EFFECT OF BACTERIAL AND CYANOBACTERIAL CULTURE ON GROWTH, QUALITY AND YIELD OF *Agaricus bisporus*

HOSSEIN RIAHI, A. ESKASH, Z. SHARIATMADARI
Faculty of Biosciences, Shahid Beheshti University G, C.
Evin,Tehran,
Iran
H-Riahi@cc.sbu.ac.ir

ABSTRACT

In the mushroom growing process, *Agaricus bisporus* function of casing soil is to provide an environment for fruit formation. Presence of *Pseudomonas* species in casing soil is important for mushrooms formation and development. They can represent up to 50 percent of the total bacteria. The result of this study showed that total number of bacteria and population of pseudomonas species increased dramatically after casing. The maximum number of bacteria was recorded at the primordia formation stage. Population of pseudomonas in casing soil was increased with addition of bacterial inoculum to the casing soil. There was a significant difference of mushroom yield as compared to the control.
Until now most research and applications of cyanobacteria have been conducted with green plants growing. Findings of this research strongly supported that the production of promoting substances such as auxins, sugars and vitamins by the algae may be partly responsible for the greater mushroom growth and yield. Inoculation of cyanobacteria into the casing soil significantly increased mushroom yield and quality.

Keywords: *Agaricus*; *Pseudomonas*; Primordial; Cyanobacteria; Phytohormones

INTRODUCTION

Following colonization of mushroom mycelia in pasteurized compost, a 1.5 inch layer called casing soil is applied on top of the compost surface. The casing soil enhances the retention of irrigation water on production beds and promotes mushroom fruit body formation. An important process during mushroom growing is pinning. Addition of casing is necessary for shifting the vegetative phase to the reproductive phase by pin formation. The casing soil supports an active, aerobic bacterial flora [2] among them fluorescent *Pseudomonas* spp. play an important role in initiation of pinning and mushroom fruit body development. There is a controversy regarding populations of pseudomonads in casing soil. Samson [12] demonstrated that fluorescent pseudomonads may represent up to 50 percent of the total bacteria in casing samples, whereas Doores et al. [1] indicated that they represented only 2 percent of the total casing bacteria. Miller et al. [7] showed that populations of casing bacteria changed over the *A. bisporus* growth cycle. *Pseudomonas putida* has been identified as an important species involved in fruiting body initiation [3, 10]. Inoculation of bacterial culture on mushroom growing media, besides the effect on pin formation, caused an increase of mycelial growth rate up to 1.6 fold, promoted the rate of radial hyphal extension, and suppressed the frequency of branching [6].

Soil is a habitat of some terrestrial blue-green algal species that are beneficial organisms for soil fertility by fixing atmospheric nitrogen, binding soil particles, helping to maintain moisture and preventing erosion. These bacteria supply substances that promote the growth of plants. There are many reports that cyanobacteria produce phytohormones such as cytokinin, auxin and auxin-like substances in soil [5, 13, 14, 15, 16]. Other plant growth regulator (PGR) substances such as amino acids, sugars, vitamins that may have a positive influence on growth of vascular plant are produced by cyanobacteria [5, 8, 9]. Until now most research and applications of cyanobacteria have been conducted with green plants. This is a first attempt to study the effect of cyanobacteria on mushroom. Moreover, cyanobacteria may enhance production of secondary metabolites. These phenomena may be controlled with or mediated by hormones [11, 13]. Cyanobacteria are another group of microorganisms that might have a positive effect on mushroom yield.

MATERIALS AND METHODS

In this work two experiments were conducted. The first study concerned the bacterial population in casing soil and effects of *Pseudomonas patuda* used as bacterial inoculum, on yield and quality of mushroom. A similar objective was followed in the second trial but algal culture was used for inoculation.

Experiment 1. Isolation and cultivation of *Pseudomonas* species from casing soil. After colonization of compost with mushroom mycelium, the substrate was covered with casing soil. Sampling was carried out in Malard mushroom research farm. To determine *Pseudomonas* population, casing soil was sampled periodically until primordia formation. Specified amount of the dilutions transferred on to sterile plates of nutrient agar (NA) and Chromagar Pseudomonas (PS822) media. Inoculated plates were incubated at 30^{o}C for 24h. Colonies that appeared at the end of incubation were counted, the unit expressed in terms of colony-forming units per gram (CFU/g) of original sample. The isolates were further subjected to standard biochemical tests. Bacterial identification of isolates was carried out by comparing the results obtained with *Bergey's Manual of Determinative Systematic Bacteriology*. The predominant *Pseudomonas* species isolated from casing soil was tested to study the growth of mycelium and pin formation.

Inocula were prepared by growing the selective strains in nutrient broth (NB) medium. After incubation at 30^{o}C for 18h, the densities of culture were determined at OD600. Then cultures were diluted further in serum until final bacterial cell numbers were 10^8 cells/ml. Bacterial suspensions (5 L) were sprayed on 42 m^2 of casing soil at time of casing.

Protein contents of fruit bodies were calculated from the nitrogen content (N×6.25) as determined by micro-Kjeldahl method [4].

Experiment 2. Cyanobacteria cultivation. Among the cyanobacteria isolated from different paddy fields one heterocyestous cyanobacteria (Nostoc) was selected because it showed a high growth rate. Isolates were grown in BG11 nitrogen free medium in a 2 L container at 24°C and a 12/12 h light/dark cycle with artificial illumination (2000-2500 Luxes) and constant stirring and aeration. After three weeks, the culture was harvested and used as inoculum. Addition of algal culture was carried out at the primordia formation stage, before second and third flushes.

Mushroom production. The compost formulation consisted of wheat straw, chicken manure and gypsum prepared in conventional yard and pasteurization tunnels. Mushrooms were grown in controlled and standard growing room. After colonization of compost with *A. bisporus* (commercial strain Sylvan A15) mycelium, the substrate was covered with 4-5 cm of casing soil. Mushroom yield was determined over a 3-weeks production period. Mushrooms were weighted after removal of stipes.

Statistical analysis. Data were subjected to analysis of variance (ANOVA) and means were compared using LSD test ($P<0.05$ and $P<0.01$). A completely randomized design was used with four replications for each treatment.

RESULT AND DISCUSSION

The results of this study showed that the total aerobic bacterial populations ranged from 7.5 to 7.8 Log of colony-forming units (CFU)/g of casing soil while fluorescent *Pseudomonas* ranged from 6.8 to 7.2 Log CFU/g. Periodic sampling of casing soil indicated that number of bacteria increased gradually with growth of mushroom mycelium into the casing soil. With the initiation of pin formation the number of bacteria increased dramatically. The maximum number of bacteria was recorded at the first flush primordia formation stage between day 18 and day 20 (Fig. 1).

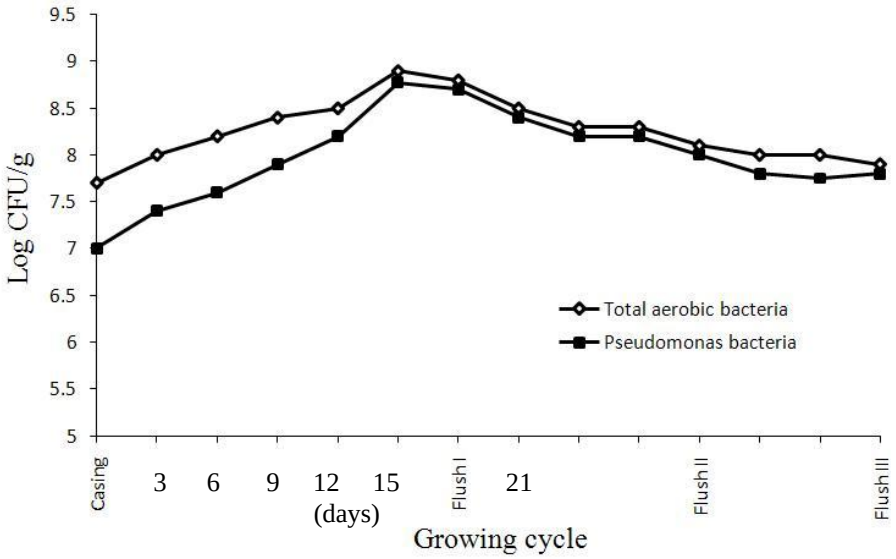

Figure 1. Total numbers of aerobic bacteria in the casing soil at different cropping stages.

Among the different strains of *Pseudomonas* isolated from soil adhering to primordia, only one was selected. Casing soil inoculated with bacterial culture (*Pseudomonas putida*) promoted faster and more uniform size of primordia. Mushroom yield increased 0.3 kg/m^2 in first break and 2.1 kg/m^2 in second break, but decreased it by 0.4 kg/m^2 in the third break. Addition of bacteria culture to the casing soil also improved quality of mushrooms. Dry matter of mushrooms increased with 0.5 percent in the first break, 0.6 percent in the second break and 0.7

percent in the third break. Protein content of treated mushrooms also increased with 3 to 5 percent in three breaks compared to untreated mushrooms (Table 1).

Table 1. Effect of bacterial culture on yield, dry matter and protein content of mushroom

	First picking			Second picking			Third picking		
Sample	Yield Kg/m^2	D.M %	Protein %	Yield Kg/m^2	D.M %	Protein %	Yield Kg/m^2	D.M %	Protein %
Control	11.8	8.2	37	7.9	7.9	34	4.8	7.5	32
Test	12.1 ns	8.7 *	40*	10 * *		8.5 * *	4.4 ns		8.2 * *
				39 * *			37 * *		
Diff.	0.3	0.5	3.0	2.1	0.6	5.0	- 0.4	0.7	5.0

* * significance < 1 % level, * significance < 5 % level, ns: not significant

Casing soil was irrigated with algal culture at the primordia formation stage, before second and third breaks. There was a significant difference in mushroom yield in three flushes treated with cyanobacterial culture as compared to the control. Mushroom yield was increased 0.661 kg/m^2 in first, 2.2 kg/m^2 in second flush and 0.053 kg/m^2 in the third flush as compared to the control. The result also showed that inoculation of casing soil with cyanobacteria had a positive effect on quality of mushroom. Dry matter content of mushrooms increased in the first flush. Dry matter was measured at 9.75 % in treated and 8.35 % in untreated mushroom. However, the amount of dry matter and protein declined in second and third flush as compared to the first flush. There was a slight difference in dry matter in treated and untreated mushroom in second and third flushes. Addition of algal culture had a positive effect on protein content of mushroom. The amount protein was increased significantly in first flush, but there was a slight difference between treated and control in second flush. Whereas, in the third flush protein content of treated mushroom was less than control (Table 2).

Table 2. Effect of algal culture on yield, dry matter and protein content of mushroom

Sample	First Flush			Second Flush			Third Flush		
	Yield Kg/m^2	D.M %	Protein %	Yield Kg/m^2	D.M %	Protein %	Yield Kg/m^2	D.M %	Protein %
Control	12.59	8.35	48.1	7.58	8.3	45.1	2.75	7.40	41.1
Test	13.2 *		9.75 * *	9.78 * *		8.6 *	2.80 ns		7.50 ns
	51.9 * *			45.5 ns			39.1 ns		
Diff.	0.61	1.4	3.8	2.2	0.3	0.4	0.05	0.10	-2.0

* * significance < 1 % level, * significance < 5 % level, ns: not significant

CONCLUSION

In cultivation of *A. bisporus*, compost and casing soil are two major elements. Studies have demonstrated that populations of pseudomonas in the casing layer on which the mushroom fruit body develops is very important. In most of the studies, total bacterial populations ranged from 8.0 to 8.5 log CFU/g casing material. With a slight difference, this result is same as other workers. The majority of bacterial population in casing was attributed to pseudomonas species. In this study they present more than 80 present of the bacterial population in the casing layer. The result showed that there is a close relation between growth of mycelium and number of bacteria in casing soil. Sampling of casing soil at different periods of mushroom growing cycle revealed that number of bacteria increased simultaneously with increase growth of mycelium into casing soil. At the pining stage, populations of pseudomonas species especially *P. putida* were important since they are playing a key role in fruiting body formation. We conclude that inoculation of native *P. putida* isolated from casing soil at the primordia formation stage will be very efficient for increasing mushroom yield and quality.

In the second experiment the application of cyanobacterial culture on casing soil and its effect on mushroom yield and quality was investigated. Irrigation of casing soil with cyanobacterial culture increased yield, dry matter and protein content of mushrooms. Production of promoting substances such as auxins, sugars and vitamins by the algae are the main factors for this issue.

ACKNOWLEDGEMENTS

We are very much thankful to the managers and staff of Malard mushroom company – especially Mr. Kabei, Judy, Soltan Shah, Marzoughi and Ebrahimi for their assessment. Without their help we would not be able to carry out this research. The authors are also grateful to the University of Shahid Beheshti for financial support.

REFERENCES

[1] Doores S., Kramer M., Beelman R. (1986). Evaluation and bacterial populations associated with fresh mushrooms (*Agaricus bisporus*), in Developments. In: Proceedings of Intl. Symp. Scientific. Technical. Aspects of Cultivating Edible Fungi. Wuest P.J., Royse D.J. & Beelman R.B. Eds. 10, 283-294.

[2] Hayes WA., Nair NG. (1976). Effects of volatile metabolic by-products of mushroom mycelium on the ecology of the casing layer. *Mushroom Science*. IX: 259-268.

[3] Hayes WA., Randle PE.,Last FT. (1969). The nature of the microbial stimulus affecting sporophore formation in *Agaricus bisporus* (Lange) Sing. *Ann. Appl. Biol*. 64: 177-187.

[4] Jackson ML. (1973). Total nitrogen was estimated by the micro Kjeldahl digestion method. Soil Chemical Analysis. Prentice Hall of India Pvt. Ltd, New Delhi.

[5] Karthikeyan N., *et al.* (2007). Evaluating the potential of plant growth promoting cyanobacteria as inoculants for wheat. *Eur. J. Soil Biol*. 43(1): 23-30.

[6] Kim MK., Math RK, Cho KM., Shin KJ., Kim JO.,Ryu JS., Lee YH. &Yun HD. (2008). Effect of *Psudomonas* sp. P7014 on the growth of edible mushroom *Pleurotus eryngii* in bottle culture for commercial production. *Bioresource Technol*. 99: 3306-3308.

[7] Miller N., Gillespie JB., Doyle OPE. (1995). The involvement of microbiological components of peat based casing materials in fructification of *Agaricus bisporus*. *Mushroom Sci*. 14(1): 313-321.
[8] Misra S., Kaushik B.D. (1989a). Growth promoting substances of cyanobacteria. Vitamins and their influence on rice plant. *Proc. Indian Sci. Acad*. *B55*: 295-300.
[9] Misra S., Kaushik B.D. (1989b). Growth promoting substances of cyanobacteria II. Detection of amino acids, sugars and auxins. *Proc. Indian Sci. Acad*. *B55*:499-504.
[10] Rainey PB., *et al.* (1990). A model system for examining involvement of bacteria in basidiome initiation of *Agaricus bisporus*. *Mycol. Res*. 94: 191-195.
[11] Saker M., Shanab S., Khater M. (2000). In vitro studies on Ambrosia maritime. I-Morphogenic responses and algal toxins elicitation. *Arab J. Biotech*. 3(2): 217-224.
[12] Samson R. (1986). Variability of fluorescent Pseudomonas populations in composts and casing soils used for mushroom cultures. In: Proceedings of Intl. Symp. Scientific. Technical. Aspects of Cultivating Edible Fungi. Wuest P.J., Royse D.J. & Beelman R.B. Eds. 10, 19-25.
[13] Shanab S. (2001). Effect of fresh water cyanobacterial extracts on alkaloid production of the in vitro *Solanum elaeagnifolium* tissue culture. *Arab J. Biotech*. 4(1): 129-140.
[14] Stirk MA., Ördog V., Van Staden J & Jäger K. (2002). Cytokinin and auxin-like activity in Cyanophyta and microalgae. *J. Appl. Phycol.* 14: 215-221.
[15] Tarakhovskaya ER., Maslov YI. & Shishova MF. (2007). Phytohormones in algae. *Russ J. Plant Physiol*. 54(2):186-194.
[16] Whitton BA. (2000). Soil and rice-fields. In: Whitton B.A. and Potts M. (eds), The Ecology of Cyanobacteria. Kluwer Academic Publishers, Dordrecht, pp. 233-255.
[17] Wood DA. (1976). Primordium formation in axenic cultures of *Agaricus bisporus* (Lange) Sing. *J. Gen. Microbiol*. 95: 313-323.