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Isolation and evaluation of native cellulose degrading microorganisms for efficient bioconversion of weed biomass and rice straw

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Abstract

Cellulose decomposing microorganisms (CDMs) are important for efficient bioconversion of plant biomasses. To this end, we isolated seven fungal isolates (*Aspergillus wentii*, *Fusarium solani*, *Mucor* sp., *Penicillum* sp., *Trichoderma harzaianum*, *Trichoderma* sp.1 and *Trichoderma* sp.2) and three bacterial isolates (bacterial isolate I, II and III) from partially decomposed farm yard manure, rice straw and vermicompost, and evaluated them for decomposition of rice straw (*Oryza sativa*), *Ipomoea carnea* and *Eichhornia crassipes* biomass. CDMs inoculation, in general, reduced the composting period by 14-28 days in rice straw, 14-34 days in *Eichhornia* and 10-28 days in *Ipomoea* biomass over control. Of the 10 CDMs tested, *Mucor* sp. was found to be the most effective as *Mucor*-inoculated biomass required minimum time, *i.e.* 84, 68 and 80 days respectively for composting of rice straw, *Eichhornia* and *Ipomoea* biomass as against 112, 102 and 108 days required under their respective control. CDMs inoculation also narrowed down the C:N ratio of the composts which ranged from 19.1-22.7, 12.9-14.7 and 10.5-13.1 in rice straw, *Eichhornia* and *Ipomoea* biomass respectively as against 24.1, 17.1 and 16.2 in the corresponding control treatments. *Aspergillus wentii*, *Fusarium solani*, *Mucor* sp., and *Penicillum* sp. were found most effective (statistically at par) in reducing C:N ratio and causing maximum loss of carbon and dry matter in composted materials. These benefits of CDMs inoculation were also accompanied by significant increase in NPK contents in the composted materials.

Key words

Biowaste recycling, Cellulolytic microorganisms, Compost quality

Introduction

Crop productivity in northeast India lags far behind the national average mostly due to impaired soil health and very low input of essential plant nutrients (Manoj-Kumar, 2011). Since application of chemical fertilizers is not very popular in the region, farmers depend mostly on the organic sources of plant nutrients. Nonetheless, inadequate and inappropriate use of organic sources often leaves the soil impoverished and the crops are nutrient-starved. Plant biomasses such as crop residues, weed biomass *etc.* are abundantly available in- and around farmland in northeast India (Hazarika *et al.*, 2006). However, such bioresources are hitherto not efficiently exploited for production of quality organic manure and are mostly wasted. Therefore, development of appropriate technology for rapid conversion of such biomasses into quality organic manure (compost) is essential for sustaining soil health and crop productivity in the region (Rajkhowa and Manoj-Kumar, 2013). Composting is a microbiological process, accomplished by cumulative action of bacteria, fungi, actinomycetes and protozoa which are present in the raw materials or introduced in the system by using inocula. One possible way is to improve the traditional method of composting by hastening the process through inoculation of cellulolytic microorganisms. Importance of cellulose degrading microorganisms (CDMs) for hastening the decomposition of plant biomasses is well established (Singh and Sharma, 2002; Abdulla and El-Shatoury, 2007). Inoculation of CDM culture during composting may be particularly useful for hastening the decomposition of cellulose and lignin-rich plant biomasses such as rice straw (*Oryza sativa*). The rate of decomposition primarily depends on the strain of the microorganisms and type of biowaste used. Species richness and their efficiency also depend on prevailing climatic conditions. Since information about the efficient strains of CDMs particularly in northeastern region of India is very limited, the present study was conducted to isolate and evaluate some potential native cellulose-decomposing fungi and bacteria for efficient bioconversion of weed biomass (*Ipomoea carnea* and *Eichhornia crassipes*) and rice straw (*Oryza sativa*), which are abundantly available in northeast India.

Materials and Methods

Selection of plant biomass and isolation of cellulose decomposing microorganisms : The plant biomass *viz.*, rice straw (*Oryza sativa*), *Eichhornia crassipes* and *Ipomoea carnea* were selected based on their availability and abundance in- and around farm land in northeast India. These plant biomasses were collected in sufficient quantity and heaped under the sun for partial wilting. They were chopped manually into 2-4 cm sizes to be used further in the study. Cellulose decomposing fungi and bacteria were isolated from partially decomposed farm yard manure (FYM), rice straw and vermicompost. Two hundred grams of each of the sources were kept in incubator for three days for growth of the microorganisms involved in decomposition process. The substrate-degrading microorganisms were isolated using serial dilution technique. Briefly, 0.1 ml of the solution from each dilution was placed in Petri plate containing Omeliansky medium (Omeliansky, 1902). The Petri plates were kept in incubator and growth of the microorganisms was observed at six-hourly intervals. The isolate that was most abundant and produced visible colony first was used for further examination. The bacterial and fungal isolates were purified by continuous subculture. The purified culture was preserved on agar slant and was identified. The Omeliansky broth was made selective for cellulose degrading bacteria and fungi by adjusting the pH at 7.0 and 4.5, respectively. Seventy-two hours old cultures were used for inoculation of the substrate.

Screening of cellulose decomposing microorganisms : Pot studies were conducted to study the relative efficiency of seven fungal isolates (*Aspergillus wentii*, *Fusarium solani*, *Mucor* sp., *Penicillum* sp., *Trichoderma harzaianum*, *Trichoderma* sp.1 and *Trichoderma* sp.2) and three bacterial isolates (bacterial isolate I, II and III) for composting of rice straw (*Oryza sativa*), *Ipomoea carnea* and *Eichhornia crassipes* biomass. Five hundred grams of each of the chopped biomasses was taken in earthen pots to which broth culture of respective CDMs containing 10^8–10^9 cfu ml^{-1} were added. Each pot received 2 ml of this suspension. At the end of composting period, the end product (*i.e.* compost) was analyzed for carbon (C), nitrogen (N), phosphorus (P) and potassium (K) content. Carbon was determined following the procedure described by Waggoner (1972), and the N, P and K content was estimated using the procedure described by Jackson (1973). The fungal population was estimated by the dilution-plate method (Johnson and Curl, 1972) using a Rose-bengal agar medium and a 10^3-fold dilution in water. The fungal isolates were characterized based on their cultural, morphological and spore characteristics and identified by consulting taxonomic monographs including those of Subramanian (1971) and Domsch *et al.* (2007). The bacterial population was determined using nutrient agar medium and 10^5-fold dilution (Waksman, 1922). Efficiency of CDMs was also evaluated in terms of percentage loss of dry matter and carbon in the end product. The dry matter loss (%) in the composted material was estimated by the following equation:

[(initial dry matter – final dry matter)/ (initial dry matter)] x100.

Carbon loss (%) was calculated by the following formula:

[(initial C content – final C content)/ (initial C content)] x100.

Experimental data were analyzed using standard statistical procedure of Gomez and Gomez, (1984). Comparison of mean values among the different treatments was done by using least significant difference (LSD) test. A 5% probability ($P < 0.05$) was used as the level of significance.

Results and Discussion

Efficiency of seven fungal isolates (*Aspergillus wentii, Fusarium solani*, *Mucor* sp., *Penicillum* sp., *Trichoderma harzaianum*, *Trichoderma* sp.1 and *Trichoderma* sp.2) and three bacterial isolates (bacterial Isolate I, II and III) for bioconversion of different plant biomasses (the biochemical properties of which have been shown in table 1) was evaluated in terms of changes in number of days required for composting, C: N ratio of the

Table 1 : Biochemical properties of plant biomasses used for composting

Particulars	*Ipomoea carnea*	*Eichhornia crassipes*	(*Oryza sativa*) Rice straw
Nitrogen (%)	1.90	1.50	0.65
Phosphorus (%)	0.75	0.72	0.12
Potassium (%)	2.50	2.20	0.96
Carbon (%)	56.40	48.20	40.40
C:N	29.6:1	32.1:1	60.2:1

compost, loss (%) in C and dry matter weight of composted material and nutrient (NPK) content in the compost.

Inoculation of CDMs significantly reduced the time required for composting plant biomasses (Table 2, 3 and 4). The CDMs inoculation, in general, reduced the composting period by 14-28 days in rice straw, 14-34 days in *Eichhornia* biomass and 10 -28 days in *Ipomoea* over control. Of the 10 CDMs tested, *Mucor* sp. was found most effective as *Mucor* inoculation reduced the composting period down to 84, 68 and 80 days for composting of rice straw, *Eichhornia* and *Ipomoea* biomass compared to 112, 102 and 108 days required under their respective control. The efficiency of *Aspergillus wentii*, *Fusarium solani*, *Trichoderma harzianum* and *Trichoderma* sp.1 was at par in decomposing different plant biomasses as they took almost similar time for decomposition. All the bacterial isolates, even though significantly reduced the composting period over the uninoculated control, took relatively more time for composting than the fungal isolates. The utility of CDMs in enhancing decomposition of biowaste has been reported in other studies also (Singh and Sharma, 2002; Abdulla and El-Shatoury, 2007; Mahanta *et al*., 2012) which is justifiably ascribed to their ability to degrade the cellulosic component of the substrate that are otherwise not so easily decomposed in the composting process.

The initial C: N ratio of different plant biomass (as substrate) is shown in Table 1, and the final C: N ratios of the resultant compost in table 2, 3 and 4. The initial C: N ratio was lowest in *Ipomoea* biomass (29.6: 1) and highest in rice straw (60.2: 1). Irrespective of the plant biomasses used, CDMs inoculation narrowed down the C: N ratios of the resulting compost which ranged from 19.1-22.7, 12.9-14.7 and 10.5-13.1 in rice straw, *Eichhornia* and *Ipomoea* compost respectively as against 24.1, 17.1 and 16.2 in the corresponding control treatments. This indicates that the inoculated CDMs could efficiently utilize the plant biomasses, thereby accelerating the process of decomposition bringing down the C: N ratios. Similar results have been reported by other workers also (Singh and Sharma, 2002; Abdulla and El-Shatoury, 2007). Nandi *et al*., (2000) reported faster decomposition of cereal straw and

Table 2 : Efficiency of cellulose decomposing microorganisms (CDM) in composting of rice straw (*Oryza sativa*) and nutrient content in resultant compost

CDM inoculation	Composting period (days)	C:N ratio	Carbon loss (%)	Dry matter loss (%)	Total N (%)	Total P (%)	Total K (%)
Uninoculated	112	24.10	37.4	48.5	1.05	0.80	0.92
Aspergillus wentii	88	19.10	45.7	54.2	1.15	0.90	1.02
Fusarium solani	86	19.10	45.7	54.4	1.16	0.92	1.04
Mucor sp.	84	19.30	45.2	53.8	1.15	0.92	1.04
Penicillium sp.	95	19.80	45.0	52.7	1.12	0.84	1.01
Trichoderma harzianum	95	20.50	44.0	52.6	1.10	0.84	0.98
Trichoderma sp.1	88	21.20	42.8	51.8	1.09	0.82	0.94
Trichoderma sp.2	96	21.50	41.3	51.1	1.10	0.88	0.98
Bacterial Isolate I	97	22.00	42.3	51.4	1.06	0.82	0.99
Bacterial Isolate II	96	22.50	39.8	51.2	1.08	0.90	0.94
Bacterial Isolate III	98	22.70	38.7	50.5	1.09	0.88	0.96
LSD (0.05)	5	1.35	2.35	1.2	0.05	0.06	0.06

Table 3 : Efficiency of cellulose decomposing microorganisms (CDM) in composting of *Eichhornia crassipes* biomass and nutrient content in resultant compost

CDM inoculation	Composting period (days)	C : N ratio	Carbon loss (%)	Dry matter loss (%)	Total N (%)	Total P (%)	Total K (%)
Uninoculated	92	17.10	48.2	49.5	1.38	0.98	1.68
Aspergillus wentii	74	12.90	59.3	56.3	1.52	1.15	1.86
Fusarium solani	70	13.10	59.1	57.1	1.50	1.18	1.88
Mucor sp.	68	13.20	60.3	57.5	1.45	1.22	1.92
Penicillium sp.	74	13.70	58.5	57.1	1.46	1.16	1.84
Trichoderma harzianum	76	14.10	59.3	56.8	1.39	1.08	1.75
Trichoderma sp.1	79	13.90	59.1	56.5	1.42	1.06	1.79
Trichoderma sp.2	82	13.30	58.5	55.9	1.60	1.06	1.74
Bacterial Isolate I	84	14.50	57.9	53.5	1.40	1.04	1.78
Bacterial Isolate II	86	14.70	58.1	52.8	1.38	1.02	1.74
Bacterial Isolate III	88	14.20	57.6	52.4	1.44	1.05	1.78
LSD (0.05)	4.26	1.51	3.3	2.2	0.07	0.08	0.09

Table 4 : Efficiency of cellulose decomposing microorganisms (CDM) in composting of *Ipomoea carnea* biomass and nutrient content in resultant compost

CDM inoculation	Composting period (days)	C : N ratio	Carbon loss (%)	Dry matter loss (%)	Total N (%)	Total P (%)	Total K (%)
Uninoculated	108	16.20	43.8	48.3	1.96	1.02	2.03
Aspergillus wentii	84	11.20	57.9	54.0	2.22	1.32	2.32
Fusarium solani	86	11.90	55.7	53.5	2.25	1.29	2.46
Mucor sp.	80	10.50	58.2	54.8	2.28	1.42	2.55
Penicillium sp.	88	12.00	54.0	53.2	2.16	1.30	2.48
Trichoderma harzianum	93	12.20	53.2	52.8	2.14	1.35	2.24
Trichoderma sp.1	96	12.50	52.1	49.8	2.16	1.26	2.10
Trichoderma sp.2	94	13.10	50.5	48.8	2.13	1.28	2.20
Bacterial Isolate I	98	12.80	50.3	50.0	2.19	1.20	2.15
Bacterial Isolate II	97	12.50	51.9	50.2	2.17	1.24	2.30
Bacterial Isolate III	88	12.10	54.3	51.4	2.14	1.22	2.22
LSD (0.05)	4.3	1.83	2.6	2.9	0.11	0.09	0.09

reduction in C: N ratio following inoculation of straw with CDMs. Fungal isolates such as *Trichorus spiralis*, *Chaetonium* sp., *Paeciliomyces fusisporus*, *Trichoderma viride*, *Aspergillus* and *Penicillium* sp. were used efficiently in hastening the decomposition of cereal crop residue, straw, banana leaves and *Jamun* leaves (Guar, 2001). In the present study, inoculation of *Aspergillus wentii*, *Fusarium solani*, *Mucor* sp., and *Penicillium* sp. recorded statistically similar and significantly lower C: N ratios in the composted material from rice straw, *Eichhornia* and *Ipomoea* biomass.

Inoculation of CDMs resulted in loss of C to the extent of 45.7, 60.3 and 58.2 % in rice straw, *Eichhornia* and *Ipomoea* biomass respectively as against 37.4, 48.2 and 43.8 % under control (Table 2, 3 and 4)**.** Among the CDMs tested, *Aspergillus wentii*, *Fusarium solani*, *Mucor* sp., *Penicillium* sp. and *Trichoderma harzianum* were statistically at par and recorded significantly higher loss of C in rice straw. *Mucor* sp. resulted in maximum loss of C in *Eichhornia* (60.3%) and *Ipomoea* (58.2%) biomass which was statistically at par with the other inocula such as *Aspergillus wentii, Fusarium solani*, *Penicillium* sp., *Trichoderma harzianum*, *Trichoderma* sp.1, *Trichoderma* sp.2 and bacterial isolate II. The dry matter loss in rice straw was maximum (54.4 %) when inoculated with *Fusarium solani* and was at par with *Aspergillus wentii* and *Mucor* sp. followed by *Penicillium* sp. and *Trichoderma harzianum.* The loss was minimum (48.5%) in control (without CDM inoculation). The dry matter loss in *Eichhornia* biomass varied from a maximum of 57.5% in *Mucor*-inoculated treatment, and lowest (49.5%) in control. The dry matter loss in *Ipomoea* followed the similar trend. The observed increase in carbon and dry matter loss in composted material consequent to CDMs inoculation further establishes their role in hastening the decomposition process during composting of plant biomasses (Singh and Sharma, 2002; Mahanta *et al*., 2012).

Irrespective of the strains used, CDMs inoculation increased N content in the compost compared to uninoculated control (Table 2, 3 and 4). The N content in rice straw, *Eichhornia* and *Ipomoea* compost ranged from 1.05-1.16, 1.38-1.56 and 1.96-2.28%, respectively. *Fusarium solani*, *Aspergillus wentii*, *Mucor* sp., *Penicillium* sp., *Trichoderma harzianum*, *Trichoderma* sp.2 were at par and found superior to other strains in this regard. Similarly, CDMs inoculation also increased P and K contents in the compost obtained from different plant biomasses. Irrespective of the substrates used, *Mucor* inoculation yielded highest P and K content in the resultant compost. The increased nutrient content in compost due to CDM inoculation may be attributed to the higher rate of nutrient mineralization (Gaur, 2001; Talukdar, 2008).

To conclude, CDMs inoculation significantly reduced the composting period and improved the quality of compost in terms of reduced C: N ratios and improved NPK contents. Based on their efficiency as reported in our study, these native cellulolytic microorganisms, either alone or in consortia, could be used for efficient bioconversion of plant biomasses (such as weed biomass and rice straw) into quality compost. This will help in meeting the nutritional requirement of crops and improving the status of soil health in an otherwise low-input agriculture of northeast India.

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