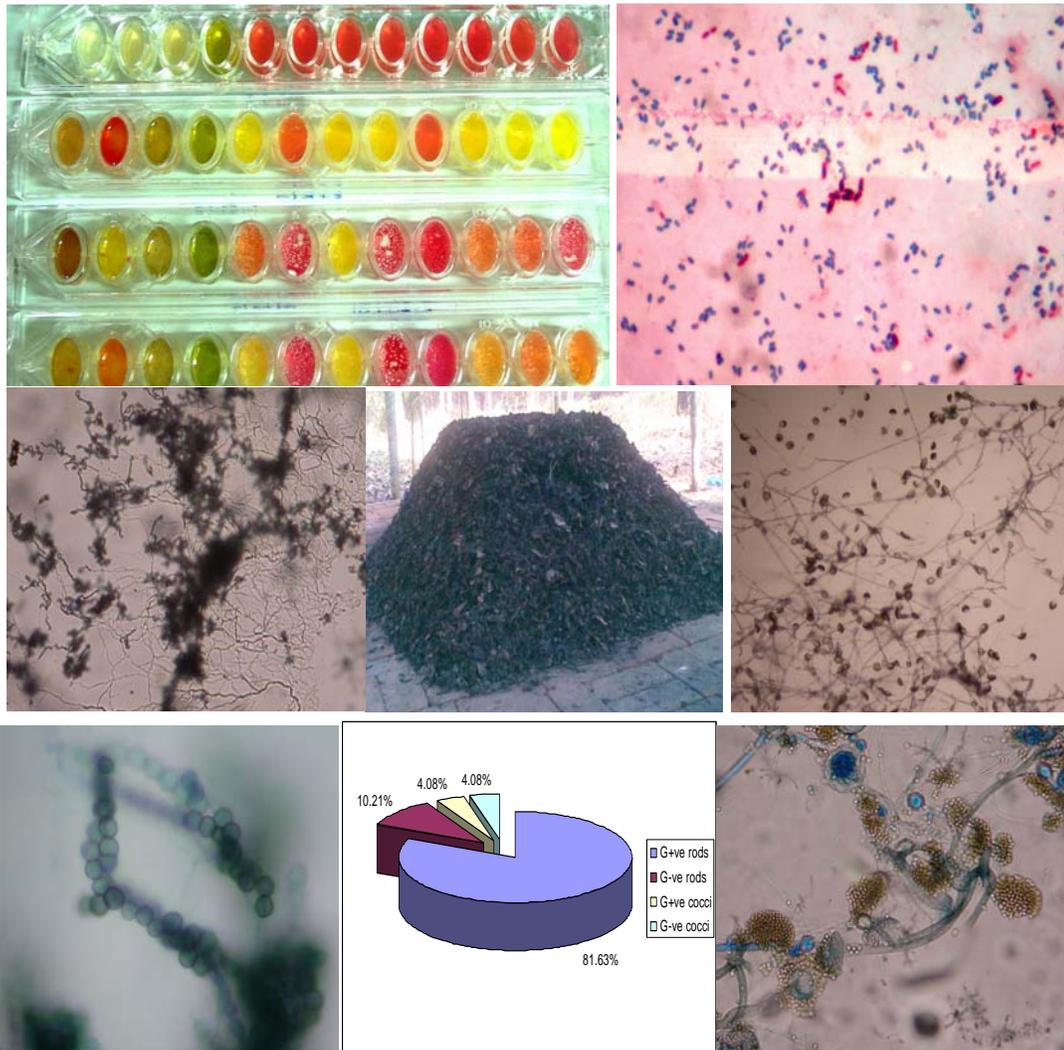


DEVELOPMENT OF MICROBIAL INOCULANTS FOR AEROBIC COMPOSTING



M. Balasundaran



Forest Genetics and Biotechnology Division

Kerala Forest Research Institute

Peechi 680653, Thrissur, Kerala

May 2009

KFRI Research Report No. 324

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**Biotechnology Discipline
Division of Sustainable Natural and Plantation Forest Management
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KFRI Research Report No.
(Final Report of the Project KFRI 390/03)

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Abstract of the Project Proposal

1. Project No. : KFRI 390/03
2. Title : Development of microbial inoculants for aerobic composting
3. Principal investigator : Dr. M. Balasundaran
4. Research Fellow : Haseena, A.
5. Date of commencement : April 2003
6. Duration : 3 Years
7. Funding Agency : KFRI Plan Fund
8. Objectives : To develop suitable inoculum as consortium of microorganisms for composting different organic waste materials
9. Practical utility : The consortium of microorganisms will serve as a ready made inoculum for composting industry

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ABSTRACT

Rapid decomposition of solid organic waste materials into compost can be achieved by adding inoculum of decomposer microorganisms. Thermophilic microorganisms are responsible for quick biodegradation process. The present study was carried out to characterize thermophilic microorganisms present in five different organic waste materials and to identify a consortium of microorganisms suitable for degrading different types of waste materials.

The five organic materials selected were forest weed, ayurvedic factory herbal waste, tea factory waste, coir pith and sawdust. Statistically significant differences were observed between the bacterial, actinomycete and fungal populations of the compost samples. The density of each of the microbial population depended upon the nature and chemical characteristics of the raw material used. One hundred and twenty eight thermophilic microorganisms comprising 49 bacteria, 62 actinomycetes and 17 fungi were selected for detailed study. Taxonomic identification of the microorganisms was performed through morphological, biochemical and molecular characterization (G+C content, and 16S rDNA sequencing and sequence analysis). The biochemical characterization of the bacterial and actinomycete isolates included Indole test, Methyl Red test, Voges Proskeur test, Citrate utilization and the utilization of the various carbohydrates like glucose, adonitol, arabinose, lactose, sorbitol, mannitol, rhamnose and sucrose. The bacterial isolates were analyzed for the motility pattern using hanging drop method. Only 4.1 per cent of the isolates were non motile. Majority of the bacterial isolates (61.2%) and actinomycete isolates (59.7%) had an optimum temperature for growth at 50-55°C; but the fungal isolates had an optimum temperature at 50-60°C.

More than 69 per cent of the bacterial isolates belonged to the genus *Bacillus*. The rest of the isolates belonged to *Thiobacillus* (10.2%), *Azotobacter* (4.1%), *Caryophanon* (8.2%), *Lactobacillus* (2%), *Cellulomonas* (2%) and *Sporosarcinia* (4.1%). Most of the actinomycete isolates were *Streptomyces* sp. (37.1%), *Streptoverticillium* sp. (19.4%), and *Thermomonospora* sp. (19.4%). Six isolates were *Saccharomonospora* sp. (9.7%), two were *Kibdellosporangium* sp. (3.2%) and three were *Kitasatoporia* sp. (4.8%). *Actinomadura* sp., *Rhodococcus* sp., *Faenia* sp. and

Thermoactinomyces sp. were 1.61 per cent each. *Aspergillus* sp. (29.4%). *Humicola* sp. and *Torula* sp. (17.6 % each) were the major fungal species, followed by species belonging to the genera *Penicillium* (5.9%), *Thermomyces* (11.8%), *Chaetomium* (11.8%) and *Mucor* (5.9%). Thirteen different genera and 29 species were identified from weed waste, whereas 11 genera and 17 species were identified from ayurvedic factory herbal waste. Eight genera and 13 species were found in tea waste, 11 genera and 25 species in coir pith and 10 genera and 16 species in sawdust. The total bacterial isolates from the five sources comprised 7 genera and 22 species; actinomycetes, 11 genera and 33 species; and fungi, 7 genera and 16 species.

All the 128 isolates were analyzed for their ability to produce five enzymes, namely, amylase, catalase, cellulase, phenol oxidase and xylanase. Actinomycetes showed maximum enzyme producing ability when compared to that of bacterial and fungal isolates. Fourteen isolates were selected for testing their suitability as microbial consortium for quicker composting, based on their biochemical characteristics and ability to produce enzymes. These included 2 bacteria, 3 fungi and 9 actinomycetes. The isolates did not show mutual antagonism. For testing the consortium, broth culture-raised microbial inoculum was applied to waste materials such as forest weeds, herbal waste, saw dust and coir pith and provided optimum conditions for aerobic composting. The microbial assay during composting process showed that out of the 14 cultures inoculated, populations of 6 cultures were predominant and they were identified as suitable to form the consortium. These isolates were *Streptoverticillium viridoflavum*, *S. reticulum*, *Streptomyces celluloflavus*, *S. albicans*, *Bacillus subtilis* and *Humicola* sp.

Application of the microbial inoculum to forest weeds, herbal waste and coir pith enhanced the speed of composting considerably to 19 days, 22 days and 26 days, respectively. The C/N ratio of the final compost samples showed that compost from forest weed (C/N ratio = 9.8), herbal waste (C/N ratio = 10.3), and coir pith (C/N ratio = 18.6) were of better quality. The sawdust underwent only partial degradation till 50th day and the C/N ratio was reduced up to 275.5 only. It is concluded that the consortium of microorganisms is suitable for quick composting of forest weeds, herbal waste and coir pith. For composting of saw dust, initial application of nitrogen along with isolates having lignin degradation ability may have to be used.

5. CONCLUSIONS

Aerobic thermophilic bacteria, actinomycetes and fungi are responsible for decomposition of lignocellulosic waste materials into compost. Such microorganisms generate heat through exothermic metabolic activity, and rapid growth and multiplication. These microorganisms are able to survive in the compost heap up to 60-70⁰ C. Thermophilic aerobic micro-organisms are physiologically very active. They are capable of producing several thermostable enzymes responsible for decomposition of cellulose and to a great extent lignin into simpler compounds. However, high C/N ratio of some of the waste materials such as saw dust (C/N>400) and coir pith (C/N >100) are the limiting factor for the microbial activity. In the present study, it was possible to decompose coir pith through consortium of microorganisms. But, Saw dust is recalcitrant to a great extent. Decomposition of saw dust can be done through a compatible combination of aerobic microorganisms and wood decaying basidiomycetes. If needed, nitrogen can be added externally to increase the C/N ratio. Further studies have to be made on this topic.

The consortium of thermophilic microorganisms developed through the present study is capable of speeding up aerobic composting method. These organisms can be exploited commercially for converting all types of lignocellulosic materials other than woody waste materials into compost. Some of the actinomycetes and bacteria capable of producing enzymes such as amylase, cellulase, catalase, etc. can be commercially exploited.

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1. INTRODUCTION

In agricultural fields and industrial areas, large amount of hard, decomposable organic materials such as rice straw, saw dust, chipdust, bark, scrap wood, and other plant materials are found discarded. Although, these waste materials are composed of organic components such as cellulose (30-75%), lignin (15-40%) and hemicellulose (7-25%), these can be degraded efficiently and profitably used as organic fertilizers. Composting is a microbial decomposition process in which easily degradable and putrescent organic waste materials are converted into a stable material, compost (Gray *et al.*, 1971).

The process of composting can be either aerobic or anaerobic. In anaerobic composting, organic materials are filled in pits and allowed to remain for several months without allowing fresh air into the organic matter. Anaerobic composting is characterized by the production of foul smelling gases and the process proceeds at a slower rate by the action of anaerobic microorganisms. Aerobic composting proceeds when oxygen is allowed into the composting system and is normally carried out in open air or in windrows. It is a thermogenic solid state fermentation process, carried out through a succession of microbial populations beginning with mesophilic microorganisms. The release of temperature through microbial respiration favors the suppression of mesophilic microorganisms as well as the growth and multiplication of thermophilic microorganisms. The major advantage of aerobic composting over anaerobic composting is that it is fast and the decomposition process is completed within eight to twelve weeks and foul smelling gases are not produced.

The composting process, mediated by microbial activity, is affected by physical and chemical environment inside the compost heap which include temperature, aeration, moisture content, C/N ratio and pH (Boulter *et al.*, 2000). At each thermal stage, specific microorganisms predominate and play a primary role in the reduction and conversion of organic waste in response to temperature. The major active group of microorganisms responsible for aerobic composting is of thermophiles. Aerobic composting will proceed even in the absence of deliberate addition of thermophilic microbial inoculum. This is because native thermophiles occurring on the raw materials will be functioning as the inoculum *in situ*. However, inoculation with more efficient microorganisms may prove beneficial and make the process of biodegradation quick and economically viable. Hence, the main objective of the present project is development of suitable microbial inoculum as consortium of microorganisms for composting different lignocellulosic waste materials.

2. REVIEW OF LITERATURE

Composting is a thermogenic, solid state fermentation process, carried out by a succession of microbial populations beginning with mesophilic bacteria, actinomycetes and fungi followed by thermophiles and ending again with mesophiles (Johri *et al.*, 1999). Composting process creates stable, soil-enriching humus and concentrates the Nitrogen (N), Phosphorous (P), Potassium (K), Calcium (Ca) and Magnesium (Mg) contents (Eneji *et al.*, 2001).

Aerobic composting involves a process of biological decomposition and stabilization of organic substrates under conditions that allow multiplication and activity of thermophilic microorganisms as a result of biologically produced heat, to produce a final product that is stable, free of pathogens, pests and plant seeds, useful in agriculture and forestry as manure (Balasundaran *et al.*, 1999; Saravanan *et al.*, 2003). High temperature within waste heap undergoing composting has been considered as consequence of microbial activity, whereby heat is liberated through respiration of microbes and built up within the pile (Tiquia and Tam, 2000).

Raw materials used for composting

A wide variety of waste materials such as sewage sludge, organic refuse and leaves, industrial wastes resulting from brewing, antibiotic fermentation, herbal medicine industry and food processing, tree barks, agricultural residues, abattoir residues and animal manure can be composted (Johri *et al.*, 1999).

Microorganisms involved

Macdonald *et al.* (1981) noted that the composting process is brought about by several organisms such as bacteria, fungi, actinomycetes and protozoa and may also involve invertebrates such as nematodes, potworms, earthworms, mites and various other organisms. Hudson (1986) described succession in the aerobic process, noting that the composition of active microflora of composting wastes normally shifts from predominant mesophile in the early stages of thermogenesis to thermophiles at the peak of the heating cycle. Several studies have reported the presence of thermophilic bacteria in hot compost (Beffa *et al.*, 1996b).

Thermophilic microorganisms involved in composting

Thermophiles are found in materials or situations that have been naturally or artificially heated such as compost piles or sun heated soils (Tansey and Brock, 1972). The number of mesophilic microorganisms increase in the first few days of composting but decrease drastically when the temperature rises to 50° C-70° C. According to Cooney and Emerson (1964) thermophilic microorganisms require a maximum temperature for growth at or above 50° C and a minimum temperature for growth at or above 20° C. A greater predominance of thermophiles may be found in materials or situations that have been naturally or artificially heated such as compost piles or sun heated soils (Tansey and Brock, 1972). Thermophiles are reported to contain proteins which are thermostable and resist denaturation and proteolysis (Kumar and Nussinov, 2001). Specialized proteins known as chaperones are produced by these organisms, which help, after their denaturation to refold the proteins to their native form and restore their functions (Everly and Alberto, 2000). The cell membrane of thermophiles is made up of saturated fatty acids, which provides a hydrophobic environment for the cell and keeps the cell rigid enough to live at elevated temperatures (Herbert and Sharp, 1992).

Several biochemical tests can be used for the identification of microorganisms involved in composting. Biochemical tests such as catalase production and fermentation of glucose, which are easy to perform, can be used for the preliminary differentiating purposes of microorganisms (Barrow and Feltham, 1993). A number of biochemical tests such as Methyl Red test, Voges Proskauer test, citrate utilization, catalase production, starch hydrolysis, tryptophan hydrolysis etc. has to be performed to characterize microorganisms. Wali *et al.* (1979) reported that there is a higher rate of breakdown of soluble proteins in thermophilic fungi as compared to that of mesophiles. Thermophilic fungi are known to produce thermostable proteases, lipases, amylases, cellulases, xylanases, lactases, trehalases and other extracellular enzymes (Johri *et al.*, 1985; Satyanarayana *et al.*, 1992).

Enzymes production by thermophiles involved in composting

Several thermophiles were observed to grow on starch, cellulose, hemicellulose, lignocellulose, lignin and pectin, but their ability to degrade lignin is doubtful (Johri *et al.*, 1999). The capacity of the microorganisms to assimilate organic matter depends on their ability to produce the enzymes needed for the utilization of the substrate. Extracellular enzymes of thermophiles, particularly cellulases and xylanases are

ABSTRACT

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glycoproteins which are associated with varied amounts of carbohydrate decomposition (Yoshioka *et al.*, 1987). Since no single organism produces all the enzymes necessary for the degradation of all types of organic waste materials, there is a need to use a consortium of microorganisms which can act synergistically for the rapid conversion of organic waste materials. Kumar *et al.* (2007) developed a fungal consortium consisting of *Aspergillus nidulans*, *Scytalidium thermophilus* and *Humicola* species to compost paddy straw and found it effective in converting paddy straw into nutritionally rich compost, thereby leading to economical and environmentally friendly disposal of crop residue.

3. MATERIALS AND METHODS

Collection of samples

Compost samples were collected from five different organic materials which were either undergoing composting or biodegradation. The organic materials were mixed weed, herbal waste from ayurvedic medicine factory and tea leaf waste from tea factory, all in the initial phase of exothermic composting process, and degrading coir pith and saw dust. The temperatures of the compost heap of the first three samples were 55-70⁰C while collecting the samples. These samples were used for the isolation of bacteria, fungi and actinomycetes.

The weed compost comprised of mixed forest weeds undergoing Berkely method of aerobic composting. The major plant species was *Eupatorium odoratum*. The samples containing weed materials undergoing composting were collected from central nursery of Kerala Forest Department located at Chettikulam in Chalakudy Forest Division. Large quantities of compost is produced in central nurseries of the Kerala Forest Department for using it as a major component of potting medium in root trainers for raising seedlings.

Ayurvedic factory waste sample comprised the herbal waste rejected after extracting herbal drug by an Ayurvedic medicine factory (Oushadhi) located in Thrissur. Composting of herbal waste was carried out by supplementing the waste with 4 kg of urea, 3 kg of lime and one basketful of cowdung per tonne of waste.

Tea waste compost originated from tea leaf waste after extraction of instant tea and also from waste tea leaf fragments from tea factory located at Munnar in Idukki District. The tea leaves were subjected to Berkeley method of composting after addition of 4 kg of urea, 3 kg of lime and one basketful (about 5 kg) of cowdung per tonne as applied to herbal medicine waste.

Coir pith sample comprised incompletely decomposed coconut husk, free of fibers. Coir pith, accumulated in coir factory premises was left in open for natural decomposition. Enough moisture was provided regularly in order to encourage decomposition. Microbial inoculum or nitrogen or other supplements were not added.

Sawdust compost comprised partially decomposed sawdust which accumulated in saw mills for several months. The saw dust is formed as waste while sawing hardwood as well as soft wood timber species. The sawdust was exposed to air and

moisture for several months. It decomposed slowly and the colour of the sample turned to grayish black due to decomposing.

Sample collection methodology

Samples from weed compost, herbal waste compost and tea waste compost were collected when the temperature inside the compost heap was above 55⁰ C. Samples were collected from different points inside each heap of the above three sources and from partially decomposed, brownish black coloured coir pith heap and partially decomposed grayish black saw dust pits. The temperature inside coir pith heap and sawdust pit was ambient temperature. The following table (Table 1) provides the details of the locality of sample collection.

Table 1. Details of the locality of the sample (compost) collection

Sample collected	Locality of collection
Partially decomposed weed	Central nursery, Kerala Forest Department Chettikulam, Chalakudy, Kerala.
Partially decomposed Ayurvedic factory (herbal) waste	Oushadhi factory, Pharmaceutical Corporation (IM) Ltd, Kutanelur, Thrissur, Kerala.
Partially decomposed tea waste	Central nursery, Kerala Forest Department, Devikulam, Idukki District, Kerala (supplied by Tata Tea factory for compost preparation).
Partially decomposed coir pith	Cooperative coir factory, Pattikkad, Thrissur, Kerala.
Partially decomposed saw dust	Different saw mills in Paravattani and Ollur, Thrissur, Kerala.

Analysis of the moisture content of samples

The compost samples were analyzed for moisture content before analyzing the microbial community in the samples.

Isolation of microorganisms

Dilution plate technique was adopted for enumeration of the microbial population in the compost. One gram of the sample was added to 99 ml of sterile distilled water in a 250 ml conical flask to get 10⁻² dilution. There were five replicates for each compost

sample. The flasks for each sample were shaken uniformly for 5 min. 10 ml of the 10^{-2} dilution samples were transferred to 90 ml water to get 10^{-3} dilution. The process was repeated to get 10^{-5} dilution for each sample. The flasks were shaken uniformly at each dilution for each sample.

For the isolation of aerobic thermophilic organisms, spread plate technique was used. 0.1 ml of the desired dilution (10^{-4} dilution for fungi and actinomycetes, and 10^{-5} dilution for bacteria) was added to the surface of solid agar medium. The sample dilution placed on the surface of the solid agar medium was then uniformly spread over the agar surface using an L shaped glass rod. The agar plates were then incubated in an inverted position at 50°C in an incubator, wrapping the plates with parafilm to prevent dehydration of the medium. Five replica plates were inoculated for each sub sample of each compost sample.

For the enumeration and isolation of bacteria, nutrient agar was used as the isolation medium and starch casein agar for actinomycetes. For the enumeration and isolation of fungi, Rose Bengal agar was used. The period of incubation varied for different organisms. Two days incubation was given for bacterial isolates, one-week incubation for fungal isolates and ten days incubation for actinomycetes. The number of microbial colonies growing in each plate was enumerated using a colony counter. Each colony was considered as a colony forming unit (cfu). The total cfu was calculated by taking the average count of the organisms from 25 plates and multiplying the number of cfu with the dilution factor used for isolation.

Screening of microorganisms for further studies

The colonies from the plates were purified by subculturing them to new plates containing the respective media. Pure cultures of bacteria, actinomycetes and fungi were transferred to agar slants of nutrient agar, starch casein agar and rose Bengal agar respectively, and properly labeled. Cultures were then used for morphological and physiological characterization. Avoiding duplicate cultures, the microorganisms selected for detailed study included 62 actinomycetes, 49 bacteria and 17 fungal cultures.

Bacteria

The bacterial colonies were scrutinized for form, elevation, colour, margin, pigmentation and colony size and tested for Gram's stain reaction, motility, catalase and amylase production.

Actinomycetes

All the actinomycete cultures were primarily screened for aerial mycelial character, reverse side colour, form, elevation and margin. The spore chain morphology was studied by the cover slip insertion procedure (Balasundaran, 1992) and the aerial and substrate mycelial morphology observed under high power of microscope (Williams *et al.*, 1989). The colonies were further screened for catalase and amylase activity.

Fungi

All the fungal isolates were subjected to preliminary screening for colony morphology such as aerial mycelium, reverse side colour, pigment production and size of the colony. The isolates were stained using Lactophenol cotton blue stain to observe the conidial chain morphology under high power objective.

Colony morphology

The cultures were observed in broth medium for turbidity, flocculancy, pellicle formation and sediment formation.

Motility of the bacteria

A 24-hour broth culture of each bacterial isolate was prepared to detect the motility adopting hanging drop method. Bacteria were differentiated into highly motile, slow moving, vibrating and non-motile, depending up on the speed of motility.

IMViC and carbohydrate utilization

The organisms to be identified were purified and the pure cultures were used for biochemical characterization. The saturated bacterial broth of pure cultures was used for inoculation. For studying the biochemical characteristics, KB001 HiIMViC Biochemical Test Kit (HIMEDIA, Bombay) was used. Each kit contained 12 wells. The first four wells for IMViC (Indole, Methyl red, Voges-Proskauer tests and Citrate utilization tests) and the rest of the wells for carbohydrate utilization tests (glucose, adonitol, arabinose, lactose, sorbitol, mannitol, rhamnose, sucrose). By surface inoculation method, inoculated each well with 50µl of the saturated culture broth. The kit was then closed and incubated at 45° C.

Optimum temperature for growth

For analyzing the optimum temperature for growth of the microorganisms isolated, the pure culture of each of the isolates was inoculated on the media of isolation (nutrient agar for bacteria, starch casein agar for actinomycetes, and potato dextrose agar for fungi). The inoculated Petri plates were then incubated at temperatures 40° C, 45° C, 50° C, 55° C, 60° C, 65° C, 70° C, 75° C and 80° C. The incubation period for bacterial isolates was 24 hours and for the actinomycete and fungal isolates 4 days. After incubation, the diameter of the colony was recorded and the data analyzed to determine the optimum temperature for maximum growth of the isolates.

Screening the isolates for enzyme production

Catalase

The isolates were grown over nutrient agar slants. 5 ml of 3 per cent hydrogen peroxide was poured over the growth on the agar slant. The isolates secreting catalase enzyme produced effervescence and based on the quantum of the bubbles, the efficiency of the cultures were graded as maximum, medium, low and no enzyme production.

Amylase

Czapek-Dox agar medium was used for the detection of starch hydrolysis. The medium was inoculated with the isolates as a spot. The plates were then incubated for 5 days at 50° C and then flooded with Lugol's Iodine. The appearance of clear zone around the microbial growth was taken as the hydrolysis of starch produced by the amylase enzyme.

Cellulase

The isolates were grown in cellulose agar medium and incubated for one week. The plates were then flooded with 0.1 per cent congo red solution for 10 min and then de-stained with 1M NaCl solution for half an hour. The appearance of clear zone around the growth showed the production of cellulase enzyme.

Xylanase

The xylan agar medium was used to grow the isolates. The inoculated plates were incubated for one week. The plates were then flooded with 1 per cent congo red solution for 10 min and then de-stained using 1N NaOH solution. The presence of clear zones around the colonies was considered as xylanase production.

Phenol oxidase

The method of Giltrap (1982) was used to determine the ability to degrade soluble phenolics. A stock solution was prepared by dissolving 0.5g tannic acid in 10 ml of distilled water and adjusted the pH to 4.7 with 1M NaOH. The solution was sterilized by filtration through a 0.2µm Millipore filter, and added to cool autoclave sterilized MMN agar (minus malt extract and containing 5g/L glucose), and mixed thoroughly prior to pouring. Plates were inoculated with the microbial isolates and incubated at 45⁰ C. Fungi capable of degrading tannic acid turned the color of the agar from pinky grey to brown through the 'Bavendamm reaction'. The intensity of the coloration gave an indication of relative degradative ability of the isolate.

Taxonomic identification of the microorganisms

The isolates were identified based on morphological, physiological and molecular characterization. The bacterial and actinomycete isolates which were efficient in production of the enzymes cellulose, amylase, xylanase, catalase, etc. were identified up to species level. For the identification of bacteria and actinomycetes, Bergey's Manual of Determinative Bacteriology 8th Edition (Buchanan and Gibbons, 1975), The Proteobacteria, (Garrity *et al.*, 2004) and Bergey's Manual of Systematic Bacteriology (Williams *et al.*, 1989), and for fungi, Illustrated Genera of Imperfecti Fungi (Barnett and Hunter, 1998) and Thermophilic Fungi (Johri *et al.*, 1999) were used. Wherever needed, the identification of bacteria and actinomycetes was confirmed up to generic level based on the Guanine + Cytosine content of the genome of the organisms adopting the method illustrated by Johnson (1985). The species level identification was confirmed based on PCR amplification and sequencing of the 16S rDNA of the organisms and comparing with the known sequences of identified organism available in the NCBI web site (Rintala *et al.*, 2002; Sachi *et al.*, 2002).

Selection of microorganisms for consortium

From the total of 128 isolates, 14 isolates were selected for testing their suitability for preparing the consortium of microorganisms to be used for enhancing the speed of composting. The selection was based on the ability of the isolate to produce specific enzymes having definite role in biodegradation. The microorganisms showing maximum enzyme activity were selected as the suitable isolates for increasing the degradation rate of substrate.

Testing for antagonism between the selected isolates

In order to avoid antagonistic organisms within the consortium, the isolates were tested for mutual antagonistic properties. All the 14 isolates selected for the consortium were inoculated on Czapek-Dox agar medium by cross streak method (Johnson *et al.*, 1959). After inoculation, the plates were incubated at 50⁰ C for 4 days. The plates were analyzed for zone of inhibition of microbial growth due to the antagonistic activity of the isolates.

Preparation of mass culture of the selected isolates

The isolates which did not show antagonism between them were selected and these microorganisms were mass cultured in suitable culture broth. Nutrient broth was used for the multiplication of bacteria, starch casein broth for the actinomycetes, and potato dextrose broth for fungi. The broth cultures were kept for incubation in an incubation shaker for 5 days at 50⁰ C. The turbid broth cultures were used for analyzing the microbial count.

Testing the isolates for composting waste materials

Four waste raw materials were selected for testing the efficiency of the isolates in enhancing speed and compost quality. These were weed materials, Ayurvedic herbal waste materials, coir pith and sawdust. For preparing weed waste, weeds were collected from KFRI campus, chopped to small size (1-2.5 cm) and inoculated with a mixture of equal quantity (cfu) of the 14 cultures at the time of initial stacking. The herbal waste materials were collected from Ayurvedic (Oushadhi) medicine factory at Kuttanellur, Thrissur. Coir pith was collected from co-operative coir factory, Alpara, Thrissur and sawdust from sawmills at Paravattani, Thrissur.

Three replicates were kept for analyzing the activity of the consortium. The aerobic composting was carried out in 35 l capacity plastic bucket; and 3/4th of the bucket was filled with the waste materials. Water was sprinkled on the waste materials so as to maintain moisture content of 50-55 per cent. The consortium of the 14 cultures which contained a microbial count of 5x10⁴ to 5x10⁵ cells per ml of each culture was added to the waste materials as inoculum. Everyday, the temperature was measured and when the temperature was close to 45⁰ C, the compost was aerated. Maintenance of

moisture content, measurement of temperature and aerating the samples were continued for three to four weeks. The resulting composts were used for further analysis.

Analysis of the compost

Besides estimating the microbial community in the waste materials during the process of composting, finished compost was analyzed for the mineral components, the moisture content. The microbial population and the type of the microorganisms active in each of the compost samples was estimated through dilution plate technique..

4. RESULTS AND DISCUSSION

In this chapter, the results of the study on the microbial source, characterization of the isolated micro-organisms, their taxonomic identification and the strategies adopted for development of a suitable consortium are described.

Moisture content of the raw materials used for composting

The moisture content of the five samples showed varying results. Partially degraded sawmill waste showed maximum moisture content (60%); the weed and Oushadhi factory waste also had high moisture (>50%). Coir pith (46%) and tea waste (42%) had lower moisture content compared to the other three composting materials (Fig.1).

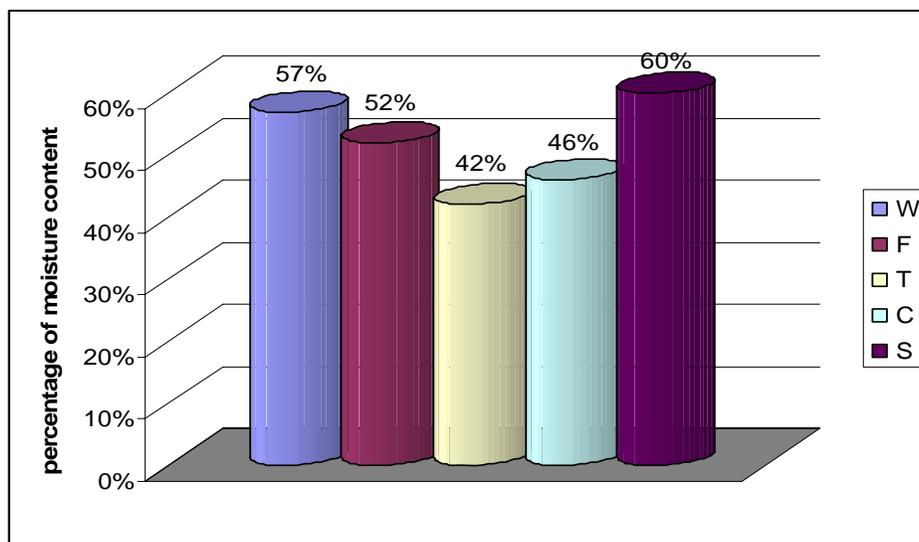


Fig. 1. Moisture content of the five different samples (W: Weed compost, F: Oushadhi factory waste compost, T: Tea waste compost, C: Coirpith, S: Saw dust)

Microbial population

The populations of bacteria, actinomycetes and fungi present in the five sources (samples) are provided in Table 2 and Figs. 2, 3 and 4 as colony forming units (cfu) per gram of sample.

Table 2. Average microbial population (cfu) per one gram dry weight of the samples

S. No.	Samples	Bacteria (cfu/g dry weight)	Actinomycetes (cfu/g dry weight)	Fungi (cfu/g dry weight)	Total (cfu/g dry weight)
1	Weed compost	99.2×10^5	58.6×10^4	65.2×10^3	105.7×10^5
2	Ayurvedic herbal waste compost	20.2×10^6	96.6×10^4	91.7×10^3	212.6×10^5
3	Tea waste compost	34.6×10^4	12.8×10^5	43.6×10^3	16.7×10^5
4	Coir pith	45.6×10^3	12.2×10^3	24.4×10^2	82.2×10^3
5	Saw dust	12.6×10^3	10.6×10^3	48.5×10^2	28×10^3

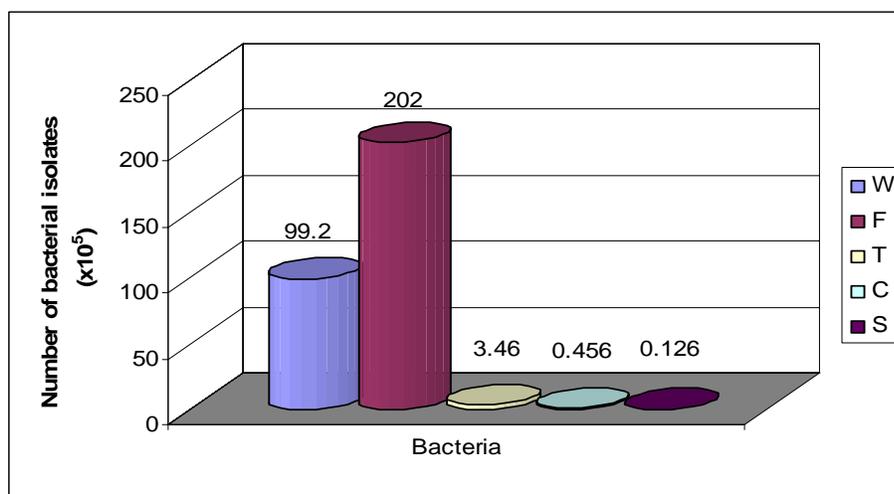


Fig. 2. Bacterial population (cfu/g sample) in the five samples (W: Weed compost, F: Herbal waste compost, T: Tea waste compost, C: Coir pith, S: Sawdust)

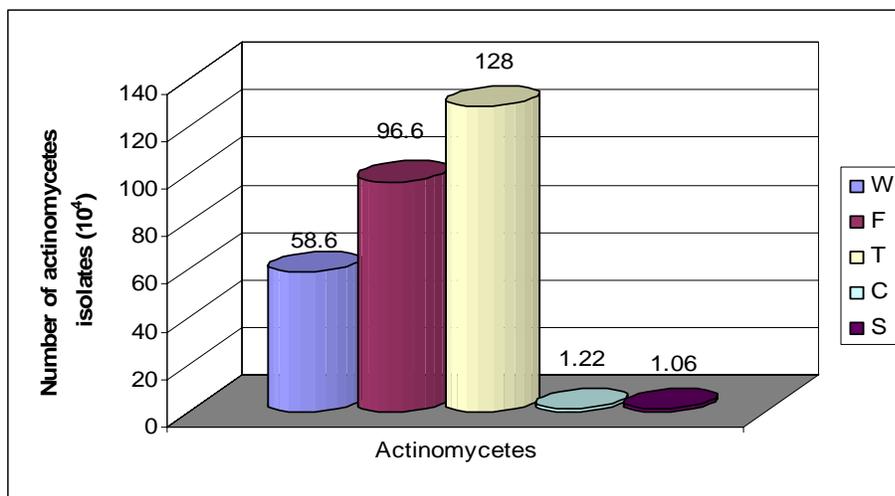


Fig. 3. Actinomycetes population (cfu/g sample) in the five compost samples (W: Weed compost, F: Herbal waste compost, T: Tea waste compost, C: Coir pith, S: Sawdust)

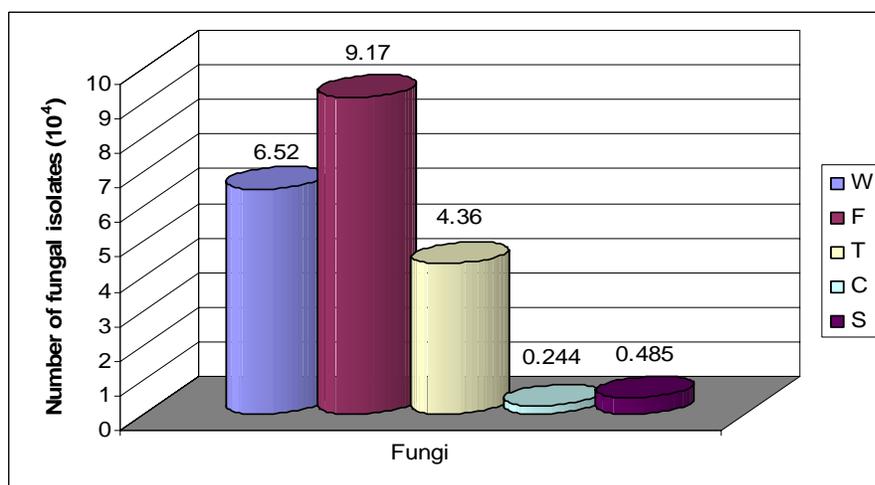


Fig. 4. Fungal population (cfu/g sample) in the five compost samples (W: Weed compost, F: Herbal waste compost, T: Tea waste compost, C: Coir pith, S: Sawdust)

The plate count of the bacterial population was the highest when compared to the fungal and actinomycete population in all except tea waste compost sample. The Ayurvedic (herbal medicine) waste compost showed the highest bacterial population. This may be because of the ingredients such as jaggery, sugar, etc. found in the herbal waste. In tea waste compost, actinomycetes population was the highest followed by fungal and bacterial population. The fungi showed the lowest number of colonies in all the four types of samples.

Bacterial, actinomycete and fungal isolates selected for detailed study

The total numbers of bacterial, actinomycetes and fungal isolates obtained as pure cultures from the five sources are provided in Table 3. Among the 360 pure cultures, 157 were bacteria, 159 actinomycetes and 44 were fungi. The isolates were maintained as pure cultures on agar slants. These included identical cultures from the same source or from different sources. Hence, only one representative isolate from identical colonies, having the same morphological characters and simple biochemical characters such as amylase and catalase production was selected for detailed characterization. Thus, 128 isolates comprising 49 bacterial isolates 62 actinomycetes and 17 fungal isolates were selected for detailed characterization.

Table 3. Number of bacteria, actinomycetes and fungi isolated as pure culture from all the five sources and their percentage (in parenthesis) out of the total number of each group of cultures from all the sources, and the number of isolates selected for detailed study

Source of culture Isolation	Bacteria		Actinomycetes		Fungi		Total Number of isolates selected
	Total isolates	Isolates selected for detailed study	Total isolates	Isolates selected for detailed study	Total isolates	Isolates selected for detailed study	
Weed compost	47 (29.6%)	18 (36.7%)	32 (20.4%)	16 (25.8%)	7 (15.9%)	5 (29.4%)	39 (30.6%)
Oushadhi factory waste compost	24 (15.1%)	13 (26.5%)	23 (14.6%)	6 (9.7%)	9 (20.5%)	3 (17.6%)	22 (17.2%)
Tea waste compost	39 (24.5%)	3 (6.2%)	10 (6.4%)	8 (12.9%)	13 (29.5%)	2 (11.8%)	13 (10.2%)
Coir pith	31 (19.5%)	11 (22.4%)	40 (25.5%)	16 (25.8%)	12 (27.3%)	6 (35.3%)	33 (25.8%)
Saw dust	18 (11.3%)	4 (8.2%)	52 (33.1%)	16 (25.8%)	3 (6.8%)	1 (5.9%)	21 (16.4%)
Total	159 (100%)	49 (100%)	157 (100%)	62 (100%)	44 (100%)	17 (100%)	128 (100%)

Colony morphology of the isolates

All the isolates were observed for the pattern of colony morphology in agar plates and in broth cultures.

Colony morphology of bacteria other than actinomycetes

The colony size of most isolates was found to be medium and in circular form. The margin was entire, but the elevation of the colonies varied. Almost all the isolates appeared in cream to yellow colors. None of the bacteria produced pigment. In broth culture, fine turbidity was observed in all the bacterial isolates and there was no flocculence, pellicle formation or sediment formation.

Colony morphology of actinomycetes

The morphological characters were observed in agar plates for aerial mycelium, reverse color, form, elevation, margin and pigment production. The aerial mycelial morphology of majority of the isolates gave a powdery appearance because of the conidial mass over the surface. Most of the mature isolates were gray or white in color. The reverse color of most of the actinomycetes was cream to yellow, while a few showed orange and brown reverse colors. Most of the actinomycetes colonies were circular in shape. The elevation and margin of the colonies were highly variable. There were 3 pigment producing actinomycetes. All the actinomycetes exhibited flocculancy in broth culture when incubated in shaker.

Colony morphology of fungi

Most of the isolates were fast growing. A majority of them exhibited cottony appearance in agar plates and a few with powdery aerial mycelium due to conidial formation. The aerial mycelial color was white, green or black. Two of the fungal isolates produced soluble pigments. All the fungal isolates also had uniformity in appearance in broth.

Microscopic appearance

Bacteria

Among the bacteria from all the five sources, 81.7 per cent of the isolates were Gram positive rods and 10.21 per cent of the isolates were Gram negative rods. Gram positive and Gram negative cocci were 4.08 per cent each. Majority (71.43%) of the bacterial isolates were spore forming and 28.57 per cent of the bacterial isolates were non spore

formers. About 51 per cent of the bacterial isolates showed high motility, while 8.16 per cent showed slow movement and 36.74 per cent showed vibration. Only 4 per cent of the isolates were non motile.

Actinomycetes

The actinomycetes showed chains of spores on the aerial mycelium which appeared as *Rectiflexibles* (straight to flexous spore chains), *Retinaculiaperti* (looped spore chains) and *Spirales* (spores arranged in spirals) spore chains. Some isolates exhibited clusters of single spores, or single spores arranged separately on aerial hyphae. Single spores on substrate mycelium were also observed.

IMViC and sugar fermentation

IMViC and sugar fermentation by bacteria other than Actinomycetes

Analysis of IMViC and the fermentation of sugar by the bacterial isolates showed that majority of the isolates could utilize glucose (79.59%) and sucrose (71.43%). Only a few organisms were found to utilize sorbitol (12.25%). Indole was utilized by very few of the organisms (2.04%).

IMViC and sugar fermentation by actinomycetes

In the case of actinomycetes, a few isolates (27.42%) were found to utilize indole, and majority of the isolates could utilize glucose and arabinose (75.81%) as their carbon source.

Optimum temperature for growth

Bacteria

About 61 per cent of the bacterial isolates had the optimum temperature of 50-55°C for growth while 16.33 per cent of the isolates had the optimum temperature of 45-50°C. Only 10 per cent of the isolates showed an optimum temperature of 60-65°C.

Actinomycetes

In the case of actinomycetes majority of the isolates (59.68%) had an optimum temperature between 50-55°C. While 33.87 per cent of the isolates showed an optimum temperature between 55-60°C, 6.45 per cent of the total actinomycetes had an optimum between 45-50°C.

Fungi

The fungal isolates had an optimum temperature varying from 35 to 65° C. 23.53 per cent of the isolates had an optimum temperature between 35-45° C and 58.82 per cent of the isolates had the temperature optimum between 50-60° C. 17.65 per cent of the isolates had temperature optimum from 60-65° C.

Taxonomic identification

Bacterial isolates

A tentative identification of the bacterial isolates up to generic level was done based on the colony morphology, staining reaction and the microscopic characteristics exhibited by the microorganisms (Fig. 5). Majority of the isolated bacteria (69.39%) belonged to the genus *Bacillus*. The next major group was *Thiobacillus* which constituted 10.21 per cent of the total isolates. *Caryophanon*, *Cellulomonas*, *Azotobacter*, and *Sporosarcinia* were also identified in small numbers.

Actinomycetes isolates

The actinomycete spore chain morphology and the colony characteristics were used for the identification of the genera. The tentatively identified genera included *Streptomyces*, *Streptoverticillium*, *Thermomonospora*, *Kidbellosporangium*, *Saccharopolyspora*, *Thermoactinomycetes*, *Kitasatosporia*, *Actinomadura*, *Rhodococcus* and *Faenia* (Fig. 6). The *Streptomyces* was the major group as it formed 37.09 per cent of the total isolates. *Streptoverticillium* and *Thermomonospora* also were frequent. 19.36 per cent of the total isolates were identified under each of these genera.

Fungal isolates

In the case of fungal isolates also, both macroscopic and microscopic characters were analyzed for the tentative identification of the genera. *Aspergillus* sp. was the major group found among the fungal isolates (29.42%) (Fig. 7). *Humicola* and *Torula* isolates were 17.65 per cent each while *Thermomyces* and *Chaetomium* constituted 11.77 per cent each. *Penicillium* and *Mucor* were present in 5.87 per cent each only.

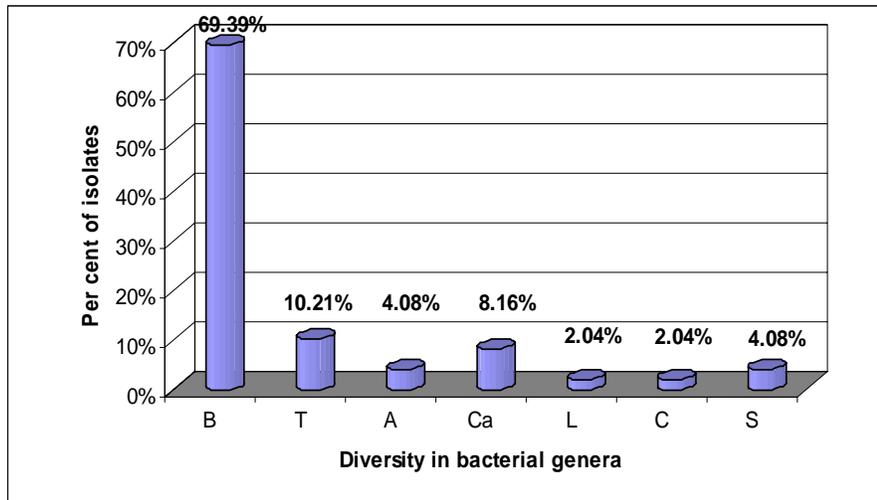


Fig. 5. Per cent of bacterial isolates belonging to different genera from all the sources (B: *Bacillus*, T: *Thiobacillus*, A: *Azotobacter*, Ca: *Caryophanon*, L: *Lactobacillus*, C: *Cellulomonas*, S: *Sporosarcinia*)

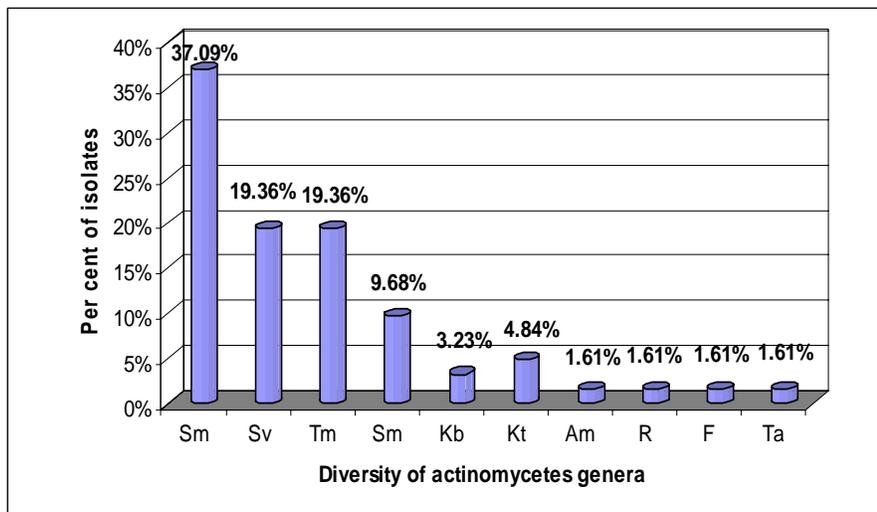


Fig. 6. Per cent of actinomycetes isolates belonging to different genera from all the sources (Sm: *Streptomyces*, Sv: *Streptoverticillium*, Tm: *Thermomonospora*, Kb: *Kibdello sporangium*, Kt: *Kitasatosporia*, Am: *Actinomadura*, R: *Rhodococcus*, F: *Faenia*)

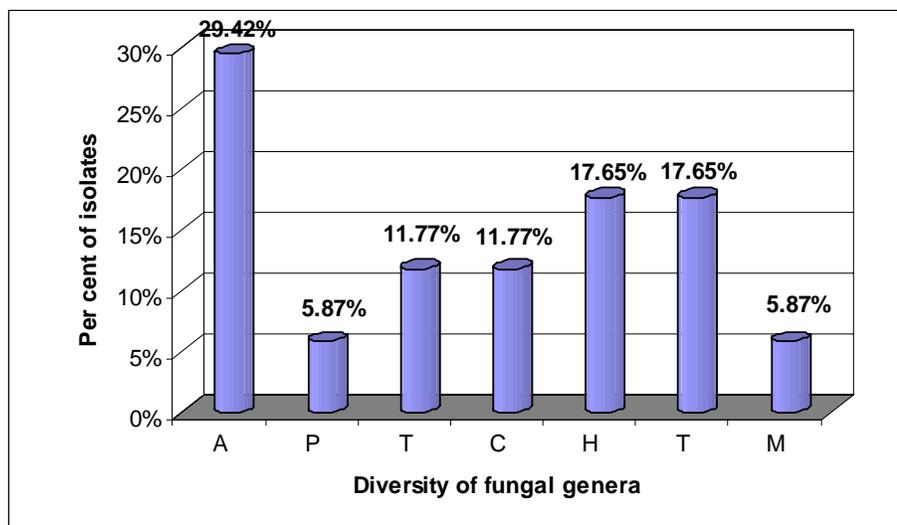


Fig. 7. Per cent of fungal isolates belonging to different genera from all the sources (A: *Aspergillus*, P: *Penicillium*, T: *Thermomyces*, C: *Chaetomium*, H: *Humicola*, T: *Torula*, M: *Mucor*)

Plate assay for enzyme production

Bacteria

Majority (95.9%) of the bacterial isolates was catalase positive; 61.2 per cent of them were starch hydrolyzing isolates (Table 4). Cellulose was degraded by 69.42 per cent of the isolates and 38.8 per cent of the isolates were xylanase producing ones. Phenol oxidase was produced by 63.3 per cent of the isolates.

Actinomycetes

Almost all the actinomycetes were enzyme producing. Isolates from weed compost, coir pith and sawdust were the most efficient ones. 95.2 per cent of the actinomycete isolates were amylase producing, 69.42 per cent isolates were cellulase producing and 95.9 per cent were catalase positive (Table 5). Xylanase was produced by 93.5 per cent of the actinomycetes. Phenol oxidase was produced by only 67.7 per cent of the actinomycetes.

Fungi

The fungal isolates also had a good ability of enzyme production. Phenol oxidase was found to be produced the main enzyme by the fungal isolates (70.6%) (Table 6). Isolates having the ability to produce all the five enzymes in significant quantity were found to be less in number.

Table 4. Number and per cent of enzyme producing bacteria out of the bacterial isolates selected for detailed study from each of the five sources of origin

Origin of Isolate	Number of bacteria selected for detailed study	Enzymes				
		Amylase	Cellulase	Catalase	Phenol oxidase	Xylanase
Weed compost	18	13 (72.2)	14 (77.8)	18 (100)	10 (55.6)	8 (44.3)
Oushadhi herbal waste compost	13	8 (61.5)	10 (66.9)	12 (92.3)	10 (76.9)	4 (30.8)
Tea waste compost	3	1 (33.3)	1 (33.3)	2 (66.6)	1 (33.3)	2 (66.7)
Coir pith	11	6 (54.5)	5 (45.5)	11 (100)	6 (54.5)	2 (18.2)
Saw dust	4	2 (50)	4 (100)	4 (100)	4 (100)	3 (75)
Total	49	30 (61.2)	34 (69.4)	47 (95.9)	31 (63.3)	19 (38.8)

Table 5. Number and per cent of enzyme producing actinomycetes out of the actinomycete isolates selected for detailed study from each of the five sources of origin

Origin of Isolate	Number of actinomycetes selected for detailed study	Enzymes				
		Amylase	Cellulase	Catalase	Phenol oxidase	Xylanase
Weed compost	16	16 (100)	16 (100)	16 (100)	8 (50)	16 (100)
Oushadhi herbal waste compost	6	5 (83.3)	6 (100)	6 (100)	4 (66.7)	6 (100)
Tea waste compost	8	8 (100)	8 (100)	8 (100)	4 (50)	6 (75)
Coir pith	16	16 (100)	16 (100)	16 (100)	12 (75)	15 (93.7)
Saw dust	16	14 (87.5)	14 (87.5)	16 (100)	14 (87.5)	15 (93.7)
Total	62	59 (95.2)	60 (96.8)	45 (100)	42 (67.7)	58 (93.5)

Table 6. Number and percentage of enzyme producing fungi out of the fungal isolates from each of the five sources of origin

Origin of Isolate	Number of fungi selected for detailed study	Enzymes				
		Amylase	Cellulase	Catalase	Phenol oxidase	Xylanase
Weed compost	5	5 (100)	5 (100)	5 (100)	4 (80)	4 (80)
Herbal waste compost	3	2 (33.3)	3 (100)	3 (100)	2 (66.7)	2 (66.7)
Tea waste compost	2	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
Coir pith	6	6 (100)	5 (83.6)	6 (100)	4 (66.6)	6 (100)
Sawdust	1	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)
Total	17	15 (88)	16 (94)	17 (100)	12 (70.6)	15 (88.2)

Selection of isolates for consortium

From the 128 microbial cultures, 14 cultures were selected to form the consortium based on their ability to produce of enzymes. The selected isolates comprised 2 bacterial isolates, 3 fungal isolates and 9 actinomycetes. From the enzyme analysis, it was concluded that actinomycetes were more efficient in enzyme production than fungi and bacterial isolates. Hence, for the selection of isolates to form suitable consortium, more numbers of actinomycetes were included in the test (Table 7).

Table 7. The list of species selected for testing their suitability to develop consortium

S. No	Isolate	Isolate No.	Species
1	Bacteria	Wb2	<i>Bacillus subtilis</i>
2		Cb6	<i>Bacillus stearothermophilus</i>
3	Fungi	Cf3	<i>Humicola sp</i>
4		Sf1	<i>Aspergillus sp.</i>
5		Wf1	<i>Aspergillus fumigatus</i>
6		Wa9	<i>Streptomyces celluloflavus</i>

7	Actinomycetes	Ca2	<i>Streptomyces albicans</i>
8		Wa6	<i>Streptomyces purpureus</i>
9		Ca6	<i>Streptomyces sulfonofaciens</i>
10		Sa7	<i>Streptovercillium viridoflavum</i>
11		Fa6	<i>Streptovercillium reticulum</i>
12		Wa8	<i>Streptovercillium cinnamoneum</i>
13		Ca3	<i>Thermomonospora alba</i>
14		Wa3	<i>Thermomonospora curvata</i>

Antagonistic property of isolates

Tests of the antagonistic property by cross streak method showed that there was no antagonism between the selected 14 isolates. All the selected 14 isolates could grow without any inhibition from any of the other isolates.

Mass culture of isolates

The broth cultures incubated for mass multiplication of the selected bacterial isolates showed a uniform fine turbidity in the broth. 10^4 to 10^5 cells per ml of these cultures were mixed together in equal quantities for inoculating to the test materials kept for composting.

Testing the consortium for composting efficiency

A period of three weeks of composting resulted in good compost in the case of weed and herbal waste. For coir pith compost, 26 days were required to get satisfactory compost. Composting did not progress to a satisfactory level for sawdust even after two months.

Temperature inside compost heap and speed of composting

The temperature of the waste materials undergoing composting was recorded daily. In the case of weed and herbal waste, the temperature inside the compost heap increased beyond 50°C while in the control, coir pith and sawdust buckets, the temperature was below 50°C (Table 8). The temperature inside coir pith compost and sawdust compost reached up to 40°C on 4th day while temperature in control buckets was 35°C only. The temperature inside weed compost stabilized at 30°C consistently on 19th day; the

temperature inside sawdust remained at 30°C on 50th day. All the usual activities such as aeration by turning and maintaining sufficient moisture content (55%) were done once in 3 days. The composting was stopped when the temperature inside the compost was close to ambient temperature.

Table 8. Effect of inoculation of consortium of thermophilic microorganisms on composting speed

Compost sample	Max temperature on 4 th day (°C)	No of days taken to complete composting	No of turnings carried out
Weed compost	50	19	9
Herbal waste compost	51	22	9
Coir pith	40	26	9
Sawdust	40	50	9

Mineral components

Table 9 shows the total percentage of C, N and S contents and C/N ratio of the final compost. The percentage of total nitrogen, carbon and sulphur components varied in all the four samples. The highest percentage of total nitrogen was shown by weed compost (3.77%) closely followed by Oushadhi factory herbal waste compost (3.57%); the sawdust showed the lowest percentage of N (0.14%). The C/N ratio was lowest for weed compost (9.78) while the ratio for herbal waste compost was 10.31. Coir pith had a C/N ratio of 18 which is acceptable for coir pith compost. But in case of sawdust the C/N ratio was 275 and the consortium was not found suitable.

Microbial population count

The microbial population per gram of the four samples drawn on 10th day of composting was estimated. Table 10 below gives the number of bacterial, actinomycetes and fungal colonies surviving in the compost samples. The total number of actinomycetes was found to be higher as compared to the bacterial and fungal isolates. This could be due to the large number of conidia formed by the actinomycetes

and the competitiveness of actinomycetes in composting materials. The total count of thermophiles was found to be maximum in the Ayurvedic herbal waste compost.

Table 9.The mineral components of the final compost

Sl. No	Sample Name	C%	S%	H%	N%	C/N	Initial C/N ratio of waste materials
1	Weed compost	36.87	0.47	5.55	3.77	9.78	20-45
2	Ayurvedic herbal compost	36.81	0.30	5.78	3.57	10.31	35-245
3	Coirpith compost	11.92	0.14	9.34	0.64	18.63	100-150
4	Sawdust compost	38.57	0.14	6.85	0.14	275.5	400

Identity of individual colonies was determined based on the morphology of bacteria, actinomycetes and fungi. It was found that among the six isolates, two *Streptomyces* species, two *Streptoverticillium* species, one *Bacillus* species and one *Humicola* species were found in higher numbers than other species in the consortium.

Table 10. Colony count of the consortium of microorganisms present in the materials undergoing composting on 10th day

Sl. No	Samples	Actinomycetes (cfu/g)	Bacteria (cfu/g)	Fungi (cfu/g)
1	Weed compost	53 x 10 ⁶	97.2 x 10 ⁴	3.2 x 10 ²
2	Ayurvedic herbal waste compost	47.2 x 10 ⁶	93.6 x 10 ⁴	2.2 x 10 ²
3	Coir pith compost	56.2 x 10 ⁶	23.2 x 10 ⁴	2.8 x 10 ²
4	Sawdust compost	44 x 10 ⁶	87.2 x 10 ⁴	5.4 x 10 ²

A consortium of microorganisms has been developed in such a way that the same can be used for composting a variety of raw materials. So, the most suitable isolates will be those which have the ability to decompose a variety of substrates. Thermophilic actinomycetes have a higher ability to produce several enzymes when compared to the thermophilic fungal and other bacterial groups. Thermophilic actinomycetes play an important role in habitats where decomposition of organic matter takes place at elevated temperatures in composts (Korn-Wendisch *et al.*, 1995). In the present study also, the maximum enzyme producing isolates were found to be actinomycetes. The test consortium of microorganisms had 9 actinomycetes, 3 fungi and 2 bacterial isolates. But only six isolates comprising four actinomycetes namely, *Streptomyces celluloflavus*, *Streptomyces alboflavus*, *Streptoverticillium viridoflavum*, *Streptoverticillium reticulum*, one bacterium namely, *Bacillus subtilis* and one fungus namely, *Humicola* sp. are finally recommended for inclusion in the consortium. For bringing down the number of organisms to six in the final consortium, the practical problem of using 14 organisms and the higher competitiveness of the selected six organisms were considered. *Humicola* sp. was included in the final consortium because of its high performance in biochemical characteristics. Fungal consortium of *Aspergillus* and *Humicola* sp. had been used by Kumar *et al.* (2007) for the conversion of paddy straw in nutrient rich compost.

The carbon, nitrogen and sulphur analysis of the compost samples gave an idea of the quality of the compost samples produced. The C/N ratio of the compost which gives a major insight into the quality of the compost was also calculated for all the four compost samples obtained after composting test. The C/N ratio of weed compost was found to be 9.78 and that of ayurvedic factory herbal waste compost was 10.31, indicating the high quality of composts. For coir pith, the selected consortium was found suitable to reduce the C/N ratio from 100-200 to 18.6. The coir pith compost had a lower quality to that of the weed and herbal factory waste compost. But the final product had a dark color and earthy odor typical of good quality compost. The C/N ratio of weed compost, ayurvedic herbal waste compost and coir pith compost showed that these three composts produced with the help of the consortium had a C/N ratio indicative of good quality compost. But in the case of sawdust the C/N ratio was too high (275) indicating that the consortium developed would not be suitable for the development of sawdust compost.

In the present study, the microorganisms which have the ability to produce polyphenol oxidase in high quantity were selected. According to Uma *et al.* (2004), the ability of an organism to produce polyphenol oxidase and laccase can accelerate the degradation of lignin. The laccase production ability of the microorganisms has not been measured in the selected thermophiles with high polyphenol oxidase activity, but phenol oxidase producing ability of the isolates could accelerate the degradation of lignin as reported by Bending and Read (1997).

For sawdust compost, the consortium was not found suitable. The ideal C/N ratio of the raw materials for composting is generally considered to be around 30:1 (Holber *et al.*, 1997). The C/N ratio of sawdust is 100-500:1. The higher C/N ratio indicated that sufficient quantity of nitrogen for optimal growth of the microbial populations is unavailable in sawdust; so the compost remained relatively unaffected and the degradation took place at a much slower rate (Rymshaw *et al.*, 1992). For quicker degradation of sawdust sufficient quantity of nitrogen has to be added so as to drastically decrease the C/N ratio.

5. CONCLUSIONS

Aerobic thermophilic bacteria, actinomycetes and fungi are responsible for decomposition of lignocellulosic waste materials into compost. Such microorganisms generate heat through exothermic metabolic activity, and rapid growth and multiplication. These microorganisms are able to survive in the compost heap up to 60-70⁰ C. Thermophilic aerobic microorganisms are physiologically very active. They are capable of producing several thermostable enzymes responsible for decomposition of cellulose, and to a great extent, lignin into simpler compounds. However, high C/N ratio of some of the waste materials such as sawdust (C/N>400) and coir pith (C/N >100) are the limiting factor for the microbial activity. In the present study, it was possible to decompose coir pith through consortium of microorganisms. But, sawdust is recalcitrant to a great extent. Decomposition of sawdust can be done through a compatible combination of aerobic microorganisms and wood decaying basidiomycetes. If needed, nitrogen can be added externally to increase the C/N ratio. Further studies have to be made in this line.

The consortium of thermophilic microorganisms developed through the present study is capable of speeding up aerobic composting process. These organisms can be exploited commercially for converting all types of lignocellulosic materials other than woody waste materials into compost. Some of the actinomycetes and bacteria capable of producing enzymes such as amylase, cellulase, catalase, etc. can be commercially exploited.

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