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Development and assessment of microbial consortium for composting of organic waste

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Abstract
Fungi, bacteria and actinomycetes play unique and important roles during composting, mixed cultures of microorganisms enhance the rate of lignocellulose degradation due to their synergistic activity through utilization of intermediate degradation products commonly known as microbial consortium. Among bacterial isolates B-8, B-34, B-39 and B-43, among actinomycetes isolates A-1, A-26, A-29 and A-31 were found compatible with each other. From the isolates B-39, B-43, A-1 and A-31 having highest CM Case and endoglucanase activity were used for development of liquid consortium on specifically developed media. Solid consortium of fungi developed on boiled sorghum grains were found to be viable up to 90 days. Liquid consortium of bacteria and actinomycetes were found viable up to 180 days on the basis of cellular colony count. Banana substrate was found to be degraded early in 105 days, followed by sugarcane waste in 128 days, banana + cotton + sugarcane in 144 days, cotton waste in 148 days and banana + cotton + sugarcane waste *i.e.* uninoculated control took maximum 171 days for decomposition. At maturity of compost slight variation was found in all substrates in case of pH, EC, organic matter content, N, P, K, C:N ratio and micronutrients as compared to uninoculated control.

Keywords: compatible, composting, consortium, isolates

Introduction
Composting is not only a waste treatment technique but also a recycling method as the end product can be used in agriculture as fertilizer. The decomposition process is carried out by various microorganisms including bacteria, fungi and actinomycetes. Composting of agricultural residues through the action of lignocellulolytic microorganisms is easier to manage and recycle the lignocellulosic waste with high economic efficiency. The recycled material when applied to soil, improves soil fertility and health. Nutritional balance during composting is mainly defined by the C/N ratio. Microorganisms require an energy source (degradable organic-C) and N for their development and activity. Optimal conditions for rapid, aerobic decomposition also include moisture content between 45% to 60% by weight, available oxygen concentration greater than 5%, feedstock particle size no greater than 1 inch and pH between 5.5 to 8.5. Though fungi, bacteria and actinomycetes play unique and important roles during composting, mixed cultures of microorganisms enhance the rate of lignocellulose degradation due to their synergistic activity through utilization of intermediate degradation products commonly known as microbial consortium. This consortium is applied to organic waste helps in faster decomposition. Use of prepared compost facilitates low input agriculture system in to profitable and sustainable one and inoculation of microbial cultures in to it improves its quality and productivity.

Material and Methods
Consortium preparation
Two compatible isolate of each bacteria, fungi and actinomycetes were selected on the basis of their compatibility for preparation of consortium. Sorghum grains were used as carrier for preparation of solid fungal consortium and liquid consortium of bacteria and actinomycetes were prepared on specifically developed medium.

Shelf life of consortium
The stored sorghum grain based and liquid consortium was further tested for its shelf life by recording cfu count at monthly interval up to seven months by serial dilution technique. The procedure adopted is as given below,

i. One gram or ml sample of consortium was suspended in 9 ml of sterilized water blank.
ii. Serial dilutions were made from 10^{-1} to 10^{-8}. One ml aliquot of dilutions from 10^{-3} to 10^{-8} was transferred to sterilized petriplates separately.

iii. For count of bacteria, fungi and actinomycetes, nutrient agar, Potato dextrose agar and Starch Casein agar medium was used. The sterilized medium before solidification (45 ^{0}C temperature) was poured in each petriplate and mixed the contents in plates by rotating the plates gently.
iv. After solidification, plates were kept inverted at 27±1 ^{0}C in BOD incubator for 4 to 7 days. All the plates were observed for the appearance of bacterial, fungal and actinomycetes colony.

The population of bacteria, fungi and actinomycetes was estimated by counting the number of colonies of the respective organisms per plate by a colony counter and computing the average number of particular groups per gram of carrier by using the following formula.

$$\text{Cfu/g of carrier} = \frac{\text{Average plate count}}{\text{Wt. of sample}} \text{ x dilution factor}$$

Assessment of maturity of compost

Physico-chemical analysis of substrates was carried out by following methods,

i. Ph- Samples were taken in 100 ml beaker and diluted 1:10 (1 part sample in 10 parts of distilled water) and placed on shaker for 1 hr. The samples were centrifuged at 4000 rpm for 30 min. and filtered through Whatman No.1 filter paper. pH of the suspension was measured potentiometrically using a combined glass electrode.
ii. Organic carbon content of substrates was determined by ignition method (Bremner *et al.*1970) [3].
iii. Total nitrogen content of the substrates was determined by modified Kjeldhals method (Piper, 1966) [21].
iv. Total phosphorus and potassium content was estimated by following the procedure given by Jackson (1973) [13].
v. Maturity of compost was recorded on the basis of pre-established maturity and stability parameters of compost (Ranalli *et al.*2001; Goyal *et al.*2005 and Raj and Antil, 2011) [24, 9, 22].

Results and Discussion

Compatibility studies

Among the 15 fungal isolates, F-9, F-31, F-13 and F-12 found compatible within themselves whereas isolate F-7, F-15 and F-2 were found to be non compatible.

Among the bacterial and actinomycetes isolates, B-8, B-34, B-39, B-44, A-1, A-26, A-29 and A-31 found compatible within themselves.

Carrier based solid consortium of efficient cellulolytic fungal isolates was preapred on boiled Jowar grains by using two fungal isolates F-9 and F-31 and liquid consortium was prepared by using bacterial isolates B-39 and B-43 and actinomycetes isolates A-1 and A-31 showing highest carboxymethyl cellulase acticity and endoglucanase activity for efficient decomposition of waste on specifically developed liquid media. The results shown close confirmity with Maiyappan *et al.* (2010) [17] who prepared consortium of effective microorganisms on the basis of *in-vitro* compatibility studies. Nine compatible strains basically identified were grouped under the genus *Bacillus* spp, *Streptomyces* spp, *Azotobacter* spp and *Frauteria* spp. Similar observations have been reported by earlier workers (Anandraj and Leema Rose Delapierre, 2010; Jain *et al.* 2011; Mishra *et al.* 2013 and Gupta *et al.* 2013) [2, 14, 19, 10] with bacteria, fungi and actinomycetes. Dawar and Gaffar (2003) [4] used 8 substrates *viz.*, rice grain, sorghum grain, cotton cake, wheat straw, rice straw, saw dust sugarcane bagasse and wheat bran for mass multiplication of bioagents like *T. harzianum* and *Gliocladium virens*. Good growth of these bioagents was observed on sorghum grain followed other carriers. Similar results have been reported by earlier workers (Zaidi and Singh, 2004; Gaur *et al.* 2005) [31, 7] using different carriers.

Table 1: Compatibility among potential fungal bacterial and actinomycetes isolates isolated from waste samples

Combinations (Fungi)	Compatibility	Combinations (Bacteria and Actenomycetes)	Compatibility
F-9 × F-31	Compatible	A-1×A-31×B-39×B-43	Compatible
F-7 × F-9	Non - Compatible	A-29×A-31×B-39×B-43	Compatible
F-7 × F-31	Non - Compatible	A-29×A-1×B-39×B-43	Compatible
F-15 × F-9	Non - Compatible	A-26×A-31×B-39×B-43	Compatible
F-15 × F-31	Non - Compatible	A-26×A-1×B-39×B-43	Compatible
F-13 × F-9	Compatible	A-1×A-31×B-34×B-43	Compatible
F-13 × F-31	Compatible	A-1×A-31×B-34×B-39	Compatible
F-12 × F-9	Compatible	A-1×A-31×B-8×B-43	Compatible
F-12 × F-31	Compatible	A-1×A-31×B-8×B-39	Compatible
F-2 × F-9	Non - Compatible	A-29×A-26×B-34×B-8	Compatible
F-2 × F-31	Non - Compatible		

Table 3: Development of media for liquid consortium of bacteia and actinomycetes

Ingredients	M1	M2	M3	M4	M5
Peptone	3 g	4 g	5 g	6 g	7 g
Beef extract	0.5 g	1 g	1.5 g	2 g	2.5 g
Yeast extract	0.5 g	1 g	1.5 g	2 g	2.5 g
Starch	5 g	7.5 g	10 g	12.5 g	15 g
Casein	0.1 g	0.2 g	0.3 g	0.4 g	0.5 g
NaCl	2.5 g	5 g	7.5 g	10 g	12.5 g
KNO3	1 g	1.5 g	2 g	2.5 g	3 g
K2HPO4	1 g	1.5 g	2 g	2.5 g	3 g
MgSO4 7H2O	0.03 g	0.04 g	0.05 g	0.06 g	0.07 g
CaCO3	0.01 g	0.01 g	0.02 g	0.02 g	0.03 g
FeSO4 7H2O	0.01 g	0.01 g	0.02 g	0.02 g	0.03 g
Glycerol	5 ml	7.5 ml	10 ml	12.5 ml	15 ml

Trehalose	0.25 g	0.50 g	0.75 g	1 g	1.25 g
Mineral Oil	10 ml	10 ml	10ml	10 ml	10ml
Distilled Water	1000ml	1000ml	1000ml	1000ml	1000ml

Shelf life study / longevity of consortium

The shelf life of carrier based consortium of fungi on Jowar grains was found upto 90 days (11.3 x 10^8 cfu/g) and later on the fungal population found to be decreased due to lack of food material in substrate. While in case of liquid medium viable cell count of bacteria was found up to 210 days (8.3 x 10^8 cfu/ml) and for actinomycetes it was up to 180 (7.3 x 10^8 cfu/ml) days respectively.

It is revealed from the results that the solid consortium for fungi can be stored at ambient conditions safely upto minimum 90 days and liquid consortium upto minimum 180 days. Jeyrajan *et al.* (1993) [15] assessed population of *Trichoderma* in four carriers amongst which talc recorded maximum population after 120 days of storage (80x10^6 cfu/g) followed by lignite (70x10^6 cfu/g). While in case of *Bacillus subtilis* peat soil was found to be the best. Similar results have been recorded by Ramakrishnan *et al.* (1994) [23] and are in confirmity with the results obtained.

Table 4: Shelf life of solid and liquid consortium

Microorganism	Carrier / Medium	Microbial Count (cfu/g/ml) / Days interval						
		30	60	90	120	150	180	210
Fungi	Sorghum grains	41.3	27.1	11.3	-	-	-	-
Bacteria	Liquid medium	55.9	44.7	37.5	24.1	17.7	11.3	8.3
Actinomycetes	Liquid medium	47.7	39.3	29.1	17.6	11.6	7.3	-

Assessment of maturity of compost

Days required for compost maturity

The average number of days required for compost maturity were varied from 105 to 171 days for all substrates. It was found that banana substrate decomposed after 105 days followed by sugarcane waste 128 days, cotton waste 148 days, banana + cotton + sugarcane compost within 144 days and the banana + cotton + sugarcane compost without microbial consortium took maximum 171 days for decomposition.

Table 5: Average days required for composting of substrates by using solid and liquid consortium

Substrate	Days required for maturity with consortium	Days required for maturity without consortium
Banana	106	121
Cotton	148	177
Sugarcane	128	150
B + C + S	144	171

Gaur (1982) [6] investigated the effect of four mesophilic fungi, *Aspergillus niger*, *Aspergillus* spp., *Trichoderma viride* and *Penicillium* spp. on composting of jowar stalk, wheat straw and jamun leaves. Due to inoculation, the period of composting was reduced by one month. Sarkar *et al.* (2011) [27] prepared eleven different consortia of the bacterial strains for degradation of kitchen waste. The maximum reduction in composting time observed was 65% in consortia no. 12 and 55% in consortia no. 7. Reduction in composting period due to inoculation of cellulolytic microorganisms have also been reported by Shinde and Rote (1983), Gawade (2001), Raut *et al.* (2008) and Iqbal *et al.* (2010) [29, 8, 25, 12].

pH

The pH of banana+cotton+sugarcane compost was found 7.63, followed by 7.78 in cotton waste, 7.86 in banana waste, 7.98 in sugarcane and it was found 8.45 in banana + cotton + sugarcane compost without microbial consortium.

The present results are in conformity with the results of research workers who revealed from the studies that the composting material gradually decomposes with time and stabilizes and finally the pH stays between 7 and 8 (Ranalli *et al.* 2001; Adebayo *et al.* 2011; Himanen and Hanninen, 2011 and Sarker *et al.* 2013) [24, 1, 11, 28].

C:N ratio

In all the substrates, decrease in C:N ratio over control was recorded. The C:N ratio was found minimum in banana compost followed by sugarcane compost, banana+cotton+sugarcane compost, cotton compost and it was maximum in uninoculated control.

Reduction in C:N ratio over initial on decomposition was recorded by several research workers. Goyal *et al.* (2005) [9] observed that the initial C:N ratio of wastes used for composting ranged from 13.9 to 51.1. As the decomposition progressed, there was decrease in C:N ratio. After 90 days of composting the C:N ratio of end product varied from 11.7 to 28.3. Similar results have also been reported by Limtong *et.al.* (1990), Ravankar *et al.* (2000), Mishra *et al.* (2001), Gade *et al.* (2010) and Raj and Antil (2011) [16, 26, 18, 5, 22] who reported that there was a decrease in C :N ratio as the decomposition progressed.

Organic matter

Organic matter content of all type of compost varied from 15.09 per cent to 25.58 per cent. It was found minimum in banana+cotton+sugarcane compost, followed by banana compost, sugarcane compost, cotton compost and it was maximum in uninoculated control.

The results obtained were similar to Patil (1994) [20] who prepared compost from wheat straw and found that organic carbon per cent was 17 and total N,P,K were in the tune of 0.54, 0.12 and 1.45 per cent respectively.

Mineral components of compost at maturity

Differences in nutrient status were recorded at maturity within different types of compost. Total nitrogen content ranged from 0.53 to 0.88 per cent. It was found maximum in cotton

compost followed by sugarcane compost, banana compost, banana+sugarcane+cotton compost and it was found least in uninoculated control. Phosphorus content ranged from 0.35 to 0.67 per cent. It was found maximum in cotton compost followed by uninoculated control, banana compost, sugarcane compost it was found least in banana+sugarcane+cotton compost. Potash content ranged from 0.33 to 0.74 per cent. It was found maximum in sugarcane compost followed by cotton compost, banana compost, banana+sugarcane+cotton compost and it was found least in uninoculated control.

Calcium content ranged from 1.03 to 1.92 per cent. It was found maximum in sugarcane compost followed by cotton compost, banana compost, banana+sugarcane+cotton compost and it was found least in uninoculated control.

Magnesium content ranged from 498 to 530 ppm. It was found maximum in sugarcane compost followed by banana compost, banana+sugarcane+cotton compost, cotton compost and it was found least in uninoculated control.

Sulphur content ranged from 0.09 to 0.19 per cent. It was found maximum in cotton compost followed by banana+sugarcane+cotton compost, sugarcane compost, banana compost and it was found least in uninoculated control.

Iron content ranged from 305 to 335 ppm. It was found maximum in sugarcane compost followed by banana compost, cotton compost, banana+sugarcane+cotton compost and it was found least in uninoculated control.

Manganese content ranged from 83 to 179 ppm. It was found maximum in sugarcane compost followed by cotton copost, banana+sugarcane+cotton compost, banana compost and it was found least in uninoculated control.

Zinc content ranged from 19 to 39 ppm. It was found maximum in sugarcane compost and cotton compost followed by banana+sugarcane+cotton compost, banana compost and it was found least in uninoculated control.

The present results are thus in conformity with the work done by earlier research workers. Patil (1994) [20] prepared compost from wheat straw and found that total N, P, K were in the tune of 0.54, 0.12 and 1.45 per cent, respectively. Verma *et al.* (1999) [30] prepared compost from different organic material like soybean trash and paddy straw and observed that compost had 1.68 per cent of N and 0.43 per cent. Sarker *et al.* (2013) [28] estimated the nutrient status of compost prepared from sugarcane press mud by microbial consortium. At the end of composting period, the N was found to be 2.34% in press mud compost while phosphorous and potassium content was 1.15% and 1.37 and slight variation was also recorded in micronutrient content. The nutrient content of compost showed the better nutrient levels of concentration compared to control. This is probably because of quick microbial activity leading to decrease in volume of the material.

Table 5: Assessment of maturity of compost prepared by using solid and liquid consortium

Parameters	Units	Substrates				
		Banana	Cotton	Sugarcane	B + C + S	B + C + S Control
pH	(1:10)	7.86	7.78	7.98	7.63	8.45
EC	dSm-1	0.519	0.572	0.459	0.772	0.956
Organic carbon	%	17.83	25.22	18.36	15.09	25.58
Total Nitrogen	%	0.82	0.88	0.83	0.66	0.53
Total Phosphorus	%	0.39	0.67	0.36	0.35	0.42
Total Potassium	%	0.44	0.58	0.74	0.41	0.33
Calcium	%	1.18	1.57	1.92	1.07	1.03
Magnesium	ppm	517	512	530	519	498
Sulphur	%	0.10	0.19	0.11	0.12	0.09
Iron	ppm	332	328	335	327	305
Manganese	ppm	161	175	179	163	83
Zinc	ppm	32	39	39	33	19
C:N ratio	-	12.61:1	16.62:1	12.83:1	14.58:1	27.99:1

Summary and Conclusions

Among the fungal isolates F-9, F-12, F-13 and F-31 found compatible and F-9 and F-31 having highest CMCase and endoglucanase activity were used for development of fungal consortium on boiled sorghum grains. Among bacterial isolates B-8, B-34, B-39 and B-43, among actinomycetes isolates A-1, A-26, A-29 and A-31 were found compatible with each other. From the isolates B-39, B-43, A-1 and A-31 having highest CMCase and endoglucanase activity were used for development of liquid consortium on specifically developed media comprising of ingredients required for growth and development of bacteria and actinomycetes. Carrier based consortium of fungi developed on boiled sorghum grains were found to be viable up to 90 days. Liquid consortium of bacteria and actinomycetes were found viable up to 180 days on the basis of cellular colony count.

Different crop substrates such as banana, cotton and sugarcane were used for decomposition with microbial consortia. Banana substrate was found to be degraded early in 106 days, followed by sugarcane waste in 128 days, banana + cotton + sugarcane in 144 days, cotton waste in 148 days and banana + cotton + sugarcane waste *i.e.* uninoculated control took maximum 171 days for decomposition. At maturity of slight variation was found in all substrate compost in case of pH, EC, organic matter content, N, P, K, C:N ratio and micronutrients as compared to uninoculated control. Differences in nutrient status were recorded at maturity within different types of compost. Total nitrogen (0.53 to 0.88 per cent), phosphorus (0.35 to 0.67 per cent) and Sulphur (0.09 to 0.19 per cent) content was found maximum in cotton compost. Potash (0.33 to 0.74 per cent), Calcium (1.03 to 1.92 per cent), (Magnesium ranged from 498 to 530 ppm) and Iron (305 to 335 ppm) and Manganese (83 to 179 ppm) content was found maximum in sugarcane compost. Whereas Zinc (19 to 39 ppm) content was found maximum in sugarcane compost and cotton compost.

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