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Bacterial inoculants and their effect on the pinning, yield and false truffle disease incidence in *Agaricus bitorquis*

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ABSTRACT: The summer white button mushroom *(Agaricus bitorquis)* with little difference in its biology and temperature requirements for cropping from *Agaricus bisporus,* requires almost the same cultural practices for its cultivation as *Agaricus bisporus.* Bacterial inoculants either prepared as broth culture or on some suitable carrier have not got significant importance in mushroom cultivation baring some reports in *Agaricus bisporus,* where their mixing in compost at spawning or inoculation in casing soil at casing have shown significant enhancement in mushroom yield. The present study was carried out with broth culture of *Bacillus megaterium, Alcaligenes faecalis, Bacillus circulans-I, Bacillus circulans-II, Bacillus thuringiensis* and commercial biofertilizer' Azotobacter', where the broth cultures were inoculated in casing at the time of casing, while the commercial biofertilizer' Azotobacter' was mixed in compost at spawning at 1.0 %. The mixing of 'Azotobacter' at spawning did not show any significant effect on the yield of Agaricus bitorquis. However, the broth culture of *Alcaligenes faecalis, Bacillus circulans-II* and *Bacillus thuringiensis* resulted in significantly higher yield than the uninoculated treatment. There was no significant difference in the pinning initiation and first harvest time (days post casing) among different treatments. The higher yield in case of three broth culture inoculation treatment in casing was largely due to more harvest during the first four weeks of the cropping cycle. The false truffle disease incidence also varied in different treatments and it was higher in *Bacillus megaterium, Bacillus circulans-I* and *B. circulans-II* inoculated treatments. However, up to 6th week of cropping the disease did not appear in control, 'Azotobacter' mixed, *Alcaligenes faecalis* and *Bacillus thuringiensis* inoculated treatments. The bacterial inoculation treatments also did not show any visible deleterious effect on the mushroom quality.

1 INTRODUCTION

Sporophore initiation and its further development in full size fruitbody requires a complex set of biochemical reactions completed in several steps. The casing material which is put on the spawn run compost provides the appropriate fruiting inducing environment. The physical, chemical and biological nature of the casing material not only determines the mycelium run in the casing material but also affects the total mushroom yield. The microbial cells being an important part of the biological system of the casing material act as the source of nutrient for the growing mushroom mycelium, and also provide the stimulants for the sporophore initiation & its further development in full size fruitbody. Investigations have been undertaken to identify the organisms stimulating fruiting of *A. bisporus,* viz; streptomycetes (O' Donoghue 1962, O' Donoghue & Ryan 1991), *Bacillus psilocybe* (Urayama 1962), *Arthrobacter terregens, Bacillus megaterium* and *Rhizobium* sp. (Park & Agnihotri 1969), *Pseudomonas putida* (Hayes et al. 1969, Miller et al. 1995), *Alcaligenes faecalis* (Ahlawat & Verma 1999).

However, such type of studies have not seen carried out in *Agaricus bitorquis* which is a very important mushroom especially for the tropical countries like India. The mechanism of interaction between *Agaricus* mycelium and other microbes present in the casing material and their mode of affecting mushroom yield is still a matter debate. The present work was undertaken to examine the effect of various bacterial inoculants prepared from the bacteria originally isolated from mushroom substrates and casing materials on the pinning initiation, yield and false truffle disease incidence in *Agaricus bitorquis,* which is very prone to this disease being grown under mesophilic temperature range of 20 to 25° C.

2 MATERIALS AND METHODS

2.1 *Bacterial inoculant*

Initially 50 bacterial isolates were isolated from both the casing materials and the mushroom substrates prepared by using different types of base materials. The four bacteria showing stimulation in mushroom mycelial growth under *in vitro* conditions were identified by the Institute of Microbial Technology, Chandigarh, India as *Bacilllus megaterium, Alcaligenes faecalis, Bacillus circulans-*\ and *Bacillus circulans-\l.* Two other bacteria, one having the potential of atomospheric nitrogen fixation in free condition *(Azotobacter* sp.) and the other a common bioinsecticide *(Bacillus thuringiensis)* were obtained from authentic sources in India. All the five bacteria were multiplied in nutrient broth having ph 7.2 on rotatary shaker maintained at a temperature of 25 + 2 °C, while the *Azotobacter* in the form of commercial biofertilizer was mixed in compost at spawning at 1.0%.

2.2 *Interaction under in vitro conditions*

Bacterial inoculants from different bacteria containing about 10 9 - 10 10 cells ml'l were mixed in casing material at the time of casing to give a final bacterial count of 10 7 - 10 8 cells gm -1 of the casing material. The bacteria mixed casing was applied on the spawn run bags of 10 kg capacity at the time of casing, keeping spawn run bags applied with uninoculated casing material as the control treatment. The bags were arranged in the cropping room by following random block design. The crop of the summer white button mushroom *(Agaricus bitorquis)* was raised by following the standard cultural practices (Dhar 1998). Two crops of summer white button mushroom were taken in the month of May to July, 1998 and July to Sept, 1999 under natural growing conditions.

2.3 *Pinning initiation and mushroom yield*

The bags maintained in the cropping room were kept under watch for the pinning initiation. Pinning initiation time was calculated in days (days post casing). The yield data were taken up to 4th week during the first crop and up to 6th week during the second crop. The mushroom yield was calculated in Kg/q of compost both on weekly basis and up to completion of the crop. The yield data were statistically analyzed for their statistical significance.

2.4 *False truffle disease incidence*

False truffle disease caused by *Diehliomyces microsporus* in *Agaricus bitorquis* is taken as the biggest hurdle in popularization of this mushroom. It is because of the temperature requirements of both the mushroom and the pathogen. The disease incidence was recorded in percentage of the bags affected from the disease after different stages of cropping. In both seasons the bags were kept up to 6 weeks of cropping and the percentage of disease incidence was calculated.

3 RESULTS AND DISCUSSION

3.1 *In vivo interactions*

The application of casing soil preinoculated with bacterial cultures resulted in delayed pinning in *Bacillus megaterium* and *Azotobacter* sp inoculation treatments both during 1st and Ilnd cropping seasons. However, the inoculation with *Bacillus circulans-1* and *Bacillus circulans-ll* resulted in little early pinning compared to the control treatment, although the effect was insignificant (Table 1). Several workers around the world have reported the stimulatory role of various bacteria on pinning initiation of *A. bisporus* (Arkan et al. 1994, Ahlawat 1998) but none has reported any stimulatory effect in *Agaricus bitorquis.*

Table 1. Effect of different bacterial inoculants in casing soil at casing on first harvest in *Agaricus bitorquis* strain NCB-13.

Table 2. Effect of different bacterial inoculation treatments on the *Agaricus bitorquis* yield.

Treatment	Yield (kg/q of compost)	
	1st Crop (4 weeks of cropping)	II nd Crop (6 weeks of cropping)
Bacillus megaterium	8.24	13.82
A Icaligenes faecalis	10.19	14.48
Bacillus circulans-l	9.32	13.39
Bacillus circulans- II	11.14	15.19
Bacillus thuringiensis	10.56	14.91
Azotobacter sp.	8.48	12.92
Control	8.34	11.23
CD (0.05)	1.74	2.03
CD (0.01)	2.29	2.67

3.2 *Yield and yielding pattern*

The yield data recorded in two different crops raised in two different seasons revealed that the inoculation of *Alcaligenes faecalis, Bacillus circulans-ll* and *Bacillus thuringienes* significantly enhanced the mushroom yield over the control treatment. However, in other inoculation treatments the difference in yield was insignificant (Table 2). The bacterial inoculation treatment which showed stimulation in mushroom yield also recorded higher yield during the first four weeks of the cropping cycle with an exception with *Bacillus circulans-ll,* where it showed a lower yield only during the IV th week of the cropping cycle (Figure 1). The yield stimulatory effects of various bacterial inoculation treatments have also been reported earlier (Curto & Favelli 1971, Arkan et al. 1994, Ahlawat 1998, Ahlawat & Verma 1999).

3.3 *False truffle disease incidence*

The summer white button mushroom requires a temperature range of 20-25°C for its pinning initiation and further development. This temperature optimum also triggers the growth of other competitor and parasitic moulds of *A. bitorquis.* The false truffle is the most common parasitic mould to *A. bitorquis* and also the biggest hurdle in popularization of this mushroom. The

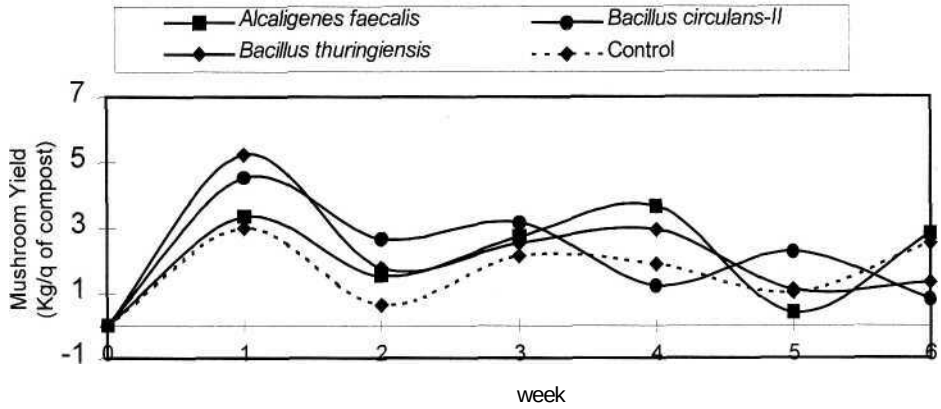

Figure 1. Effect of bacterial inoculation treatments on yielding pattern of *Agaricus bitorquis*

disease incidence data recorded in natural growing condition (without any artificial inoculation) indicated that in first crop disease symptom appeared much earlier than in the second crop. It was because of the temperature optima during the cropping season and it was 25± 2 °C during maximum part of the cropping cycle. However, in Ilnd crop disease incidence was much less and it was again because of the temperature optima (22 ± 2 °C) during the cropping cycle. Maximum disease incidence was in *Bacillus megaterium,* and *Azotobacter* sp. (37.50 and 22.22 %) inoculated bags followed by *Bacillus circulans-I* and *Bacillus circulans-ll* bags. The disease incidence was lowest in *Bacillus thuringiensis* (12.50 and 0.0 %) and *Alcaligenes faecalis(25.0* and 0.0.%) inoculated bags (Table 3). The severity of problem requires immediate attention in this direction but there has been no report of controlling this disease through bacterial inoculants.

Table 3. Effect of different bacterial inoculation treatments on the false truffle disease incidence in *Agaricus bitorquis.*

Treatment	Disease incidence(percentage of total bags)	
	1st Crop (6 weeks)	II nd crop (6 weeks)
Bacillus megaterium	37.50	22.22
A!caligenesfaecalis	25.00	00.00
Bacillus circulans-l	25.00	22.22
Bacillus circulans- II	25.00	22.22
Bacillus thuringiensis	12.50	00.00
Azotobacter sp.	37.50	22.22
Control	50.00	11.11

The stimulation in pinning initiation was not significant, this indicates that the bacterial inoculants are not playing a role during mycelial spread or during pinning initiation. The other aspect of this study where the inoculants increased the yield of mushroom seems largely due to their role as a rich source of nutrients (Sparling et al. 1982, Atkey & Wood 1983, Ahlawat & Rai 1997). The temperature optimum *for A. bitorquis* cultivation also favors the growth of all the bacteria involved in the study. On the other hand all these inoculants were found not to increase the yield of *A. bisporus.* That is because of the lower temperature requirement of that mushroom, which is not suitable for multiplication of these bacteria. These inoculants have enough scope of exploitation as a part of regular cultural practice for *A. bitorquis* cultivation.

REFERENCES

Ahlawat, O.P. 1998. Effect of bacterial inoculants on mycelial growth, pinning, yield and quality of white button mushroom *(Agaricus bisporus). J. Scientific and Industrial Research.* 57: 686-691.

Ahlawat, O.P. & Rai, R.D. 1997. Effect of " Azotobacter" and "Phosphotika" biofertilizers on spawn run, pinning and yield of the white button mushroom *(Agaricus bisporus). Mushroom Research. 6:* 69-74.

Ahlawat, O.P. & Verma, R.N. 1999. *Alcaligenes faecalis* - a potent bacterial inoculant for improving yield/quality of *Agaricus bisporus* strain U_3. *Proceedings of the International Conference on Mushroom Biology & Product. Sydney, Australia.*

Arkan, O.; Aksoz, N. & Guler, P. 1994. Interaction between fructifications of cultivated mushroom, *Agaricus bisporus* and *Pseudotnonas* sp. isolated from casing soil. *Turkish Journal of Biology* 18: 213-222.

Atkey, P.T. & Wood, D.A. 1983. An electron microscope study of wheat straw composted as a substrate for the cultivation of the edible mushroom *(Agaricus bisporus). J. Applied Bacteriology* 53: 293-304.

Curto, S. & Favelli, F.I972. Stimulative effect on certain microorganisms (bacteria, yeast, micoalgae) upon fruit body formation of *A. bisporus* (Lange) Sing. *Mushroom Science* VIII: 67-74.

Dhar, B.L. 1998. *Cultivation Technology of summer white button mushroom (Agaricus bitorquis).* National Research Center for Mushroom, Solan, India.

Hayes, W.A., Randle, P.E. & Last, F.T. 1969. The nature of microbial stimulus affecting sporophore formation in *(Agaricus bisporus)* (Lange) Sing. *Annals Applied Biology* 64: 177-187.

Miller, N., Gillepsie, J.B. & Doyle, O.P.E. 1995. The involvement of microbiological components of peat based casing materials in fructification *of Agaricus bisporus. Mushroom Science* XIV: 313-321.

O'Donoghue, D.C. 1962. New Light on fruit body initiation. *Mushroom Science* V: 247-249

O'Donoghue, D.C & Ryan, J.P. 1991. Influence of wide range of bacteria, actinomycetes and fungi on mycelial growth of *A. bisporus* (Lange) Sing, and the special fruiting requirement of *A. bisporus. Mushroom Science* X: 753-759.

Park, J.Y. & Agnihotri, V.P. 1969. Bacterial metabolites trigger sporophore formation in *Agaricus bisporus. Nature:* 222:984.

Sparling, G.P., Fermor, T.R. & Wood, D.A. 1982. Measurement of microbial biomass in composted wheat straw, and the possible contribution of the biomass to the nutrition of *A. bisporus. Soil Biology and Biochemistry* 14: 609-611.

Urayama, T. 1961. Stimulative effect of certain bacteria on *A. bisporus* (Lange) Sing. *Botany Magazine Tokyo* 74 : 56-59.