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ASSOCIATION OF FUNGI, BACTERIA AND ACTINOMYCETES WITH DIFFERENT COMPOSTS

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Abstract

In the present study the agricultural and kitchen wastes viz., potato peels, sugar cane waste, tree bark, used microbiological media, news paper, saw dust, fruit peels, grass, leaves, guar, used tea, spinach twigs, wood chips, fruit and vegetable wastes were used alone and in combinations as compost feed-stocks. Microorganisms isolated and characterized from the above composts include the species of fungi viz., *Aspergillus, Trichoderma, Mucor, Penicillium, Alternaria, Cladosporium, Monilia, Helminthosporium, Coccidioides, Scedosporium,* actinomycete viz., *Nocardia* and bacteria viz., *Bacillus, Lactobacilli, Micrococcus, Pseudomonas, Clostridium*. Of these isolates, members of the genus *Aspergillus* were most prevalent (38%) followed by *Bacillus* comprising of 20% of the total microbial isolates. The study supports the idea that composting can be useful to treat wide range of organic materials such as yard trimmings, kitchen wastes and food processing discards. In addition, the knowledge regarding species composition of the microorganisms of different composts can help to optimize compost quality standards.

Introduction

The increased production of waste in the world is of great concern at different levels of population (Woulters *et al*., 2005). Various alternatives are exercised to diminish this increase by elimination, purification and/or recycling. The modern concept of environmental management is based on the recycling of wastes. In this context, composting appears to be a safe form of treatment of some wastes and the reclamation of the nutrients contained in them (Iranzo *et al*., 2004).

Composting is a fertilizing mixture of partially decomposed organic matter from plant and animal origin (Piet *et al*., 1990). The active component mediating the biodegradation and conversion processes during composting is the resident microbial community, among which fungi play a very important role. Therefore, optimization of compost quality is directly linked to the composition and succession of microbial communities in the composting process (Taiwo & Oso, 2004; Peters *et al*., 2000). There is practically no substance existing in nature that is not used by one microorganism or another (Iranzo *et al*., 2001). It is therefore necessary to identify the microorganisms present in the different processes, as several different species of microbes are usually involved (Radajewski *et al*., 2000; Hugenholtz *et al*., 1998).

The biomass ratio of fungi to prokaryotes in compost is about 2:1 (Wiegant, 1992; Sparling *et al*., 1982). In addition, fungi use many carbon sources; mainly lignocellulosic polymers and can survive in extreme conditions. They mainly are responsible for compost maturation (Miller, 1996). They also degrade complex polymers such as polyaromatic compounds or plastics and are being increasingly applied to bioremediate soils contaminated with a wide range of pollutants (Ashraf & Ali, 2006; Minussi *et al*., 2001; Eggen & Sveum, 1999; Kastner & Mahro, 1996).

Since composting is a microbial process, compost stability and maturity are the results of microbial activity. Except for extremely mature compost, most compost contains relatively high organic matter content with potentially available organic carbon and nutrients that support microbial populations or activity (Butlera *et al*., 2001; Wu & Ma, 2001; Inbar *et al*., 1990)**.** Microorganisms absorb dissolved nutrients and water serves as a medium for distribution within the heterogeneous compost substrate (Spinosa & Vesilind, 2001). Therefore, adequate moisture is essential for microbial activity. A dry compost pile does not decompose efficiently. Likewise, the decomposition of organic matter is seriously inhibited if the moisture content is higher than optimum, as the excess moisture causes an anaerobic condition (Nakasaki & Ohtaki, 2002; Bass, 1999).

Similarly temperature also influences microbial decomposition of waste during composting. As temperature rises in the compost, decomposition speeds up. As temperature drops, composting slows down. Substantial changes occur in microbial populations and species abundance during the various temperature stages (Gupta *et al*., 1987). Mesophilic bacteria and fungi are dominant in the initial warming period and during the curing phase and thermophilic bacteria (especially actinomycetes) during the high temperature phase (Finstein & Morris, 1975). The quickly changing physicochemical conditions in composting processes, are likely to select for a succession of different microbial communities and it can be expected that temperature and the available substrates, including electron donors and acceptors, are the main factors (Paul & Clark, 1996).

Since composting methods and different substrates are associated with difference in the composition of a microbial community, monitoring of the resident microbial population in compost is essential to determine its quality and field of application (Peters *et al*., 2000). Similarly, monitoring of microbial diversity is essential to detect pathogens hazardous to humans, animals and plants and to optimize compost quality standards (Summerbell *et al*., 1994). The objective of the present study is to provide necessary information regarding the communities of microorganisms involved in composting that could be used in the field of bioremediation (Mihial *et al*., 2006; Laine & Jorgensen, 1996).

Materials and Methods

Various composts were prepared using single and combination of substrates. These substrates included potato peel, sugar cane waste, tree bark, used microbiological media, news paper, saw dust, fruit peels, grass, leaves, legume (guar), used tea, spinach twigs, wood chips, fruits and vegetables waste. These were collected from different sources as an agricultural, household especially kitchen waste. Necessary chopping and shredding was done as per requirement as it helps speed up decomposition and hasten the process of composting by increasing the surface area available for microbial action, and providing better aeration (Taiwo & Oso, 2004; Nielsen *et al*., 1997; Strom, 1985).

For the preparation of single substrate compost, 6 different chopped substrates 75% w/w (each) were filled into the compost bins, amended with the 250 g of soil as an inoculum (Table 1). In order to prepare the composts of multiple substrates, the soil (250g) was amended with combination of different chopped substrates 25% each (Table 2). Combinations of substrates were considered to makeup the acceptable C: N ratio of 25:1 to 30:1 (Hankin *et al*., 1975; Iranzo *et al*., 2004). Grass and leaves were used as bulking agents to facilitate aeration in the compost. All the test composts were run in triplicate. The single and combination of substrates were prepared and monitored for composting outside for the period of 16 weeks at the end of cooling phase. Water was added until moisture content was adjusted between 40-60% (Buswell, 1984). Proper turning was done to get homogenous

compost. As the composting progressed, the materials were regularly inspected using the traditional technique of touch and smell method. Moisture retention capacity of each treatment was maintained and the temperature of the surface, middle and depth of each treatment was noted successively. The maximum temperature reached during composting was 60ºC.

Isolation of microorganisms was carried out after 16 weeks of incubation at the end of cooling phase and standard plate count (SPC) was performed as given by Pelczar *et al.,* (2003). Ten-fold serial dilutions were made up to 10^{-5}. An amount of 0.1 ml from the diluted samples was spread on soil extract agar (pH 7; for bacteria) and soil extract with 0.1% (w/v) malt agar plates (pH 5.5; for fungi and actinomycetes) using a glass spreader. Petri plates were then incubated at ambient temperature (30-40ºC) for 24 h for bacteria and 4-5 days for fungi. The isolates were maintained on respective media slants. Prevalence of different groups of microorganisms was calculated in terms of percentage. These isolates were identified on the basis of conventional cultural and morphological characteristics (Buchanan & Gibbons, 1986; Barnett & Hunter, 1998; Barnett, 1960; Thom & Raper, 1945).

Results and Discussion

A total of 119 species of microorganisms were isolated from different composts which include the species of fungi viz., *Aspergillus, Trichoderma, Mucor, Penicillium, Alternaria, Cladosporium, Monilia, Helminthosporium, Coccidioides, Scedosporium,* actinomycete viz., *Nocardia* and bacteria viz., *Bacillus, Lactobacilli, Micrococcus, Pseudomonas, Clostridium* (Table 1& 2). A large majority (38%) of total number of isolates were members of the genus *Aspergillus*. *Bacillus* was found to be the second largest genus comprising 20 % of the total microbial isolates.

Table 1. Microorganisms isolated from composts prepared by single substrate.

Compost #	Substrate	Isolates (number)
01	Potato peel	*Aspergillus niger (6)*
		Mucor sp. *(3)*
		Penicillium rubrum (1)
02	Sugar cane waste	*Nocardia* sp. *(1)*
		Bacillus cereus (2)
03	Tree bark	*Alternaria alternata (1)*
		Monilia sp. *(2)*
		Trichoderma sp. *(4)*
		Aspergillus flavus (2)
		Lactobacilli sp. *(1)*
04	Used microbiological media	*Aspergillus tereus (2)*
		Aspergillus niger (4)
		Trichoderma sp. *(3)*
		Alternaria alternata (2)
05	News paper	*Micrococcus roseus (1)*
		Bacillus polymyxa (2)
06	Saw dust	*Nocardia* sp. *(3)*
		Pseudomonas aeruginosa (2)
		Mucor sp. *(1)*

Table 2. Microorganisms isolated from composts prepared by combination of substrates.

Compost #	Substrates	Isolates (number)
07	Used microbiological media, fruit peels, grass and leaves	*Aspergillus niger (4)* *Aspergillus microviridocitrinus(3)* *Aspergillus flavus (2)* *Bacillus licheniformis (1)* *Nocardia* sp. *(1)* *Mucor* sp. *(3)*
08	Sugar cane waste, legume (guar), grass and leaves	*Aspergillus tereus (2)* *Aspergillus niger (5)* *Helminthosporium* sp *(2)* *Lactobacilli* sp. *(1)* *Cladosporium cladosporioides (3)*
09	Sawdust, wood chips, grass and leaves	*Aspergillus nidulans (3)* *Clostridium* sp. *(2)* *Aspergillus tereus (2)* *Trichoderma* sp. *(6)*
10	Used microbiological media, fruits and vegetables, grass and leaves	*Aspergillus nidulans (2)* *Aspergillus niger (5)* *Aspergillus tereus (2)* *Coccidioides* sp. *(2)* *Penicillium* sp. (*3)* *Bacillus licheniformis (3)*
11	Used tea, spinach twigs, grass and leaves	*Aspergillus niger (4)* *Aspergillus nidulans (3)* *Aspergillus flavus (4)* *Scedosporium* sp. *(2)* *Bacillus subtilis (3)* *Bacillus cereus (7)*

Substrates used in the study were composted singly and in combinations (Table 1& 2). It was found that in comparison with the composts prepared from single composts, combination of two or more type of wastes enhanced not only the number but also the diversity of saprophytic microorganisms that play an important role in the biodegradation of such materials. The study of Nakasaki & Ohtaki (2002) explains that when a microorganism is incubated in the presence of two or more substrates, the substrates will be degraded in the order of their ease of degradation. Besides that, presence of two substrates also increased the variety of microorganisms. Fungal species were found to be numerous during both mesophilic and thermophilic phases of composting. Their importance along with actinomycetes has been reported in composting, especially during the late curing stage (Strom, 1985). Growth of fungi was apparent in the outer layers of compost when temperatures were high. This particular character suggests that compost molds are strict aerobes that grow both as white, gray or green fuzzy growth or unseen filaments on the compost surface.

In our study we managed to recycle the agricultural, household particularly kitchen waste which can be regarded as 'biowastes' through composting. Although likely perspectives with regard to thermophillic and mesophillic microorganisms have been reported by several authors during composting of these 'clean' biowastes but knowledge of the importance of specific taxonomic groups and functional groups can still be

improved (Strom, 1985; Finstein & Morris, 1975). The present study provides a comprehensive knowledge of relative composition of microorganism in composts based on different substrates. Since substrates utilized for composting are as critical as composting conditions, so they have impact on the type of active microflora. The versatility of microorganisms present in compost depends on the nature of the substrates that were subjected to composting and on the phases of composting (Dubey & Maheshwan, 2005; Hoitink *et al*., 1997).

Proper composting promotes the development of a number of saprophytic soil microorganisms. Species of *Bacillus, Enterobacter, Flavobalistinum, Pseudomonas, Streptomyces, Nocardia, Rhodococcus, Penicillium, Trichoderma* and *Gliocladium* have been reported by several authors (Gbolagade, 2006; Anastasi *et al*., 2005; Charest *et al*., 2004; Taiwo & Oso 2004; Ryckeboer *et al*., 2003; Fordyce, 1970). Dubey and Maheshwan (2005) have stated that the cellulytic fungi, such as *Aspergillus, Trichoderma, Penicillium and Trichurus* accelerate composting for efficient recycling of dry crop wastes with high C: N ratio and reduce the composting period for about one month. If these saprophytes are provided with the right conditions they can be antagonistic towards a number of important soil borne microbial pathogens, including *Rhizoctonia solani*, *Fusarium* sp., *Pythium* sp., *Phytophthora* sp., *Armillaria* sp., *Phomopsis* sp., *Sclerotinia sclerotiorum* and *Sclerotium rolfsii* (Whipps, 2001). Therefore, such microorganisms can be best utilized as biocontrol agents.

Large and diversified microbial populations were found to be present during the composting process as well as in mature compost. It has been suggested that the appearance of some microorganisms reflects the quality of maturing compost (Strom, 1985). Since the environmental conditions during composting are radically different from those experienced by organisms in most natural environments, it is possible that compost-derived organisms might have abilities not found in the microbial populations of soil and water. For this reason, further studies on microbial ecology of compost are likely to have beneficial effects, not only for the composting industry but also for uses of compost-based materials. The implication of the most prevalent microorganisms explored in the study includes the following (in ascending order of their prevalence):

***Aspergillus* sp.:** *Aspergillus* was the most common fungal genus comprising 43% of total microbial species isolated from various composts prepared in the study (Fig. 3). The isolated species from single substrate composts comprise 38% of the total viz., *A. niger, A. flavus, A. tereus* (Fig. 1) and from combination of substrates comprise 51% of the total viz., *A. niger*, *A. microviridocitrinus*, *A. flavus*, *A. tereus*, *A. nidulans* (Fig. 2). These association of *Aspergillus* sp., with different composts has been reported (Anastasi *et al*., 2005; Dubey & Maheshwan, 2005; Wouters *et al*., 2005; Iranzo *et al*., 2004; Taiwo & Oso 2004; Rickeboer *et al*., 2003; Strom, 1985; Fordyce, 1970). Our findings correspond with Anastasi *et al*., (2005) who have reported the highest load and number of species of *Aspergillus* in addition to *Penicillium* in two composts study. The findings of Strom (1985) are also in agreement with our results that the number and diversity of microorganisms is more when two or more wastes are used for composting. In our study, the species with the abundance in most of the composts was found to be *Aspergillus niger*. Its abundance can be attributed to its universal presence as a saprophyte growing on dead leaves, stored grain, and other decaying vegetation. The spores are widespread and are often associated with organic materials and soil.

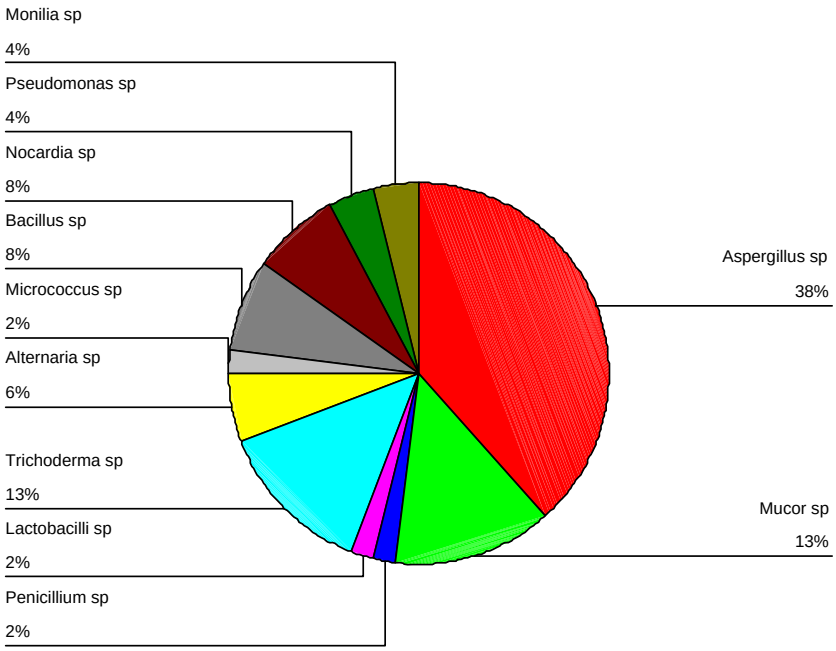

Fig. 1. Prevalence of different groups of microorganisms in composts based on single substrates.

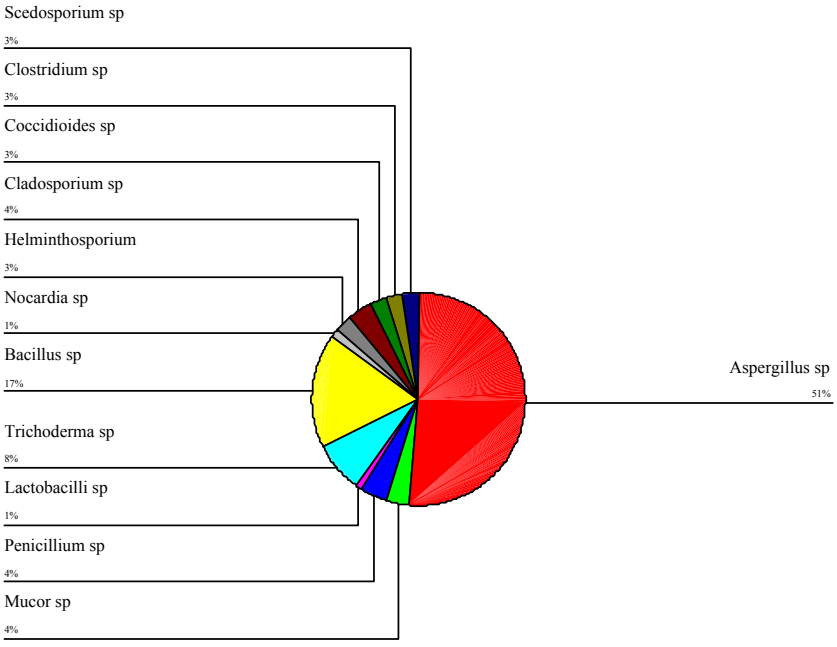

Fig. 2. Prevalence of different groups of microorganisms in composts based on multiple substrates.

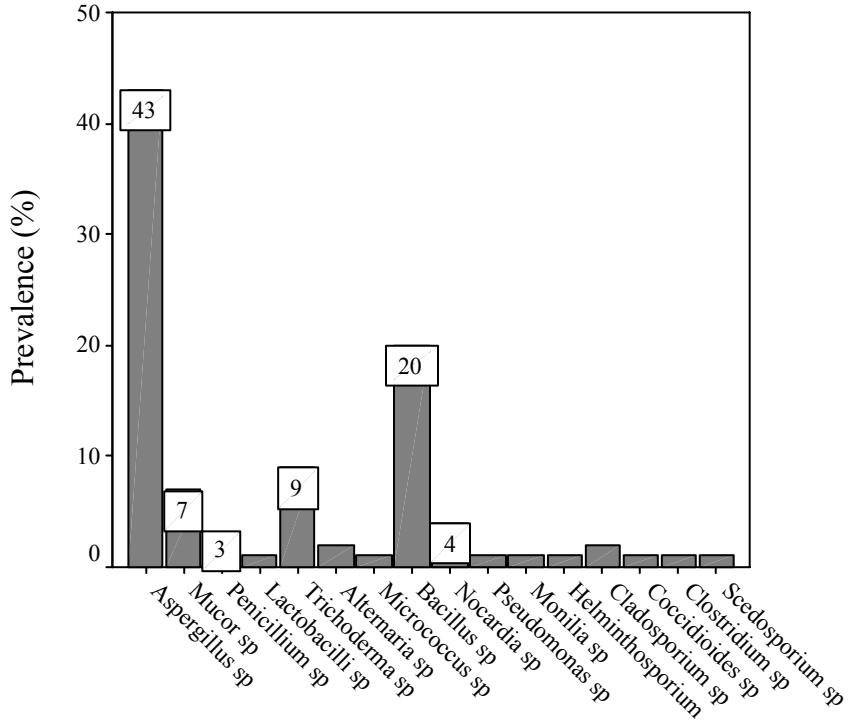

Fig. 3. Prevalence of different groups of microorganisms in composts based on various substrates.

***Bacillus* sp.:** Species of Bacillus viz., *B. licheniformis, B. subtilis, B. cereus, B. sphaericus, B. coagulans type B*, and *B. stearothermophilus* have been reported in various composts (Gbolagade, 2006; Anastasi *et al*., 2005; Charest *et al*., 2004; Taiwo & Oso 2004; Rickeboer *et al*., 2003; Boulter *et al*., 2002; Strom 1985). An overall 20% of some of these species, in addition to several others were found in the present study (Table 1 & 2; Fig. 3). *Bacillus* sp comprised the largest number with being more diverse bacterial group. Its abundance can be associated with wide temperature tolerance by forming endospores that attribute their presence in hot composting stages (Peter *et al.,* 2000). In contrast chitinolytic enzymes produced by *Bacillus cereus* appear to be involved in biocontrol of *Rhizoctonia solani* (Whipps, 2001; Chernin *et al*., 1995, 1997; Pleban *et al*., 1997).

***Trichoderma* sp.:** In our study an overall 9% species isolated from composts belong to genus *Trichoderma* (Fig. 3). Their abundance can be associated by their presence as ubiquitous soil and compost borne saprotroph, which has been exploited in the commercial production of enzymes (Cullen & Kersten, 1992), antibiotic production (Howell, 1998) and in the biological control of plant diseases caused by economically important plant pathogens such as *Rhizoctonia solani, Phytophthora* sp. and *Pythium ultimum* (Whipps & Lumsden, 2001; Whipps, 1997, 2001; De Ceuster & Hoitink, 1999; Abbasi *et al*., 1999). In our study, *Trichoderma* sp., were isolated from the composts prepared mainly from bark, sawdust, wood chips and used microbiological media depicting the source as lignocellulose, which can be used as an effective biocontrol agent in compost-amended substrates.

***Mucor* sp.:** The fourth abundantly found species comprises of *Mucor* isolated as 13% of the total microbial species from composts prepared from single substrate (potato peels and fruit peels) (Fig. 1). Their abundance can be attributed to their profuse occurrence in soil, manure, fruits, vegetables and starchy foods (Pelczar, 2003). Rickeboer *et al*., found *Aspergillus* sp., and *Mucor* sp., being the predominant fungi after thermophillic phase. The association of *Mucor* sp., with composts has also been reported by Fordyce *et al*., (1970) and Thornton *et al*., (2002).

***Nocardia* sp.:** *Nocardia* sp., was identified in the actinomycete populations being 8% of total isolates from composts based on single substrate namely sugar cane waste, sawdust and plain soil. The number was found to be more in single substrate based composts as compared with the composts based on multiple substrates (Table 1 & 2). It has been suggested that actinomycete populations increase during compost maturation (Edwards, 1995).

Conclusion

Conventional techniques were used to identify the fungal cultures however molecular techniques can be adopted to have a better understanding of active compost fungi. Anastasi *et al*., (2005) suggested that molecular techniques only complement the conventional techniques that remain indispensable for the complete study of fungal communities and provide pure cultures that can be used for further physiological characterization of each isolate. Along with the systematic characterization of fungal communities in compost, a functional analysis is needed to highlight potentials and applications. Large unexploited diversity of microorganisms awaits discovery. Several fungal, bacterial and actinomycetes strains from these composts are now being investigated to evaluate their capability to degrade some petroleum hydrocarbons and to decolourize several synthetic dyes in order to reveal their potential application in bioremediation.

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