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INFLUENCE OF BACTERIA ON THE MYCELIAL GROWTH AND BIOLOGICAL EFFICIENCY OF OYSTER MUSHROOM *PLEUROTUS FLORIDA* PF05

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Abstract

Oyster mushroom *Pleurotus florida* was cultivated in poly bags employing wheat straw as substrate. The effect of bacterial culture (BC) and culture filterate (CF) of a bacterial isolate (*Glutamicibacter arilaitensis* MRC119) was investigated on the growth rate, ACC deaminase, fruitbodies and the biological efficiency of oyster mushroom. The growth rate of mycelia was faster when treated with culture filtrate and bacterial culture in comparision to untreated control. The fastest radial and lineal growth rate achieved were as 8.97 mm/d, 8.11 mm/d respectively with the treatments of CF and BC on 20th day, while control showed 4.98 mm/d. The highest ACC-deaminase (687.76 IU/g) production with CF while BC showed as 513.67 IU/g. However, the highest number of fruiting body were recorded as 41.32 and 32.23 with CF and BC treatments, which were higher than control (17.32) sets. The treatment with CF resulted highest yield of biological efficiency (256.45%) followed by 204.65% with BC and 154.87% of control. Therefore, the use of mushroom growth promoting bacterial culture and its culture is recommended for better biological efficiencies.

***Keywords*:** Mushroom cultivation, oyster mushroom, bacteria, fruiting body and biological efficiency.

Introduction

Mushroom have big advantage of their cultivation in small spaces of a landless farmer's house and may generate appropriate income that aids in family support (Mamiro and Mamiro, 2011; Tesfay *et al*., 2020) and the supply of proper food. Supplementing the cultivation substrates with various additives, shorten the crop period and also increases mushroom productivity (Curvetto *et al*., 2002; Naraian *et al*., 2009, 2010; Carrasco *et al*., 2018). However, the excess supplementations with harsh chemicals cause loss in the quality and texture of mushrooms. Thence, there is a thrust need to search a new inoffensive biological alternative to improve the sustainable cultivation of mushroom and can enhance the fruit body yield of mushrooms.

Many microorganisms have been shown to promote the yield of *P. ostreatus* and other cultivated mushrooms, the biological properties of substrate appears very important for induction of fructification. Growth and development of mushrooms are particularly affected by bacteria pseudomonads (Grewal and Rainey, 1991). The beneficial bacterial microflora positively affect growth of fungal mycelia (Mohammad and Sabaa, 2013). Moreover, bacteria also initiate primordia formation and stimulate growth of fungi (Cho *et al*., 2003). Young *et al*. (2012) reported bacteria such as *Pseudomanas resinovorans, Microbacterium esteraromaticum* significantly improved yield and biological efficiency of button mushroom. Many bacteria have the ability to produce ACC deaminase enzyme which has potential to degrade ACC into a-ketobutyrate and ultimately increase mushroom growth (Dong *et al*., 2010).

Kim *et al*. (2008) revealed that *Pseudomonas* sp. P7014 enhance the growth of oyster mushroom (*Pleurotus eryngii*) in bottle culture. Besides; Cho *et al*. (2003) also found the similar result of growth promotion with culture of edible fungus *Pleurotus ostreatus* and fluorescent pseudomonads. The *Arthrobacter* sp. improves the growth plus yield of mushroom (Young *et al*., 2012; Zhou *et al*., 2017) and the bacterium *Arthrobacter arilaitensis* presently named as *Glutamicibacter arilaitensis* (Sutthiwong *et al*., 2018; Cleary *et al*., 2018). Therefore the present work was conducted to investigate the effect of a bacteria *Glutamicibacter arilaitensis* MRC119 (GenBank accession: MN626391) on the different aspects (Radial & lineal growth rate, yield and biological efficiency) of oyster mushroom *Pleurotus florida.*

Materials and Methods

Microbial strains and their maintenance

Fungal culture of oyster mushroom *Pleurotus florida* and growth promoting bacteria *Glutamicibacter arilaitensis* MRC119 were obtained from Mushroom Training &

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Research Center (MTRC), Veer Bahadur Singh Purvanchal University, Jaunpur, Uttar Pradesh, India. Fungal and bacterial cultures were grown and maintained respectively on potato dextrose agar and nutrient agar media. The cultures were maintained in culture tubes by sub culturing frequently at 4±1°C in refrigerators.

Radial growth test

The radial growth test of fungal mycelium was performed through co-cultivation of fungal and bacterial culture in Petri plates according to Kim *et al*. (2008). For this four day old mycelial plug (5 mm) was kept on the center of Petri plates containing bacterial lawn or supplemented with bacterial culture and incubated for 5-15 days at 22 ±1°C. The control plates were neither bacteria nor culture filtrate.

Lineal growth test

For determination of lineal growth rate overnight water soaked wheat straw was filled in the 18×150 mm test tubes autoclaved at 121°C for 40 minutes. After cooling test tubes were inoculated with bacterial inoculant (liquid culture) and by the culture filtrate. These were then inoculated by fungal inoculum putting over young mycelial plug on wheat straw and incubated at 25±1°C to investigate mycelial growth. While, control sets were not supplemented with neither bacteria and nor culture filtrate.

Assay of ACC deaminase activity

The assay of ACC deaminase activity in the samples of co-cultivation sets was performed spectrophotometrically through a modified method of Honma and Shimomura (1978) as described by Penrose and Glick (2003) measuring absorbance at 540 nm.

Cultivation of oyster mushroom

The cultivation of oyster mushroom was conducted in polybags using water soaked wheat straw sterilized with 2% (v/v) formalin and 1% (w/v) bavistin for overnight. The spawning was performed through layer-wise manner and after completion of mycelia run, polythene bags were cut opened and the beds were made naked. Thereafter cultivation beds were irrigated by sprinkling fresh water regularly thrice a day and wait for primordia. The mature fruit bodies were harvested and weighed separately.

Counting of fruiting bodies

Fully matured fruitbodies were harvested by sharp clean knife just above the surface of the substrate and count the number of fruit bodies per set separately.

Determination of biological efficiency (BE)

The biological efficiency was calculated using the following formula (% BE, Kg fresh mushroom Kg^{-1} dry substrate).

Results and Discussion

Influence of CF and BC on lineal growth rate

The lineal growth rate (mm/d) of mushroom mycelia was determined in test tube containing sterilized wheat straw treated with culture filtrate and bacterial culture and control (uninoculated). The culture filtrate treatment showed gradually increasing order of lineal growth rate as 4.72, 6.41, 8.73, 8.97 mm/d with the time of incubations on 5^{th}, 10^{th}, 15^{th} and 20^{th} days, respectively. However, bacterial culture treatment resulted in 4.45, 5.9, 7.89, 8.11 mm/d lineal growth rate respectively on 5^{th}, 10^{th}, 15^{th} and 20^{th} days. Both bacterial culture and culture filtrate showed faster growth rate as compared to untreated control sets. The highest growth rate recorded was 8.97 mm/d on 20^{th} day of culture filtrate treatment that was followed by 8.11 mm/d on 20^{th} day of bacterial culture treatment. It was observed that the culture filtrate remarkably influenced the lineal growth rate as compared with the treatments of bacterial culture and control (Table 1) Eyini *et al*. (2005) also reported similar observation that *Azotobacter* sp. synergistically increased the linear growth of oyster mushroom *Pleurotus eous*. The reason for the superior effect of CF on mycelial growth rate might be due to the existence of different types of soluble growth stimulatory substances such as indole acidic acid (IAA), siderophore and ACC deaminase activity in the aliquots of CF, which have been directly used by fungal mycelia (Zarenejad *et al*., 2012; Ebadi *et al*., 2012). However, the bacterial culture showed lesser effect because of readily available soluble growth inducing substances like growth hormones.

Influence of CF and BC on radial growth rate

The response of CF and BC on radial growth of mushroom mycelia was evaluated. The highest 8.3 mm/d of radial growth rate was recorded with the treatment of CF, which was two folds higher than the growth rate of control achieved on 20^{th} day. The second most highest lineal growth rate (7.9 mm/d) on the 20^{th} day of incubation was recorded with the treatment of BC subseqently. However the untreated control sets represented highest 4.1 mm/d on the 20^{th} day, that was lesser than the growth rates observed with subsequent response to the treatments of CF and BC (Table 2). The inoculation of CF was found to be remarkably effective on lineal growth rate of mushroom mycelia in comparison to BF. Similar results were also reported in response of bacterial culture (Kim *et al.*, 2008). Furthermore, similar results were also reported when white-rot fungus *Pleurotus florida* and a bacterium fluorescent pseudomonad co-cultivated, which positively influenced the mycelia growth (Cho *et al*., 2003).

Influence of CF and BC on ACC deaminase

The radial growth promotion effect of culture filtrate and bacterial culture was determined by measuring the length of

mycelia in response of their respective treatments. The co-cultivation of bacterial and fungal mycelia on PDA plates for 10-20 days resulted in a remarkable increases in the radial growth of mycelia compared to uninoculated. The analysis of ACC deaminase activity with the addition of bacterial inoculum and culture filtrate was recurred on 5th, 10th and 15th day of incubation. The highest enzyme activity recorded with the bacterial culture treatment (881.32 IU/g) while it was followed by 780.76 IU/g on 10th day of incubation. The untreated sets showed no activity of ACC deaminase. The treatment of bacterial culture found to be more effective in comparision to the treatment with culture filtrate (Table 3). Chen *et al*. (2013) disengaged another strain of *P. putida* UW4 creating ACC-deaminase compound amazingly advanced the hyphal development of button mushroom *A. bisporus*. In addition, several workers reported that bacteria and its culture filtrate produced ACC-deaminase enzyme which metabolized ACC into α-ketobutyrate and ammonia and regulating ethylene production (Glick *et al*., 1999; Saghafi *et al*., 2019).

Effect of CF and BC on initiation and number of fruitbodies

The effect of CF and BC on the initiation of pin heads and number of mushroom fruit bodies was found to be clearly visible in mushroom beds. The number of mushroom fruit bodies found highest (41.32) in CF treated cultivation beds compared to BC and control. The CF and BC treated cultivation sets achieved faster initiation of pinheads with rapid development and enlargement in size of fruit bodies. The addition of both CF and BC remarkably influenced the fruit body yield of oyster mushroom. The sets added with CF resulted in the highest number of fruit bodies which was higher than untreated control sets. Whereas, BC treated cultivation sets resulted in total 32.23 number of fruit bodies and it was 17.32 in control sets. Overall, addition of CF influenced both initiation of pinheads and development of oyster mushroom fruit bodies with the remarkable superiority over BC added sets (Figure 1). The similar results were reported by Riahi *et al*. (2011) at the pining stage, populations of pseudomonas species especially *P. putida* were important since they play a key role in fruiting body formation. Ebadi *et al*. (2012) found in his study that mushroom treated with bacterial culture increase the number of fruit bodies. An experiment showed that inoculation of pure bacterial cultures of fluorescent Pseudomonas spp. isolated from the mycelial plane promoted the highest primordia formation and enhanced the development of the fruit body of *P. ostreatus* (Cho *et al*., 2003).

Influence of CF and BC on biological efficiency

A group of microorganisms including bacterial culture and its culture filtrate in our study remarkably improved the biological efficiency of oyster mushroom *P. florida*. The culture filtrate and bacterial culture both induces early primordia initiation and fructification compared to uninoculated. In this study we found higher biological efficiency in beds treated with culture filtrate 256.45 than bacterial culture 204.65 and control 154.87 which are clearly shown in Figure 2. The biological efficiency of mushroom found affected by the synergistic involvement of bacteria. Mushroom cultivation supplemented with *Pseudomonas putida* increased biological efficiency reported by (Mohammad and Sabaa, 2015). The inoculation of bacteria accelerated fungal mycelia, which resulted increased basidium development and increased mushroom biological efficiency (Han, 1999; Cho *et al*., 2003).

Based on the observations of the present study it can be concluded that mushroom growth promotory bacteria *Glutamicibacter arilaitensis* MRC119 and its culture filtrate played a crucial role in the production of higher yields oyster mushroom. We therefor suggest the use of mushroom growth promoting bacteria and culture filtrates for safe and ecofriendly cultivation of mushrooms escaping harmful chemicals.

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Table 1: Lineal growth rate (mm/d) of fungal mycelia in response to bacterial culture and its culture filtrate on different days of incubation

Different treatments	Lineal growth rate (mm/d)			
	5th day	10th day	15th day	20th day
Culture filtrate (CF)	4.72± 0.02	6.41± 0.03	8.73± 0.03	8.97 ±0.04
Bacterial culture (BC)	4.45± 0.01	5.9± 0.02	7.89± 0.04	8.11 ±0.03
Untreated (Control)	3.23 ±0.01	4.42 ±0.01	4.88 ±0.02	4.98 ±0.02

±, standard deviation (n=10)

Table 2: Radial growth rate (mm/d) of fungal mycelia in response to bacterial culture and its culture filtrate on different days of incubation

Different treatments	Radial growth rate (mm/d)			
	5th day	10th day	15th day	20th day
Culture filtrate (CF)	6.5±0.02	7.8±0.03	8.1±0.02	8.3 ±0.04
Bacterial culture (BC)	6.1±0.01	6.8±0.02	7.3±0.04	7.9 ±0.03
Untreated (Control)	3.1 ±0.01	3.7 ±0.02	3.9 ±0.01	4.1 ±0.02

±, standard deviation (n=10)

Table 3: ACC deaminase activity (IU) of pureculture filtrate, bacterial culture and control

Treatments	ACC deaminase activity (IU/g)		
	5th day	10th day	15th day
Culture filtrate (CF)	476.87±21	881.32±28	687.76±31
Bacterial culture (BC)	366.54±11	780.76±17	513.67±15
Untreated (Control)	nd	nd	nd

nd= not detected, ±, standard deviation (n=3)

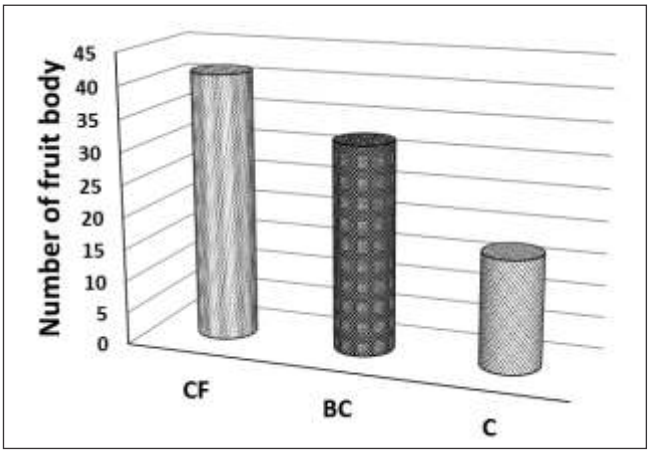

Figure 1: Total number of fruitbodies in cultivated mushroom *Pleurotus florida* treated with culture filtrate and bacterial culture

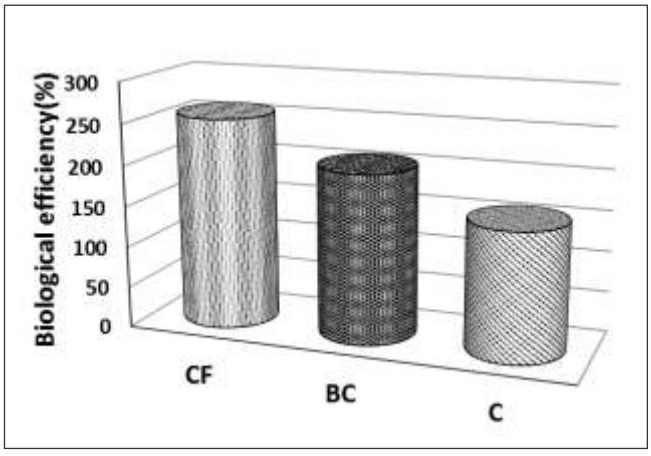

Figure 2: Biological efficiency of cultivated mushroom *Pleurotus florida* treated with culture filtrate and bacterial culture