

Indegenous cellulolytic fungi in composting of agro-industrial residue

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ABSTRACT

A variety of indigenous microbial strains were found to be involved in decomposition of agro-industrial residues for preparation of dry and wet composts. Among them four fungal isolates were found to be dominant in decomposition of the lignocellulosics of the residues. These isolates identified as *Trichoderma* sp., *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* sp. were capable of producing Cellulolytic and Xylanolytic enzymes. Agro-wastes were inoculated with the isolates as mixed culture for determining their enzymatic activity during composting where enzymatic activity was stable up to 15 days, but pH was found to be gradually dropped to 4. *Trichoderma* sp. was found to be most potential in producing cellulolytic and Xylanolytic enzymes. However, growth of *Aspergillus flavus* was faster than that of *Trichoderma* sp. in Potato Dextrose Agar medium. © 2014 Trade Science Inc. - INDIA

KEYWORDS

Agro-industrial residues;
Composts;
Lignocellulosics;
Cellulolytic enzymes;
Xylanolytic enzymes.

INTRODUCTION

Composting is a biotechnological art of decomposition of organic wastes. It is well recognized that cellulolytic fungi especially *Aspergillus* spp, *Trichoderma* spp, *Penicillium* spp, *Candida* spp. Etc. play an important role in composting of agro-industrial residues^[1]. A few bacteria have been recently reported to yield cellulase activity. Bacteria involved in cellulase production include the *Corynebacterium*, *Cellulomonas fimi*^[2], *Bacillus licheniformis*, *Brevibacillus parabrevis*, *Cellulomonas cellulans* MTCC 23, *Cytophaga hutchinsonii* NCIM 2338, *Pseudomo-*

nas aeruginosa, *Ralstonia pickettii*, *Pseudomonas* sp. and *Pseudomonas cepacea* and thermophilic bacteria *Clostridium thermocellum*^[2], *Thermoascus aurantiacus*^[3].

Even though there are many reports on cellulase producing fungi^[4] only few have high activities for commercial success^[5,6]. Filamentous fungi are preferred for commercially important enzymes production, because the level of the enzymes produced by these cultures is higher than those obtained from yeast and bacteria^[7].

Microorganisms of the genera *Trichoderma* and *Aspergillus* are thought to be cellulase producers, and crude enzymes produced by these microorganisms are

commercially available for use. The most extensively studied cellulolytic and hemicellulolytic enzyme-producing fungi are the *Trichoderma* species including *T. reesei*^[8,9], *T. viride*^[10], *T. harzianum*^[11], *T. longibrachiatum*^[12].

MATERIALS AND METHODS

Two types of locally made compost were used as sample for the isolation of the potential microbes. Standard serial-dilution agar plate method was employed for quantitative analysis of total aerobic heterotrophic microorganisms using nutrient agar medium^[13]. The plates were incubated at 37°C for 24 hours.

The cellulolytic fungus was isolated from compost. The fungal isolates were inoculated on slants of potato dextrose agar (PDA)^[14] and incubated at 30°C for 4-5 days using Heraeus incubator (BK500, Germany) to produce mycelium and spores. The cultures were kept at 4 °C in refrigerator (Italy) and sub cultured every 3-4 weeks regularly. The fungal colonies grown on PDA medium (for 4-5 days and 3 days respectively at 30°C) were studied for size, shape, colour, opacity, consistency, texture, pigment formation etc. The selected fungal mycelia and yeast cells were examined using a phase-contrast microscope (Nikon Microphot, UFX-IIA, Japan) and their microscopic features were noted. For counting the fungal spore and yeast cells in culture, Neubauer counting chamber (Germany) was used.

The number of viable cells was determined by serial dilution in saline and then spreading 0.1 ml of the appropriately diluted sample onto Nutrient Agar medium. This method assumes that each of the viable cells from an individual colony when grown on Nutrient Agar plate. The plates were incubated at 37 °C for 24 hrs and the number of colonies was counted with a colony counter.

The fungal inoculum was grown on medium containing (per liter) 10g yeast extract, 10g beef extract, 2g peptone, 1g glucose and appropriate amount of FeSO₄.7H₂O. This medium was used for enrichment of the growth of the organism. The standard mineral basal medium used for fungal growth and enzyme^[15]. It contained (per liter) 0.6 g KH₂PO₄, 0.5 g MgSO₄.7H₂O, 0.4 g K₂HPO₄, 0.74 g CaCl₂.2H₂O, 2.32 g NH₄H₂PO₄, and 1.0 g yeast extract, and 7.0 ml of trace salts solution containing (per 100 ml): 200 mg

CoCl₂.7H₂O, 500 mg FeSO₄.7H₂O, 160 mg MnSO₄.H₂O, and 140 mg ZnSO₄.7H₂O and 2.0% (w/v) sugarcane bagasse as a carbon source.

Growth in liquid culture media was carried out in 250 ml wide-mouth conical flask containing 50 ml medium, containing the substance for quantitative studies on growth and enzyme production. The strains were grown under different nutritional conditions (media, carbon and nitrogen substance, concentration of substance, etc.) and environmental conditions (initial pH) in a rotary shaker at 150 rpm for various growth periods up to 72 hrs. For evaluation of the effect of culture pH on enzyme production, the fungus *Trichoderma sp.* was cultivated in enzyme production medium with different initial pH value (4.0-6.0) at 30°C and 200 rpm for 3 days. After incubation the enzyme activity (CMCase) was determined.

After production of enzymes in shake and static cultures, the cells were separated out by centrifugation (WIFUG Lab. Centrifuge 200 E, Japan) at 10,000×g for 10 min. The supernatant was dialyzed against 0.1 M phosphate buffer (pH=6.8) overnight to remove the unwanted low molecular weight solutes of the culture supernatant. The dialyzed culture supernatant was then directly assayed or storage at 4 °C.

Activity of different enzymes such as carboxymethyl cellulase (CMCase), Xylanase activities of the culture supernatant were assayed using specific assay substrates and appropriate adjustment of incubation times, so that the estimated activities were within the optimum range of each enzyme.

The agro-waste was collected from local area. The waste contained various residues. The volume of the container was 8 liter. The weight of the waste was 6 kg. Inoculum growth media were inoculated with potential microbes and the media were incubated for 24 hrs in a shaker. Then the cultures were mixed. Then the waste was inoculated with the mixed culture. Then waste was incubated for 15 days in aerobic condition.

SPSS version 11.5 was used to analysis the data. One - way analysis of variance (ANOVA) was used to determine significant difference where (P <0.05).

RESULTS AND DISCUSSIONS

In this study two types of locally made composts were used as samples for the isolation of indigenous

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fungus strains. Nutrient agar medium was used for determining the microbial load of the compost. Total bacterial density in locally made wet compost ($8.6 \pm 0.62 \times 10^4 \text{ cfu mL}^{-1}$) was significantly higher than that of

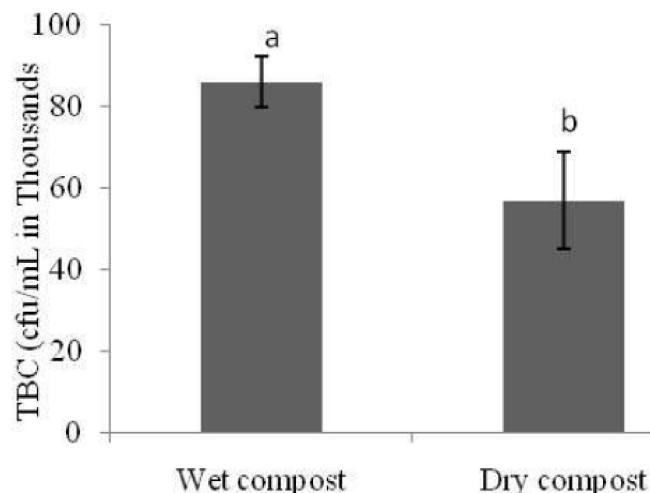


Figure 1 : Bacterial density (cfu mL^{-1}) in locally made wet and dry compost

dry compost ($4.7 \pm 1.2 \times 10^4 \text{ cfu mL}^{-1}$) (Figure 1).

Four types of colonies were found on Potato Dextrose Agar (PDA) medium. Then these different colonies were subcultured several times on PDA media to obtain pure culture of these different microbes. From the colony morphology and microscopic features the fungi were identified as *Trichoderma* sp. (Figure 2), *Aspergillus niger* (Figure 3), *Aspergillus flavus* (Fig-

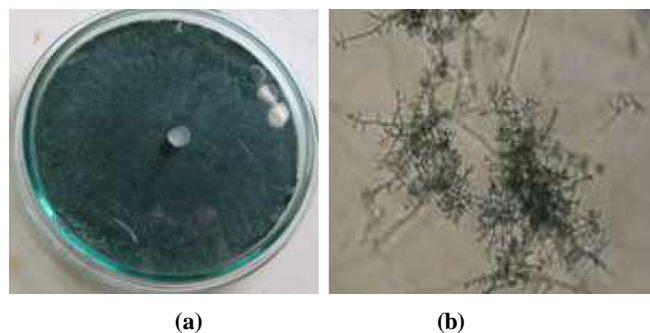


Figure 2 : (a) Seven day old culture of *Trichoderma* sp. in PDA medium and (b) Microscopic view of the mycelia of *Trichoderma* sp.

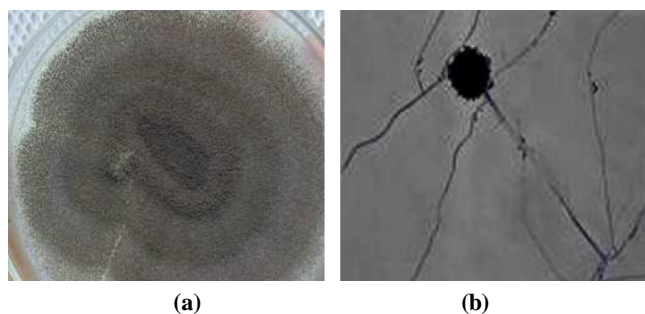


Figure 3 : (a) Seven day old culture of *Aspergillus niger* in PDA medium at 25 °C and (b) Microscopic observation of *Aspergillus niger*

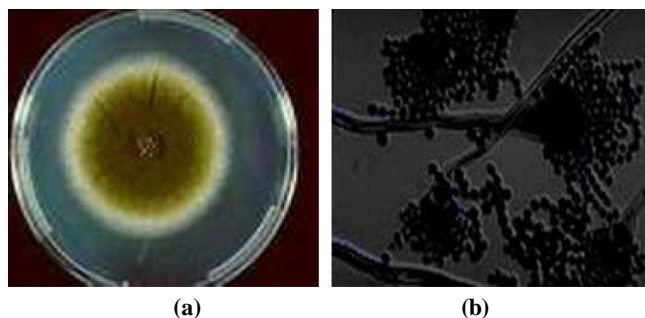


Figure 4 : (a) Seven day old culture of *Aspergillus flavus* in PDA medium and (b) Microscopic observation of *Aspergillus flavus*

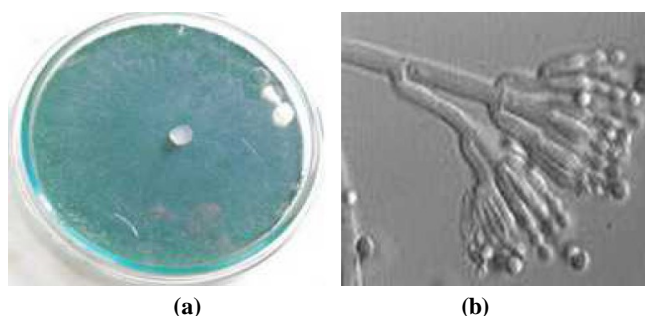


Figure 5 : (a) Seven day old culture of *Penicillium* sp. in PDA medium and (b) Microscopic observation of *Penicillium* sp.

ure 4) and *Penicillium* sp. (Figure 5).

The different enzyme activity of four isolated fungus species was determined (TABLE 1). In this study the *Trichoderma* sp. showed higher CMCase and Xylanase activity.

Agro-waste collected from local area was inoculated with *Aspergillus niger*, *A. flavus*, *Penicillium* sp

TABLE 1 : Enzyme activity of four isolated fungus species.

Substrate	Enzyme	Enzyme activity (u/ml) produced by			
		<i>Trichoderma</i> sp	<i>Penicillium</i> sp	<i>Aspergillus niger</i>	<i>Aspergillus flavus</i>
CMC	CMCase	0.91±0.01 ^{ab}	0.37±0.01 ^{bcBC}	0.41±0.01 ^{bBC}	0.40±0.02 ^{bcBC}
Bagasse	Xylanase	8.10±0.03 ^{aA}	3.87±0.01 ^{dA}	4.25±0.01 ^{bA}	4.15±0.03 ^{cA}
	CMCase	0.87±0.01 ^{ab}	0.32±0.01 ^{bc}	0.38±0.01 ^{abC}	0.37±0.01 ^{abBC}

and *Trichoderma sp.* isolates as mixed culture for determining their enzymatic activity during composting where enzymatic activity was stable up to 15 days (Figure 6) but pH was found to be gradually dropped to 4 (Figure 7).

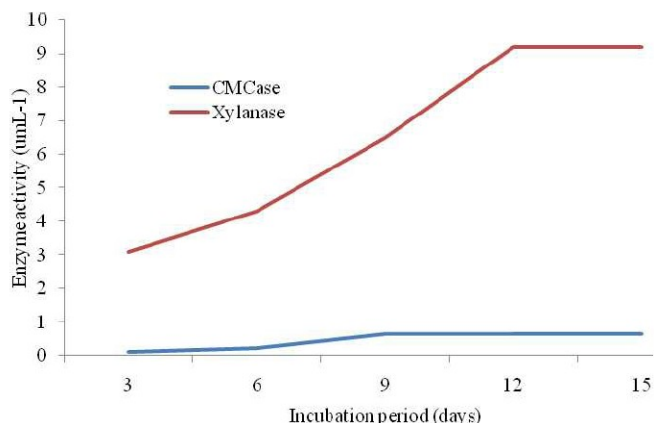


Figure 6 : Enzyme activity of mixed culture during composting of agro-waste

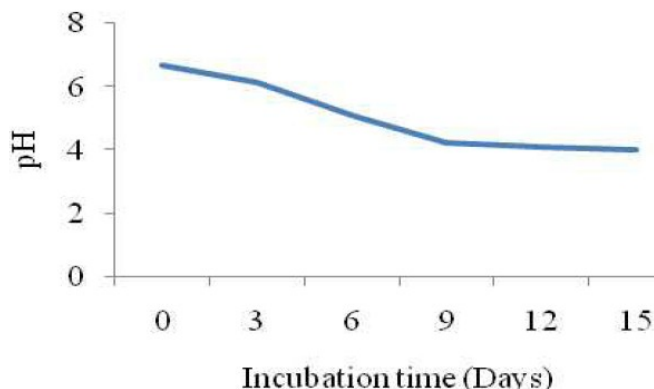


Figure 7 : Changes of pH during Composting of Agro-waste

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