	Nitrogen (%)	Protein (%)	Potassium (%)
Substrate alone	315.33	6066.9	18200.7	90.28	4.91	30.69	5.10
Casing alone	283.33	5450.58	16351.74	80.74	5.31	33.21	4.84
Substrate + casing	339.00	6529.14	19587.42	96.37	4.90	30.62	4.75
Control	265.00	5412.06	16236.18	79.59	4.51	28.21	4.38
LSD (P<0.05)	8.86	223.77	671.3	2.70	0.57	3.54	0.75
LSD (P<0.01)	12.90	325.59	976.78	3.92	0.82	5.15	1.08

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