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Selection of Effective Ligno-Cellulolytic Fungal Isolates for Recycling of Paddy Crop Residues

S.B. Gupta[1], Anup K. Singh[2], Kapil S. Thakur[3], D.K. Tamrakar[4], Tapas Chowdhury[5] and M.P. Thakur[6]

[1-5]Department of Microbiology, [6]Department of Plant Pathology,
Indira Gandhi Krishi Vishwavidyalaya, Raipur - 492 006, India.

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Three hundred five samples of partially decomposed paddy crop residues were collected from Dhamtari, Kanker, Bastar, Durg, Raipur and Mahasamund districts of Chhattisgarh plains in order to isolate and identify effective lingo-cellulolytic fungal isolates for quick recycling of paddy crop residues. In this connection, 110 fungal isolates were isolated. These isolates were mostly belonging to fungal genera *Aspergillus, Trichoderma, Penicillium, Mucor* etc. All the isolates were further tested for lignin and cellulose decomposing ability. Out of the total 110 isolates, 25 fungal isolates showed lignolysis. While 63 isolates showed cellulose degrading ability

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The total annual availability of agricultural waste in India is about 357, of which, 170 million tonnes are left out for burning or cause environmental pollution (Tiwari and Pandey, 2002). Thus, a potent source of organic carbon is lost from Indian tropical soils which requires frequent application of organic manure to sustain fertility and productivity of soils for long period. Composting/ humification has been used as a means of disposal of organic wastes. It should be improved qualitatively either by enriching with plant nutrients and nutrient mobilizing beneficial microbes. Several studies have shown that humic substances are very important for sustainable agricultural production. They are formed through polymerization or condensation of phenolic compounds derived from lignin degradation with amino acids and peptides (Kononova and Alexandrova, 1973). Adequate lignin decomposition is therefore, supposed to increase humus formation.

The beneficial effect of high C:N ratio containing organic waste like paddy crop residues on soil productivity depend upon their rate of decomposition which is mainly controlled by enzymatic activity of soil microbes. Although, soils abound in natural micro flora, yet suitable reinforcements to the existing flora are liable to promote decomposition of added plant residues. Cultures of selected microorganisms and preparations of certain enzymes have been found useful in recent year in accelerating the composting of organic wastes. Thus, research need exist in evaluating the role of natural microbes for activating the decomposition of soil-incorporated plant residues, particularly those of lignocelluloses in nature (Bharadwaj, 2003). Therefore, the present investigation was

* To whom all correspondence should be addressed.
Tel. +91-771-244901 (Ext.) 2109
Fax. No. +91- 771 -2442131, 2442302
E-mail : sbgupta_igau2002@yahoo.co.in

undertaken in order to identify and formulate suitable fungal inoculants for quick recycling of the organic wastes like paddy crop residues including paddy stubbles.

MATERIAL AND METHODS

Three hundred five samples of partially decomposed paddy crop residues (husk, stubbles etc.) were collected from Dhamtari, Kanker, Bastar, Durg, Raipur and Mahasamund districts of Chhattisgarh plains in order to isolate and select effective fungal isolates for recycling of lingo-cellulosic agricultural wastes. For isolation of fungal isolates, aliquot of appropriate dilution of the sample was inoculated on potato dextrose agar medium (PDA). The genera of these isolates were identified on the basis of morphological characteristics as described by Barnett and Hunter 1972, Dube, 1993. After purification, the isolates were preserved in PDA slants with properly marked isolate numbers.

Further lignolytic activities of all the fungal isolates were tested by spot method on low nitrogen agar medium (Glucose 1%, Malt extract 1%, Peptone 0.2%. Yeast extract 0.2%, Asparagine 0.1%, KH_2PO_4 0.2%, $MgSO_4$ 1%, Thiamine HCl 1ppm, Agar 2.0% and pH 6.5. Filter sterilized phenol red (0.02%) was spraeded on agar plates as an indicator. Plates were incubated for 6-7 days and observed for change in colour around colony from yellow to red.

Cellulolytic activity of fungal isolates were determined by using assay of Cx-cellulose (Endo-β-1, 4 gluconase) by measuring reduction in viscosity of the 0.5 percent carboxy methyl cellulose (CMC) solution. Viscometeric measurements were made with Ostwald's viscometer at different time interval of 0,15,30,45 and 60 minutes. The reaction mixture consisted of

(i) 5ml of 0.5% CMC solution.
(ii) 2ml of sodium citrate buffer at pH level of 7.0
(iii) 2ml of culture filtrate of different isolates as a source of cellulase enzyme.

The reduction in viscosity of CMC was calculated by the formula as suggested by Muse *et al.*, 1972:

$$\%\text{loss in visocity} = \frac{T_0 - T_1}{T_0 - T_w} \times 100$$

where
T_0 = Flowtime of reaction mixture at 0 minutes.
T_1 = Flowtime of reaction mixture at particular time interval
T_w = Flowtime of distilled water

RESULTS AND DISCUSSION

The sum of 110 fungal isolates were obtained from 305 samples collected form different districts of chhattisgarh plains (Table 1). Out of 110 fungal isolates, highest number of isolates belonged to genera *Aspergillus* (23) followed by *Penicillium* (12), *Mucor* (11), *Trichoderma* (9), *Rhizopus* (8), *Humicola* (7), *Paecilomyces* (6), *Alternaria* (5), *Fusarium* (5), *Sporotrichum* (5), *Chrysoporium* (5), and *Sclerotium* (5) (Barnett and Hunter 1972, Dube 1993, Gilman 1998). Donnison *et al.,* (2000) also reported that *Mucor, Trichoderma* and *Paecilomyces* fungi were commonly isolated from litter and soil. Similarly, Malibari *et al.,* (1989) mentioned that they isolated 23 fungal genera, among them the largest frequency of *Aspergillus* species (12) occurred in both rhizosphere and non-rhizosphere soil.

Further, all the fungal isolates were screened for lignolytic ability. Results indicated that out of total 110 isolates, 35 isolates mostly belonging to fungal genera *Aspergillus, Trichoderma, Chaetomium Penicillium* (Table 2, Fig. 1) and showed lignolysis as indicated by development of pinkish red colour around the microbial colony because of lignolytic enzymes. Lignolytic enzymes are produced after cessation of primary growth and during secondary metabolism of lignolytic microbes. In phenol red plates, changes of colour from yellow to red around the lignolytic microbial colonies indicate positive character of lignolysis, which is an oxidative process.

Isolates of genus *Aspergillus* was found to be most dominant lignolytic fungi followed by isolates of *Trichoderma,* among the 25 lignolytic fungal isolates (Table 2). Colombo *et al.,* (1996) also found *Aspergillus* as potential species for lignolysis of crop residues in a laboratory experiment. *C. cellulolyticum* a soft rot fungus is able to degrade lignin under aerobic conditions as reported by Schober and Trosch (2000).

The dominance of *Aspergillus* and *Penicillium* in rhizosphere and non-rhizosphere soils were also reported by Fresquez and Sabey (1989) which is in accordance with the present study. These microbes may be able to produce bioactive substances and biocatalysts, which may probably be helping in lignin degradation. (Cheetham, 1987, Steele and Stowers, 1991).

The fungal isolates were further quantitatively tested for cellulose decomposing ability by viscometeric method. Out of 110 fungal isolates tested, only 63 isolates exhibited cellulolytic activity by reduction in viscosity of the CMC reaction mixture at different time intervals (Table 3 and Fig. 1). Among them, *Aspergillus* and *Trichoderma* isolates were found

Table 1. List of fungal isolates, associated with paddy crop residues under climatic conditions of chhattisgarh plains.

S.No	No. of Isolates	Geneara	Morphological characteristics
1.	23	*Aspergillus*	Aseptate mycelium hyaline, conidiophore upright, bearing philals at apex, conidia-1 celled, globose
2.	12	*Pencillium*	Condiiophores branched septate mycelia, primary and secondary sterigmata present, phialides bottle shaped, conidia ovoid.
3.	11	*Mucor*	Asepted hyaline, branched sporangiophores bear globular sporangium
4.	9	*Trichoderma*	Condiophore hyaline, highly branched, phialides-2-3, conidia 1 celled, ovoid, borne in small terminal clusters, shows rapid growth and green patches or cushions of conidia
5.	9	*Chaetomium*	Perithecium with long beak and covered with hairs. The asci are cylindrical or oval, conidia absent, ascospores present.
6.	8	*Rhizopus*	Aseptate mycelium distinguishable into aerial hyphae called stolons is root like rhizoids. Sporangiophores short arise from junction of stolen and rhizoids. Sporangia white at early stage, turns bluish black at maturity.
7.	7	*Humicola*	Condiophores, simple or rarely with short branches, dark comidia single, brown 1-celled produce simple phialides and phialospores in chains.
8.	6	*Paecilomyces*	Condiophores and branches more divergent than in Pencillium; conidia (phialospores) in dry basipetal chains, 1-celled, ovoid hyaline.
9.	5	*Alternaria*	Septate, hyaline creeping hyphae, condiophores single or in groups, erect and unbranched. Conidia inverted club shaped elongated at the tip, borne in chains. Colonies black, green, brown rough appearance.
10.	5	*Fusarium*	Hyphae are hyaline, branched and septate conidia slimy, scattered on mycelium. Conidophores branched, septate bearing terminal phialide. Oval or comma shaped microconidia and hyaline or pale spindle shape macro conidia present.
11.	5	*Sclerotium*	Mycelium densely branched, bearing numerous olive brown to clove brown, globose sclerotia.
12.	5	*Sporotrichum*	Hyphae creeping, irregularly branched, conidiophores only as projection from side branches. Conidia borne terminally on branches, sessile, hyaline, or bright coloured.
13.	5	*Chrysoporium*	Conidiophores poorly differentitated, hyaline, conidia, (aleuriospores) hyaline, 1 celled, single or in short chains.
Total	110		

Table 2. List of promising lingo-cellulolytic fungal isolates

Ligno-cellulolytic fungal isolates			
Lignolytic isolates		Cellulolytic isolates	
Isolate Nos.	Genera	Isolate Nos.	Genera
OMF- 4,82,110, 120,**147**,513	*Aspergillus*	OMF-4,8,10,14,52,41,82,110,120, 147,470,503,513,520,535,536	*Aspergillus*
OMF-19,45,50,91,134,172	*Trichoderma*	OMF-19,45,50,88,91,93,134,157,172	*Trichoderma*
OMF-**105**,167,173	*Pencillium*	OMF-2,5,9,12,27,31,51,87,105,167	*Penicillium*
OMF-**84**,**114,146,**468	*Chaetomium*	OMF-84,114,146,165,175,442,468	*Chaetomium*
OMF-**11,115**	*Mucro*	OMF-174,184	*Chrysosporium*
OMF-174,184	*Chrysosporium*	OMF-122,144,163,452,474,509,531	*Humicola*
OMF-117,143	*Paecilomyces*	OMF-160,166,176,177,187	*Sclertium*
		OMF-182,451,510,528	Sporotrichrum

Table 3. Performance of cellulolytic fungal isolates.

S. No	Genera	No of isolates	% loss in CMC viscosity due to cellulase at different time interval			
			15 minutes	30 minutes	45 minutes	60 minutes
1.	*Aspergillus*	17	≤ 69.23	≤ 80.0	≤ 90.0	≤ 90.0
2.	*Trichoderma*	9	≤ 60.0	≤ 80.0	≤ 90.0	≤ 90.0
3.	*Pencillium*	12	≤ 50.0	≤ 50.0	≤ 66.67	≤ 80.0
4.	*Chaetomium*	5	≤ 50.0	≤ 50.0	≤ 62.50	≤ 75.0
5.	*Humicola*	7	≤ 50.0	≤ 50.0	≤ 66.67	≤ 75.0
6.	*Sporotichum*	4	≤ 50.0	≤ 50.0	≤ 50.0	≤ 75.0
7.	*Sclerotium*	5	≤ 40.0	≤ 60.0	≤ 75.0	≤ 75.0
8.	*Chrysoporium*	2	≤ 37.5	≤ 50.0	≤ 62.50	≤ 75.0
	Total	63				

to be most efficient cellulolytic fungal isolates as indicated by results obtained during assay of Cx-cellulase (Endo-β-1, 4 gluconase). Among the cellulolytic fungal isolates, OMF-45:*Trichoderma,* OMF-8:*Aspergillus,* OMF-134:*Trichoderma* showed highest cellulolytic activity at 60 minutes time interval (90% loss in viscosity of the reaction mixture) followed by OMF-88: *Trichoderma* (83.33%); OMF-470: *Aspergillus* (83.33%)OMF-5:*Pencillium* (80%), OMF-12: *Penicillium* (80%) and OMF-172: *Trichoderma* (80%). Lynch (1989) reported that *Trichoderma* spp. can produce enzymes which are effective in degradation of natural lingo-cellulose rich organic wastes such as paddy stubbles. Krol (1999) also found that *Penicillium* and *Trichoderma* fungi synthesized enzymes which catalyzed degradation of high C/N ratio containing organic matter.

CONCLUSION

It is concluded from the study that isolates of *Trichoderma* (OMF-45,OMF-134) and *Aspergillus* (OMF-8) found to be most efficient cellulolytic fungal isolates among 63 isolates taken under study. However, *Aspergillus* isolates were found to be most dominant followed by *Trichoderma* isolates among 25 lignolytic isolates studied.

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