

Effect of Maize Substrate Bacterial Isolates on Growth of *Pleurotus eoeus* and *Calocybe indica*

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ABSTRACT

An investigation was carried out to isolate and characterize the bacterial isolates from maize crop byproducts (Maize stover, Maize sheath, and Maize rind) and to study the effect of bacterial isolates on growth of *Pleurotus eoeus* and *Calocybe indica* in *in vitro* condition. The results revealed that all the bacterial isolates (Iso-S-1, Iso-S-2, Iso-S-3, Iso-Sh-4, Iso-R-5) were inhibitory to mushroom fungi.

INOCULATION of bacterial culture on mushroom growing media, caused an increase of mycelial growth rate up to 1.6 fold and promoted the rate of radial hyphal extension (Kim *et al.*, 2008). *Pseudomonas putida* has been identified as an important species involved in fruiting body initiation (Hayes *et al.*, 1969). The casing soil supports an aerobic bacterial flora, among them fluorescent *Pseudomonas* spp. play an important role in initiation of pinning and mushroom fruiting body development (Hayes and Nair, 1976). Hence these studies were conducted to know the effect of different maize substrate bacterial isolates on *Pleurotus eoeus* and *Calocybe indica* in *in vitro* condition.

Bacteria were enumerated in different substrates like Maize stover, Maize cob sheath and Maize rind by following the dilution plate count method using nutrient agar medium. The bacterial sp. like Iso-S-1, Iso-S-2, Iso-S-3 were isolated from maize stover, Iso-S-4 was isolated from maize sheath and Iso-R-5 was isolated from maize rind. The Population was calculated by counting the colonies and further dominant types of colonies were purified by streak plate method. These cultures were maintained on nutrient agar slant for further studies.

Seven days old culture *i.e.*, mycelial disc (5mm) from a *Pleurotus eoeus* and test bacteria were placed on the PDA plate opposite to each other, equidistant from the periphery and were incubated at 25°C. Likewise *Calocybe indica* and test bacteria were placed on the plate opposite to each other equidistant

from the periphery and were incubated at 30°C. After 7 days of the incubation period, radial growth of *Pleurotus eoeus* was recorded and percentage inhibition was calculated in relation with control (Hajieghrari *et al.*, 2008).

$$L = (C - T) / C \times 100$$

L = Inhibition of radial mycelial growth; C = radial growth measurement of fungus in control;

T = Radial growth measurement of fungus in the presence of bacterial species.

Note: Mycelial growth measurement: The four different radial measurements of mycelial growth from centre point of the fungal inoculum.

The data collected was subjected to statistical analysis using CRD (Completely randomized designed) (Littly and Hills, 1978).

Biochemical characters of the isolated bacteria is presented in the Table I. The effect of bacterial isolates on the growth of *Pleurotus eoeus* is presented in the Table II and Plate 1. The maximum mycelial growth of *Pleurotus eoeus* (3.20cm) on PDA plate without bacterial culture was recorded. In bacterial culture combination with mushroom fungus plate shows the inhibitory effect of *Pleurotus eoeus* by all bacterial isolates. Among the bacterial isolates highest percentage of mycelial growth inhibition was found in Iso-S-3 (49.4%).

The effect of bacterial isolates on the growth of *Calocybe indica* is presented in the Table III and

plate 1. The maximum mycelia growth of *Calocybe indica* (2.37cm) on PDA plate without bacterial culture was recorded. In bacterial culture combination with mushroom fungus plate shows the inhibitory effect of *Calocybe indica* by all the bacterial isolates. Among the bacterial isolates highest percentage of mycelial growth inhibition was found in Iso-S-3 (9.70%).

The 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 (Kim *et al.*, 2008).

In this study on contrary to Kim *et al.*, (2008) no isolated bacteria showed the growth enhancing character but they showed the inhibitory effect on

TABLE I
Morphological and biochemical characteristics of isolates

Characters	Iso-S-1	Iso-S-2	Iso-S-3	Iso-Sh-4	Iso-R-5
Shape	Cocci shape	Cocci shape	Cocci shape	Cocci shape	Cocci shape
Gram reaction	G+ve	G-ve	G-ve	G-ve	G-ve
Endospore staining	-	-	-	-	-
Catalase test	+	+	+	+	+
Gelatin hydrolysis	-	-	-	+	-
Nitrate utilization	+	+	+	-	-
Carbon source utilization (dextrose, cellulose, Lactose, maltose, arabinose, glucose, D - xylose)	+	+	+	+	+
Motility test	-	-	-	-	-
MRVP test	-	-	-	-	-

Note: ‘+’ - positive , ‘—’ - negative

Isolates: Iso-S-1, Iso-S-2, Iso-S-3 from maize stover, Iso-Sh-4 from maize sheath and Iso-R-5 from maize rind.

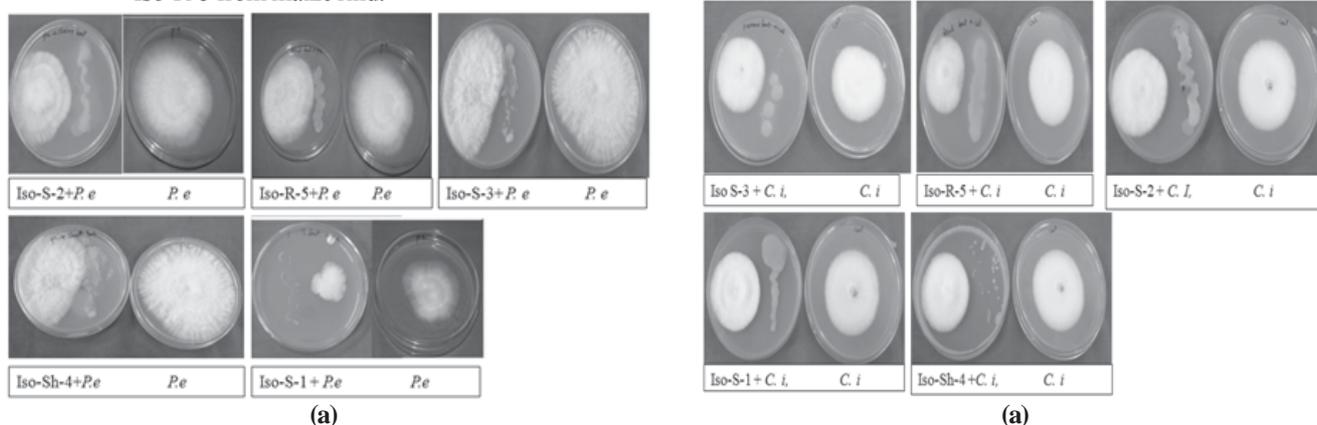


Plate 1. Compatibility of bacterial isolates and mushroom fungal species

Note: a) Compatibility test between *Pleurotus eous* and bacterial isolates
b) Compatibility test between *Calocybe indica* and bacterial isolates
P. e-*Pleurotus eous*, *C. i*-*Calocybe indica*

Isolates: Iso-S-1, Iso-S-2, Iso-S-3 from maize stover, Iso-Sh-4 from maize cob sheath and Iso-R-5 from maize rind

TABLE-II
Compatibility of bacterial isolates and
Pleurotus eoeus

Isolates	<i>Pleurotus eoeus</i> growth on PDA medium(Radius in cm)	% Inhibition of mycical growth
Control (only <i>Pleurotus eoeus</i>)		3.20
-		
Iso-S-1	2.35	26.6
Iso-S-2	2.42	24.4
Iso-S-3	1.62	49.4
Iso-Sh-4	2.53	20.94
Iso-R-5	2.43	24.06
SEm ±	0.04	
C.D at 1%	0.18	

Note: Data are mean value of four replication
PDA- Potato dextrose agar

TABLE III
Compatibility of bacterial isolates and
Calocybe indica

Isolates	<i>Pleurotus eoeus</i> growth on PDA medium(Radius in cm)	% Inhibition of mycical growth
Control (only <i>Calocybe indica</i>)		2.37 -
Iso-S-1	2.22	6.33
Iso-S-2	2.23	5.91
Iso-S-3	2.14	9.70
Iso-Sh-4	2.24	5.48
Iso-R-5	2.26	4.64
SEm ±	0.04	
C.D at 1%	0.18	

Note: Data are mean value of four replication

mushroom fungus sp., this may be due to production of antifungal compounds reported by Tharmila *et al.*, 2013. Similarly growth inhibitory ability of *Bacilli* species, *Bacillus subtilis* and *B. polymyxa* in *invitro* have been reported against wood decaying fungi (Melentev *et al.*, 2006).

All the bacterial isolates had antagonistic effect on vegetative growth of the mushroom fungal species.

REFERENCES

- HAJIEGHRARI, B., TORABI-GIGLOU, M., MOHAMMADI, M. R. AND DAVARI, M., 2008, Biological potential of some Iranian *Trichoderma* isolates in control of soil borne plant pathogenic fungi. *Afr. J. Biotechnol.* **7**: 967-972.
- HAYES, W. A. AND NAIR, N. G. 1976, Effects of volatile metabolic by-products of mushroom mycelium on the ecology of the casing layer. *Mushroom Sci.*, **9**: 259-268.
- HAYES, W. A., RANDLE, P. E. AND LAST, F. T., 1969, The nature of the microbial stimulus affecting sporophore formation in *Agaricus bisporus* (Lange) Sing. *Annu. Appl. Biol.* **64**: 177-187.
- KIM, M. K., MATH, R. K., CHO, K. M., SHIN, K. J., KIM, J. O., RYU, J. S., LEE, Y. H. AND YUN, H. D. 2008, Effect of *Pseudomonas* sp. P7014 on the growth of edible mushroom *Pleurotus eryngii* in bottle culture for commercial production. *Bioresource Technol.*, **99**: 3306-3308.
- LITTLY, T. M. AND HILLS, F. C. M., 1978, Agricultural experimentation, John Willy and Sons, INC. USA. **13**:22-26.
- MELENTEVEV, P., HELISTO, L., YU, K. M., AKTUGANOV, N. F. G. E. AND KORPELA, T., 2006, *In vitro* screening of antagonistic effect of soil borne bacteria on some selected phytopathogenic fungi. *Appl. Bioch. and Microbiol.*, **42(1)**: 70-75.
- THARMILA, S., VASANTHAKALA, R., NALINI, S. AND ARULANANTHAM, C. T., 2013, *In vitro* screening of antagonistic effect of soil borne bacteria on some selected phytopathogenic fungi. *Archives of Appl. Sci. Res.*, **5(1)**:1-4.

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