

# Evaluation of thermophilic fungal consortium for paddy straw composting

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**Abstract** Out of 10 thermophilic fungi isolated from wheat straw, farm yard manure, and soil, only three showed highest cellobiase, carboxymethyl cellulase, xylanase, and FPase activities. They were identified as *Aspergillus nidulans* (Th<sub>4</sub>), *Scytalidium thermophilum* (Th<sub>5</sub>), and *Humicola* sp. (Th<sub>10</sub>). A fungal consortium of these three fungi was used to compost a mixture (1:1) of silica rich paddy straw and lignin rich soybean trash. The composting of paddy straw for 3 months, during summer period in North India, resulted in a product with C:N ratio 9.5:1, available phosphorus 0.042% and fungal biomass 6.512 mg of *N*-acetyl glucosamine/100 mg of compost. However, a C:N ratio of 10.2:1 and highest humus content of 3.3% was achieved with 1:1 mixture of paddy straw and soybean trash. The fungal consortium was effective in converting high silica paddy straw into nutritionally rich compost thereby leading to economical and environment friendly disposal of this crop residue.

**Keywords** Carboxymethyl cellulase · Cellobiase · Filter paper activity · Farm yard manure · Paddy straw · Soybean trash · Xylanase

## Introduction

Rice–wheat cropping system is widely practiced in north-west India. As a consequence, crop residue yield of wheat and paddy range between 3.2–5.6 t ha<sup>-1</sup> and 6.2–11.8 t ha<sup>-1</sup> respectively. Paddy straw contains >9.0% silica, is rarely used as animal feed. A little portion of paddy straw is used for growing mushroom (Ross and Harris 1983; Stratsma et al. 1995) and in industries as packing material. The major portion of it is burnt in the field itself (Gupta et al. 2004). This results on one hand in a waste of renewable organic source in soil affecting C:N ratio and biota and on the other hand leads to emission of green house gases (Badrinath et al. 2006). Keeping in mind the harmful effects of burning paddy straw in the field, as well as the convenience of farmers, an economical, environment friendly and low labor intensive strategy should be adapted for effective utilization of this waste. The application of bio-degraded products of straw into soil has enormous potential to recycle nutrients and maintain soil fertility (Gaind et al. 2006). However, the presence of high lignin content makes the paddy straw less vulnerable to microbial attack. For effective utilization of lingo–cellulosic residues, several physical and chemical pre-treatments are required, that may not be convenient for farmers having small holdings. To make the process of lignin degradation economically viable, inoculation with lignocellulolytic microorganisms may prove beneficial. Since no single organism produces all the enzymes necessary for

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bioconversion of lignocellulose to optimum level, there is need to use a consortium of lignocellulolytic microorganism which can act synergistically for rapid bioconversion of agricultural residues without any chemical pretreatment. Mesophilic microbiota initiate decomposition by degrading readily available substrates present in composting mixture, but as the temperature during composting rises to 60–65°C, their population declines leading to ultimate effectiveness of the process. The decomposition rate is most rapid during the thermophilic stage, achieved with in first week of composting. Though, there may be some natural thermophilic microorganisms present in the substrate mixture, but they may not be able to execute the desired degradation of substrate due to lack of specific enzymes for cellulose and lignin degradation to optimum level. Therefore, there is need to inoculate thermophilic microorganisms capable of producing cellulolytic and lignolytic enzymes to degrade the crop waste like paddy straw. As fungi are saprophytic in nature and can easily grow on various crop residues (Ragunathan and Swami 2003), an idea of isolating thermophilic fungi having lignocellulolytic properties was subjected to exploration. This paper describes the isolation and selection of thermophilic fungi on the basis of hydrolytic enzymes activities, development of consortium of selected strains of fungi and testing of its efficacy to compost paddy straw as a primary substrate.

## Material and methods

### Substrates for isolation of fungal strains

Soil, wheat straw compost and farm yard manure were collected from Indian Agricultural Research Institute (IARI) Farm, experimental compost pits in the Division of Microbiology and Division of Agronomy, IARI, New Delhi respectively and used as supplement to isolate thermophilic fungi. Paddy straw was enriched with each of these supplements separately, moistened with Reese minimal medium (Reese and Mandel 1963) and incubated for 15 days at 50°C. Ten thermophilic fungal cultures were isolated from enriched materials, by dilution plating technique using Reese minimal medium with 1% swollen cellulose as the sole carbon source.

### Screening of fungal strains

The fungal cultures were screened qualitatively for the production of cellulase, xylanase, laccase and lignin peroxidase at 50°C. The cellulase activity was tested by zone of clearing on Reese minimal medium with acid swollen cellulose as the sole carbon source (Rautela and Cowling 1966), while the presence of xylanase was tested by the method of Kluepfel (1988) using xylan as the carbon source. Laccase production was detected by modification of the method of Hankins and Anagnostakis (1975). Peroxidase production was confirmed by following the procedure of Egger (1986).

The quantitative estimation of enzymes produced by fungal isolates involved the release of reducing sugars from filter paper (FPase), carboxy methyl cellulose (CMCase), cellobiose (cellobiase) and xylan (xylanase). The cultures were grown in Reese minimal broth supplemented with 1% cellulose, xylan and paddy straw separately as the sole carbon source. Sterilized Reese minimal broth was inoculated with 6 mm disc cut from a 7 day old fungal culture of each strain separately and incubated at 50°C. After 15 days of incubation, the broth was filtered through Whatman 44. The filtrate was stored at 4°C and assayed for FPase, CMCase, cellobiase (Ghose 1987), and xylanase (Bailey et al. 1992).

### Selection of fungi for consortium

Out of 10 thermophilic fungal isolates, the three most efficient cultures (Th<sub>4</sub>, Th<sub>5</sub>, and Th<sub>10</sub>) were used for solid state fermentation (SSF) of paddy straw under laboratory conditions. In 500 ml Schott Duran bottles, 10 g shredded paddy (2.5 cm pieces) and 30 ml of Reese minimal broth was taken, mixed thoroughly and sterilized at 1.1 kg cm<sup>2</sup> pressure for 15 min. Each of the three fungal cultures was grown separately in Petri dishes for 15 days at 50°C on Potato Dextrose Agar (PDA). A 6 mm disc of each culture was punched out and transferred aseptically to each bottle as per the schedule given in Table 2. All the bottles along with control were incubated at 50°C for 15 days. The degraded product was analyzed for dry matter loss, organic matter, organic carbon and total nitrogen by following the standard methods (Jackson 1973). Humus was estimated by the method of Kononova (1966).

### Compatibility test

All the three selected cultures were tested for their compatibility with each other. They were co-inoculated on PDA plate and incubated at 50°C for 1 week. None of the culture inhibited the growth of other culture, but grew well.

### Inoculum preparation

Inoculum of the selected fungi was raised on boiled sorghum seeds mixed with 2% CaSO<sub>4</sub> and 4% CaCO<sub>3</sub>. Flasks containing sorghum grains were inoculated with 6 mm disc of each culture, grown on PDA, and incubated at 50°C. After 2 weeks, all the three cultures were mixed in equal proportion and used as inoculum for compost preparation.

### Preparation of substrate mixture for compost production

Paddy straw and soybean trash, the primary substrates used for composting were collected from IARI farms and chopped to a size of 10–15 cm. The initial wide C:N ratio of paddy straw (88:1) was narrowed down to about 60:1 by adding 0.45% urea in one set of the treatment and by adding soybean trash in the ratio of 1:1 to the other set of treatment. About 20 kg of each substrate mixture was filled in the plastic containers having lids and inoculated with fungal consortium @ 1% (w/w) as per treatment schedule (Table 4). The inoculum was mixed thoroughly. The substrate was allowed to decompose for 3 months during summer season under natural conditions. The contents in each container were turned upside down at monthly intervals and the moisture was maintained at 60% (w/v). The composted sample were drawn at maturity and divided into two parts. One part was dried in oven at 60°C and ground to pass through 2 mm sieve. The powdered samples were analyzed for organic matter, organic carbon and total nitrogen by following the standard method (Jackson 1973). Crude protein was calculated by multiplying the nitrogen percent with 6.25. Available phosphorus was estimated by the method of Olsen et al. (1954). Total humus content (humic acid, fulvic acid and humin) in matured compost was estimated by the method of Kononova (1966) using 0.1 M sodium pyrophosphate

as an extractant followed by dialysis of filtrate under running water for 24 h.

The fungal biomass was estimated by the method of Aidoo et al. (1981). 100 mg oven dried sample was mixed with 5 ml of 2 N HCl in a screw capped tube and incubated in boiling water bath for 2 h. The contents were filtered through Whatman 42 filter paper. 1 ml filtrate was mixed with 1 ml sodium nitrite (5% w/v) and 1 ml potassium hydrogen sulphate (5%). The tubes were incubated at room temperature for 15 min. To this 1 ml of ammonium sulphamate (12.5% w/v) was added. After shaking for 5 min 1.0 ml 3-methyl-2-benzothiazole hydrazone hydrochloride (MBTH 0.5% w/w) was added and the tubes were kept in boiling water bath for 5 min. After cooling, 1.0 ml ferric chloride (0.83% w/v) was added. The contents after thorough mixing were left for 30 min for color development and OD was measured at 650 nm against reagent blank.

Another set of samples were air dried and ground to pass through 2 mm sieve. The levels of different enzymatic activities viz dehydrogenase, alkaline phosphatase, cellulase, cellobiase and xylanase were assayed in matured compost samples following the standard procedures.

### Enzyme assay

#### *Estimation of dehydrogenase*

Dried and sieved compost sample (1 g) was treated with 3% triphenyl tetrazolium chloride (TTC) in distilled water for 24 h at 28°C in darkness. The triphenylformazone (TPF) formed was extracted with 20 ml methanol by shaking vigorously for 1 min and filtered through Whatman 42 filter paper. TPF was measured spectrophotometrically at 485 nm following the method of Casida et al. (1964).

#### *Alkaline phosphatase*

About 4 ml of modified universal buffer (pH 11.0) and 1 ml *p*-nitrophenyl phosphate disodium (0.025 M) was added to 1 g compost sample and incubated at 37°C for 60 min. It was followed by the addition of 1 ml of 0.5 M CaCl<sub>2</sub> and 4 ml of 0.5 M NaOH. Mixture was centrifuged at 4000g for 5 min. *p*-nitrophenol was determined spectrophotometrically

at 400 nm and phosphatase activity expressed as  $\mu\text{moles}$  of PNP released  $\text{g}^{-1} \text{h}^{-1}$  with reference to standard curve of *p*-nitrophenol (Tabatabai and Bremner 1969).

Estimation of cellulase, cellobiase, xylanase, phenol and soluble protein

Enzyme was extracted from compost with citrate buffer of pH 7.0. A known sample (0.5 ml) of filtrate was incubated with respective substrates (filter paper strips/cellobiose/xylan) and volume was made up to 1 ml with 0.05 M citrate buffer of pH 4.8. All the tubes were incubated at 50°C for 1 h for cellulase and 30 min for CMCase cellobiase and xylanase. Reducing sugars liberated by action of enzyme was estimated by adding 3 ml dinitrosalicylic acid and keeping in boiling water bath for 16 min (Wood and Bhat 1988; Bailey et al. 1992). Enzyme concentration was represented as international unit (IU  $\text{g}^{-1}$ ). 1 IU is defined as  $\mu\text{mol}$  of product produced  $\text{ml}^{-1} \text{min}^{-1}$  for filtrate. Cellulose, hemicellulose and lignin content in the primary substrates were estimated by the method of Goering and Van Soest (1970). Phenol and soluble protein were estimated in buffer extracts of compost samples by the method Bray and Thorpe (1954) and Lowry et al. (1951) respectively.

Statistical analysis

Through out the study, three replications of each treatment were kept. The data were subjected to

analysis of variance (ANOVA) using least significance difference test and comparing the difference between the specific treatments. Test of significance was determined at  $P < 0.05$  (Panse and Sukhatme 1978).

## Results and discussion

Qualitative screening of the fungal cultures revealed that all the 10 strains expressed cellulase, cellobiase, CMCase, xylanase, and peroxidase. However, laccase was expressed by only one strain Th<sub>4</sub>.

The quantitative estimation of enzyme production in Reese's minimal medium with paddy straw (1% w/v) as the sole carbon source showed significant variation in the abilities of the strains (Table 1). Maximum activity of FPase (11.43 IU  $\text{ml}^{-1}$ ) by Th<sub>10</sub>, cellobiase (134.21 IU  $\text{ml}^{-1}$ ) by Th<sub>4</sub>, CMCase (30.36 IU  $\text{ml}^{-1}$ ), and xylanase (236.43 IU  $\text{ml}^{-1}$ ) by Th<sub>5</sub> were recorded. The most promising cultures were identified as *Humicola* sp.Th<sub>10</sub>, *Aspergillus nidulans* Th<sub>4</sub>, and *Scytalidium thermophilum* Th<sub>5</sub> at Indian Type Culture Collection Centre, Division of Mycology and Plant Pathology, IARI, New Delhi.

Preliminary analysis of samples taken after 15 days of paddy straw decomposition by solid state fermentation under laboratory conditions revealed that maximum loss of dry matter, least C:N ratio and maximum humus was achieved when inoculation was made with mixed cultures of fungi (Table 2). Therefore, the consortium with all the three fungal cultures was used for composting of paddy straw.

The physico-chemical analysis of different substrates used in the study is given in Table 3. There

**Table 1** Production of extracellular enzymes (IU/ml) by the fungal cultures using rice straw as the sole carbon source at 50°C in 15 days

Isolate no.	Source of isolation	FPase	Cmcase	Cellobiase	Xylanase
Th <sub>1</sub>	FYM	3.70	1.83	14.89	104.34
Th <sub>2</sub>	FYM	4.46	12.32	13.24	104.78
Th <sub>3</sub>	FYM	5.86	2.82	55.46	114.10
Th <sub>4</sub>	FYM	9.27	14.95	134.21	150.16
Th <sub>5</sub>	Soil	7.49	30.36	69.17	236.43
Th <sub>6</sub>	Soil	4.51	6.98	15.12	108.29
Th <sub>7</sub>	Soil	6.73	8.06	23.90	138.03
Th <sub>8</sub>	Soil	3.89	7.27	63.15	132.95
Th <sub>9</sub>	WSC	4.95	9.27	45.95	98.12
Th <sub>10</sub>	WSC	11.43	15.38	90.20	182.08
$P < 0.05$	–	1.03	2.23	7.89	0.56

FYM, farm yard manure; WSC, wheat straw compost; FPase-filterpaperase; CMCase, carboxymethyl cellulase

**Table 2** Changes in chemical parameters of paddy straw after decomposition for 15 days at 50°C by single and mixed cultures of selected thermophilic fungi

Inoculation	Dry matter loss (%)	Organic matter (%)	Carbon (%)	Nitrogen (%)	Humus (%)	C/N
No (Control)	16.00	71.03	41.18	0.53	1.0	77.69
<i>Aspergillus nidulans</i> Th <sub>4</sub>	31.37	71.20	41.18	0.62	2.47	66.42
<i>Scytalidium thermophilum</i> Th <sub>5</sub>	26.27	63.07	36.64	0.64	2.75	57.25
<i>Humicola</i> spp. Th <sub>10</sub>	23.30	64.23	37.12	0.59	0.73	62.92
<i>A. nidulans</i> Th <sub>4</sub> + <i>S. thermophilum</i> Th <sub>5</sub>	19.03	76.17	44.20	0.58	1.33	76.20
<i>A. nidulans</i> Th <sub>4</sub> + <i>Humicola</i> spp. Th <sub>10</sub>	22.00	71.33	44.18	0.56	1.62	78.89
<i>S. thermophilum</i> Th <sub>5</sub> + <i>Humicola</i> sp. Th <sub>10</sub>	19.83	73.17	42.34	0.57	1.73	74.28
<i>A. nidulans</i> Th <sub>4</sub> + <i>S. thermophilum</i> Th <sub>5</sub> + <i>Humicola</i> sp. Th <sub>10</sub>	44.73	62.10	35.96	0.60	3.93	56.6
LSD ( $P < 0.05$ )	3.14	0.63	1.47	0.04	0.15	–

**Table 3** Characteristics of substrates used for composting

Substrates	Organic carbon (%)	Total Nitrogen (%)	C:N	Phosphorus (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Paddy straw	36.8	0.42	87.6	0.031	41.3	20.4	12.1
Soybean trash	51.0	1.03	49:1	0.033	41.9	10.3	39.7
Paddy straw + Soybean trash (1:1)	50.0	0.77	64:1	0.033	41.6	15.3	25.2

was not much variation in the cellulose content of paddy straw and soybean trash, but lignin content of latter was almost three times to that of former. Hemicellulose content of paddy straw was almost double to that of soybean. Soybean due to high content of nitrogen had the most desired C:N ratio to initiate the decomposition. Therefore, it was used as an organic source of nitrogen supplement substituting urea in some treatments.

The chemical analysis of mature compost prepared from paddy straw and mixture of paddy straw + Soybean trash (1:1) is given in Table 4. The pH being an important parameter controlling the availability of nutrients like P, Fe, and Zn should get due attention while recommending the compost to be mature and safe for soil application. It was found to be in the near neutral range of 6.3–7.5, the acceptable values for the final product. Fungal inoculation resulted in lowering of pH of all the treatments compared to control, barring soybean amended fungal inoculated treatment that had slightly higher pH (7.5), compared to its uninoculated counterpart. This may be attributed to

greater production of ammonia during the process of decomposition. Electrical conductivity, a measure of dissolved salts ranged between 1.3 mS and 2.4 mS, and was far below the safe limit of 3 mS. The effect of inoculation was well pronounced in reducing the electrical conductivity.

A reduction in total organic carbon (TOC) content was observed in all the composts. This points the mineralization of organic material present in each of the substrates. Both the substrates must be having some easily mineralizable fraction of organic matter. Reduction in total carbon content was 36% in soybean supplemented paddy straw compared to 25% in urea amended and inoculated paddy straw. Though the lignin content of soybean trash was high, but fungal consortium was effective in degradation of organic matter and brought significant reduction in total organic carbon. The N content in most of the treatments improved due to a concentration effect caused by degradation of labile organic carbon compounds which reduced the weight of the composting mass. It is believed that when organic matter

**Table 4** Physicochemical characteristics of paddy straw compost prepared by using consortium of three fungal strains after 90 days

Substrates	Inoculation	pH	EC (dS/m)	OM (%)	TOC (%)	TKN (%)	C:N	Humus (%)	Available P (%)	Crude protein (%)	Fungal biomass*
Paddy straw	Uninoculated	7.0	2.4	50.03	29.0	1.54	18.8:1	2.1	0.030	9.64	3.125
Paddy straw	Inoculated	6.9	2.1	49.90	28.9	1.98	14.6:1	2.3	0.035	12.41	4.850
Paddy straw + 0.45% urea	Inoculated	6.4	1.3	47.50	27.5	2.88	9.5:1	2.9	0.042	18.04	6.512
Paddy straw + Soybean.trash	Uninoculated	6.3	1.7	60.33	35.0	2.81	12.4:1	2.8	0.035	17.59	4.503
Paddy straw + Soybean.trash	Inoculated	7.5	2.1	55.27	32.0	3.12	10.2:1	3.3	0.040	19.48	5.049
LSD ( $P > 0.05$ )		–	–	2.17	1.56	0.03	–	1.2	0.014	0.19	0.131

EC, electrical conductivity; OM, organic matter; TOC, total organic carbon; TKN, total Kjeldahl's nitrogen

\*In terms of mg of N-acetyl glucosamine/100 mg of substrate

**Table 5** Enzymatic activities in compost prepared from paddy straw and soybean trash by thermophilic fungi after 90 days

Substrates	Inoculation	Dehydrogenase ( $\mu\text{g TPF/g/h}$ )	Alkaline phosphatase ( $\mu\text{g PNP/g/h}$ )	Cellulase (IU/g)	Xylanase (IU/g)	Soluble protein ( $\mu\text{g/g}$ )	Phenol ( $\mu\text{g/g}$ )
Paddy straw	Uninoculated	1718.87	520.17	2182.55	94.12	1153.58	1477.02
Paddy straw	Inoculated	1788.79	530.07	2533.05	162.18	1422.12	1364.53
Paddy straw + 0.45% urea	Inoculated	1901.66	528.01	2456.8	124.69	1277.34	1456.36
Paddy straw + Soybean.trash	Uninoculated	1752.17	526.16	2243.42	127.8	1107.12	1549.90
Paddy straw + Soybean.trash	Inoculated	1928.58	521.50	2664.92	70.42	825.4	951.79
LSD ( $P > 0.05$ )		52.50	10.23	64.25	8.65	54.98	73.98

loss is greater than loss of  $\text{NH}_3$ , nitrogen concentration usually increases (Bernal et al. 1998).

The improvement in nitrogen and lowering of carbon percent resulted in lowering of C:N ratio, an important criterion for compost to be fully mature, as well as an adequate predictor of amendments impact on N cycling on its incorporation in soil. Though, most of the tested composts had the C:N < 20, in this study, the lowest C:N (9.54:1) was observed in compost prepared by using paddy straw amended with 0.45% urea and inoculated with fungal consortium. Unamended, but inoculated paddy straw after decomposition had comparatively higher C:N ratio (14.6:1). On the contrary, soybean supplemented paddy straw had C:N 12.4:1. This showed the importance of initial C:N in the substrate mixture used for composting. Low initial C:N ratio caused fast degradation of cellulose and hemicellulose, while high initial C:N resulted in low degradation (20%) of both cellulose and hemicellulose. These results were in agreement with the findings of Eiland et al. (2004). However, the most acceptable C:N ratio of 10.3:1 and highest humus content was achieved when paddy straw was amended with soybean trash. This showed the effectiveness of fungal consortium and supplementation with soybean trash on the degradation of paddy straw. It was also seen that by replacing urea with soybean, most desired C:N was achieved and the compost so obtained was a dark brown colored mass with no odour but with high organic value.

Highest fungal biomass (6.512 mg of *N*-acetyl glucosamine/100 mg substrate) was recorded in urea amended paddy straw followed by soybean amended treatment. High level of *N*-acetyl glucosamine was considered to be due to the higher growth of fungi in easily available nitrogen supplemented treatment (Aidoo et al. 1981).

Inoculation improved the dehydrogenase activity to a considerable extent as compared to control (Table 5). As this activity is linked to microbial respiratory process, the maximum value of dehydrogenase activity in soybean amended treatment (1928.58  $\mu\text{g TPF g}^{-1}\text{h}^{-1}$ ) showed the higher proliferation of both native and inoculated flora. This may be due to high N and P % in this treatment.

Not much variation in values of alkaline phosphatase was observed among all the treatments. An inverse relation in available P content and alkaline phosphatase was observed. This was in agreement

with the findings (Nannipieri 1990; Gaiind and Lata 2004), due to inhibition of phosphatase activity in presence of soluble phosphorous.

Xylanase activity and soluble protein content were maximum in paddy straw inoculated treatment whereas cellulase activity was maximum in soybean supplemented and fungal inoculated paddy straw (2664.92 IU  $\text{g}^{-1}$ ) (Table 5). Expression of high xylanase activity must have resulted in the maximum saccharification of lignocellulosic material in paddy straw (Bernier et al. 1983).

Highest production of phenol (1422.12  $\mu\text{g g}^{-1}$ ) was recorded in unamended, fungal inoculated paddy straw compost. This showed that lignin was degraded into monomeric phenolic compounds due to action of phenol oxidase and lignin peroxidase. However, low values of phenol in soybean amended, fungal inoculated treatment in spite of high content of humus may be attributed to the fact that fungal consortium was highly effective in enzymatic oxidation of the toxic compounds. Inoculation with specific fungi with the capability of degrading phenolic compounds also explains the phenolic reduction.

## Conclusions

The enhancement of nutritive values and reduction in volume of agricultural wastes using thermophilic fungi has also been reported by Abraham et al. (1992). The fungal consortium proved effective in converting high silica paddy straw into valuable product as compost. The supplementation of paddy straw with soybean trash and inoculation with fungal consortium yielded compost that was nutritionally comparable to compost prepared with paddy straw supplemented with urea and fungal inoculation. The compost prepared without the use of urea may be of great use in organic farming as it will be free from chemicals.

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