KAVAKA 42: 52-68(2014)

Thermophilic fungi: Diversity and significance in composting

Seema Rawat[1] and B.N. Johri[2]*

[1]*Department of Botany and Microbiology, H.N.B. Garhwal (Central) University, Srinagar, Pauri Garhwal, U.K.*

[2] *Department of Biotechnology, Barkatullah University, Bhopal, M.P.*

Corresponding author Email: bhavdishnjohri@rediffmail.com

(Submitted in December, 2013 ; Accepted on June 25, 2014)

ABSTRACT

Composting is the process of decomposition of organic matter by a mixed population of microorganisms in a warm, moist and aerobic environment. It involves complex physico-chemical interactions between the organic matter and decomposer resulting in the end product which can be utilized as fertilizer, substrates for mushroom production or biogas. The active component mediating the biodegradation and conversion process during composting is resident microbial community which exhibits a successional pattern with the change in physico-chemical conditions of compost. It begins with mesophilic microflora and culminates with thermophilic microflora. *Torula--Humicola* complex, *Myriococcum thermophilum*, *Papulospora thermophila*, *Rhizomucor miehei*, *Sporotrichum thermophile*, *Stilbella thermophila*, *Talaromyces thermophilus*, *Thermoascus aurantiacus* and *Thielavia terrestris* are some of the important components of compost. Thermophilic fungi, constituting the climax and also the dominant component, play a pivotal role in decomposition of plant residues and contribute significantly to the quality of compost mainly by providing selectivity to it. This necessitates the monitoring and characterization of the thermophilic fungal community composition, patterns and dynamics of species diversity at spatial scales. The recent use of metagenomic approaches has given new insights in identification of population structure and *in situ* functionality of each component which plays an important decisive role in successful colonization and succession in compost. The knowledge of complete spectrum of thermophilic fungi would help in manipulating compost environment and hastening the process of composting besides improving the quality of compost.

Key words: Composting, mesophilic microflora, thermophilic microflora, climax community, metagenomic approaches, *Torula–Humicola* complex

INTRODUCTION

Composting is the biological decomposition of organic matter under controlled conditions brought about by the growth of microorganisms and invertebrates. Composting has been at the forefront of the diversion and processing of organic wastes as it is a relatively simple and robust process. It is different from the natural process of decomposition of large amounts of organic material on a regular basis by microorganisms which results into humus and is a mean of nutrient turnover in the natural ecosystem. The latter takes place at a slow pace which can be accelerated by creating conditions ideal for growth and activity of the decomposers. This accelerated process of decomposition of organic matter by a mixed population of microorganisms in a warm, moist, aerobic environment, has been termed as, "composting" (Rawat and Johri, 2013). Composting stabilizes organic matter, yielding an end product that contains humus, and has a uniform crumbly texture. It can be carried out on a wide range of scales in almost any indoor or outdoor environment and in almost any geographic location. It can be implemented as a small open windrow type facility through to a large facility that uses a sophisticated in-vessel technology.

It has the potential to manage most of the organic material in the waste stream including restaurant waste, leaves and yard wastes, farm waste, animal manure, animal carcasses, paper products, sewage sludge, wood, etc. and can be easily incorporated into any waste management plan. An understanding of the composting process is important for producing a high-quality product and preventing operating problems.

It involves complex physico-chemical interactions between the organic matter and decomposer, resulting into end products: compost (soil-like material composed of more resistant residues of the organic matter, breakdown products and dead and living microorganisms along with products formed from further chemical reaction between these materials), carbon dioxide, water and heat. The finished compost can be classified as a 100% organic fertilizer containing primary nutrients as well as trace minerals, humus and humic acids, in a slow release form. Compost improves soil porosity, drainage and aeration and moisture holding capacity and reduces compaction. In addition, compost helps in buffering soils against extreme chemical imbalances; aids in unlocking soil minerals; releases nutrients over a wide time window; acts as a buffer against the absorption of chemicals and heavy metals; promotes the development of healthy root zones; suppresses diseases associated with certain fungi; and helps plants tolerate drought conditions (Johri and Rajni, 1999; Rawat, 2004).

Compost can be used in a variety of applications. High quality compost can be used in agriculture, horticulture, landscaping and home gardening. Medium quality compost can be used in applications such as erosion control and roadside landscaping. Low quality compost

can be used as a landfill cover or in land reclamation projects (Eklind *et al*., 1998; Hoitink and Boehm, 1999; Li and Jang, 1999; Odlare, 2005). Composting is a far better and an ideal eco-friendly approach for disposal and decomposition of wastes compared to landfilling and burning as latter poses environmental problems. The active component mediating biodegradation and conversion process during composting is the resident microbial community. The ease with which organic materials are composted depends on the type of decomposers, the type of organic material being composted and the composting method used.

The knowledge about the resident microorganisms in composts, their coexistence and their succession during the different stages of composting is essential for manipulating compost environment and for ensuring a high quality of the final compost product (Steger *et al*., 2007).

The microbial community changes with the change in physico-chemical conditions of compost. Fungi are the predominant component of compost. Thermophilic fungi constitute the climax community of compost which provides selectivity to compost. Thus, the optimization of compost quality is directly linked to composition and succession of microbial communities in the composting process. This necessitates the characterization of diversity and successional pattern of thermophilic fungal community. The cultivation independent approaches in recent years have given new insights into microbial community succession (Herrmann and Shann, 1997; Boggs *et al*., 1998; Klamer and Baath, 1998; Kowalchuk *et al*., 1999; Peters *et al*., 2000) and has also made it possible to define the causes of time-dependent changes in the health of microbial community on the basis of observed genetic diversity (Purohit *et al*., 2005). The study of those remaining, known as "unculturables," is important to understand the genetic diversity, population structure, and ecological roles of microbes and to find ways of utilizing them as a novel source of molecules with unique properties (Cowan *et al.*, 2005). In recent decades, the investigation by function- and sequence- based screening of the entire microbial genome collected directly from a specific environment, the so-called metagenomic approach, has gained much attention to gather otherwise inaccessible information about microbial communities from various environments as well as to obtain valuable enzymes (Lorenz *et al*., 2002; Schmeisser *et al.*, 2007).

This chapter covers various types of composting, physico-chemical aspects of composting, and structure of thermophilic fungal community and their functionality in various compost ecosystems. Readers may find some bias towards mushroom compost ecosystem which is partly due to its uniqueness with respect to conditions under which the mushroom crop is grown and relatively short time required to complete the successional cycles which are not matched elsewhere and partly due to our long research experience of this ecosystem.

COMPOSTING

Composting represents an astonishing example of solid-state fermentation (SSF) wherein a crude variety of wastes such as sewage sludge, refuse, animal manure, industrial wastes, food wastes, leaves, tree bark, agriculture residues, abattoir residues, etc. can be treated through microbial route irrespective of their suitability as feed-stock for compost production (Rawat *et al.*, 2005). Refuse (municipal solid waste) is partly compostable but poses problems due to its extreme heterogeneity as it consists of food scrapes (garbage), paper, glass, plastic, metal, sweepings, yard waste, ash, etc. The organic rich fraction after separation can, however, be composted; the whole municipal solid waste can also be passed through the composting stage (mass composting), possibly with subsequent segregation (Satyanaryana and Grajek, 1999).

The basic aims of composting according to Miller (1994) are "(1) achievement of a suitable bulk density (compost makes a more physically stable landfill and can be easily stored, transported and disposed off than the original material as bulk density of former is higher), (2) modification of complex polysaccharides and plant materials, (3) biological removal of readily available nutrients to avoid overheating, (4) building up of an appropriate biomass and a variety of microbial products, (5) establishment of selectivity, (6) conversion of nitrogen into stable organic form, and (7) sanitation i.e., killing of pathogenic microbes, larvae and weeds".

The various methods of composting are sheet composting, trench composting, enclosed channel composting, in–vessel composting, bin composting, windrow composting, and aerated static pile composting. Sheet and trench composting are long methods which allow organic material to decompose naturally on the surface of the soil (or untilled ground) and into trenches, respectively. These methods do not destroy pathogens. Enclosed channel composting produces compost in 3-6 months. In–vessel composting, bin composting, windrow composting and aerated static pile composting are faster methods of composting. The pathogens are destroyed during processing and thus these methods provide selectivity to compost. Composting can also be classified as hot and cold composting based on temperature. Hot composting is the most efficient method for producing quality compost in a relatively short time. In addition, it favours the destruction of weed seeds, fly larvae and pathogens. While hot composting, using the windrow or bin method, requires a high degree of management and hot composting, using the in-vessel method, requires a lesser degree of management. Cold composting is an ideal method for adding organic matter around trees, in garden plots, in eroded areas, etc. The time required to decompose organic matter using this method is governed, to a large extent, by environmental conditions and could take two years or more (Hultman, 2009; Rawat and Johri, 2013).

Composting is done at small scales like decomposition of domestic wastes as well as large scales like decomposition of industrial wastes. It is carried out *via* either batch or a continuous mode. Batch mode is a long process comprising of four sequential phases: the mesophilic (or moderate temperature phase), thermophilic (or high temperature phase), cooling phase and the curing phase. The initial stage of decomposition of mass of organic matter, initiated by mixing and wetting the substrates, the mesophilic stage, which lasts for a couple of days, is governed by the mesophilic microflora, which uses up the readily available nutrients. The aerobic fermentation (composting) commences as a result of growth and activity of microorganisms resulting in release of heat, ammonia and CO_2 as byproducts along with other unpleasant smelling compounds. The metabolic activity of the mesophilic microorganisms results in rise in temperature which paves way for the development of thermophilic microflora which initiates the second phase of composting. This phase of composting starts very rapidly and may last days, or weeks or even months. It is the thermophilic stage that results in maximum decomposition of organic matter besides sanitation. During the cooling stage, mesophilic microflora recolonizes the compost and degrades more resistant organic matter (Tiquia *et al*., 2002; Hiraishi *et al*., 2003). The final phase of composting is called curing, aging or maturing stage, which is long (may lasts up to several months) and is an important one since it provides a safety net for destruction of the pathogens. Uncured compost can produce phytotoxins, besides depriving soil of oxygen and nitrogen and can contain high levels of organic acids (Mathur *et al*., 1993).

In the continuous mode of composting, similar phases occur but they are not as apparent as in the batch mode and these could occur concurrently rather than sequentially. A well-designed continuous system can eliminate the need for a mesophilic stage and operate continuously at thermophilic temperatures. This mode offers a mean of decomposition of putrescible materials quickly under close process control.

Municipal compost is prepared by batch mode while garden composting is performed in a continuous mode. Mushroom compost and vermicompost, though prepared by batch mode, are significantly different from general municipal compost.

TYPES OF COMPOST

All composts viz., mushroom compost, vermicompost, industrial compost, municipal compost, garden compost, etc. are unique with respect to the raw material used, their way of preparation, physico-chemical conditions and the end product generated.

Mushroom compost is prepared very rapidly (18-24 d) and does not involve curing stage. It is prepared, either by long method (LMC) or short method (SMC), from various agro-residues viz., wheat straw/ paddy straw/ sugarcane bagasse as a base material along with other additives viz., chicken manure, calcium ammonium nitrate, urea, superphosphate, muriate of potash, wheat bran, gypsum as additives. LMC is the primitive, cheap method involving only one phase (without pasteurization) (Mantel *et al.*, 1972). SMC is a quick method constituting of a general advancement in controlled composting (Sinden and Hauser, 1950) and involves two sequential phases: phase I (an uncontrolled self-heating process initiated by mixing and wetting the ingredients as they are stacked in windrows which are periodically turned and watered at approximately two days interval) and phase II which is the indoor process of pasteurization, carried out in tunnels.

Vermicompost (also called vermicast or worm castings), obtained by the decomposition of organic materials viz., straw, shredded newspaper, saw dust and horse manure using surface feeding worms, usually red wigglers, white worms and earthworms, especially *Eisenia foetida* (redwiggler) and other microorganisms, is rich in microbial activity and plant growth regulators, and is fortified with pest repellence attributes as well. It differs from other composts as it is prepared by a mesophilic process and contains a higher content of humic acid (50-70%). It is carried out both indoor in specially designed worm boxes as well as large-scale outdoor, involving decomposition in pits, heaping above ground on polythene sheets, in tanks, in commercial biodigester and in cement rings. The organic material is sprinkled with phosphate powder and cow dung slurry and is allowed to decompose for 15-20 days. The earthworms are released once heat generated has been cooled down. The vermicompost is ready in about 2 months if agricultural waste is used and about 4 weeks if sericulture waste is used as substrate (Tognetti *et al.*, 2005; Vivas *et al.*, 2009).

Industrial compost is prepared *via* a large scale composting system involving various techniques, viz., in-vessel method; aerated static pile composting, sheet composting, anaerobic digestion and high fiber method. Municipal compost is prepared from the decomposition of yard waste, food scraps, leaves and other domestic wastes in large bins. It differs from backyard compost in the size of containers and also the factors such as flow of air and temperature are controlled more effectively.

PHYSICO-CHEMICAL ASPECTS

Composting is a very dynamic process involving quick changes in physico-chemical conditions due to microbial activity which in turn also poses a selection pressure on the succession of microbial communities and thus has a profound effect on the entire process. The essential parameters which affect composting are, temperature, ammonia, carbon dioxide, moisture and C: N ratio of the substrate. The initial phase of composting is the most dynamic part of the process and is characterized by rapid increases in temperature, large swings in pH, and

degradation of simple organic compounds (Schloss *et al*., 2003). The mass of decomposing organic materials is an exception to most ecosystems as it results into not only intensive heat production due to the metabolic activity of microorganisms but also acts as an effective retention system resulting in a significant rise in temperature. Generally, self-heating occurs when organic materials are assembled, provided there is sufficient mass, atleast one ton, for insulation, and that moisture, aeration and nutrition level are adequate (Satyanarayana and Grajek, 1999). The composting period is governed by a number of factors including, temperature, moisture, oxygen, particle size, the carbon-to-nitrogen ratio and the degree of turning involved. Generally, effective management of these factors will accelerate the composting process and allows compost to be prepared in 2-3 weeks (Johri and Rajni, 1999).

The CO_2 evolution on the surface of compost pile shows good correlation with microbial activity compared to the number of propagules. For example, it has been shown that whereas the total number of propagules is low during peak heating, CO_2 evolution is high due to higher rate of respiration of the abundant thermophilic microflora (Johri and Rajni, 1999). Respiratory CO_2 of *Scytalidium thermophilum* was documented to be the likely reason for the growth promotory effect of this fungus on mushroom yield (Weigant, 1992).

Micro-organisms require carbon (C), nitrogen (N), phosphorus (P) and potassium (K) as the primary nutrients. Carbon (C) and nitrogen (N) compounds are the components most likely to seriously limit the composting process if present in either excessive or insufficient amounts, or when the carbon-to-nitrogen (C:N) ratio is incorrect. Microorganisms in compost digest (oxidize) carbon as an energy source, and ingest nitrogen for protein synthesis. The proportion of these two elements should approximate 30 parts carbon to 1 part nitrogen by weight. C: N ratios within the range of 25:1 to 40:1 result in an efficient process. Softwood shavings, sawdust and straw are good sources of carbon. Other inexpensive sources of carbon include municipal waste and shredded newsprint or cardboard. Most manures are a good source of nitrogen. Given a steady diet at a 30:1 ratio, microorganisms can decompose organic material quickly. When the C: N ratio is too high, there is too little nitrogen and decomposition slows. When the C: N ratio is too low, there is too much nitrogen and it will likely be lost in atmosphere in the form of ammonia or nitrous oxide, and odour can be a problem. Proper blending of carbon and nitrogen helps ensure that composting temperatures will be high enough for the process to work efficiently.

Lignin is one of the main constituents of plant cell walls, and its complex chemical structure makes it highly resistant to microbial degradation (Rawat and Johri, 2013). This nature of lignin has two implications. One is that lignin reduces the bioavailability of the other cell-wall constituents, making the actual C: N ratio (viz., ratio of biodegradable C to N) lower than the one normally cited. The other is that lignin serves as a porosity enhancer, which creates favourable conditions for aerobic composting. Therefore, while the addition of lignin-decomposing fungi may in some cases increase available C, accelerate composting and reduce N loss, in other cases it may result in a higher actual C: N ratio and poor porosity, both of which prolong composting time.

Polyphenols include hydrolysable and condensed tannins. Insoluble condensed tannins bind the cell walls and proteins and make them physically or chemically less accessible to decomposers. Soluble condensed and hydrolysable tannins react with proteins and reduce their microbial degradation and thus N release. Polyphenols and lignin are attracting more attention as inhibiting factors. Rawat and Johri (2013) suggested that the contents of these two substances be used to classify organic materials for more efficient on-farm natural resource utilization, including composting. The particle size also influences composting. The ideal particle size is around 2-3 inches. In some cases like composting of grass clippings, the raw material may be too dense to permit adequate air flow or may be too moist and therefore a bulking agent (straw, dry leaves, paper, cardboard, etc.) should be added to allow for proper ventilation. Mixing materials of different sizes and texture also helps in aerating the compost. Microbial activity occurs at the interface of particle surfaces and air. The surface area of material to be composted can be increased by breaking it into smaller pieces, or by other means. Increased surface area allows the microorganisms to digest more material, multiply faster and generate more heat. Generally, the smaller the size and more fragile the particle, the greater the biological activity and rate of composting. Chopped crop residues, softwood shavings and sawdust, for example, require no further size reduction. Materials can be chopped, shredded, split or bruised to increase their surface areas.

Temperature is one of the important parameter to monitor composting efficiency, because it affects not only the biological reaction rates and the dynamic population of microbes, but also the physicochemical characteristics of composts (Namkoong *et al*., 2002; Antizar-Ladislao *et al*., 2005). Temperature is directly proportional to the biological activity within the composting system. The process of composting involves two temperature ranges: mesophilic and thermophilic. The ideal temperature for the initial composting stage is 20-45 °C, at subsequent stages with the thermophilic organisms taking over, a temperature range of 50-70 °C may be ideal. High temperatures characterize the aerobic composting process and serve as signs of vigorous microbial activities. Pathogens are normally destroyed at 55 °C and above, while the critical point for elimination of weed seeds is 62 °C. Turnings and aeration can be used to regulate temperature. The size of the compost pile is crucial for not only temperature build up but also for

maintenance of appropriate microbial equilibrium and successional pattern. High temperature is also necessary for the chemical incorporation of nitrogen into stable form within the compost (Rawat and Johri, 2013).

The natural buffering effect of the composting process lends itself to accepting material with a wide range of pH, however, the pH level should not exceed 8.0. At higher pH levels, more ammonia gas is generated and may be lost to the atmosphere. Composting may proceed effectively over a range of pH without seriously limiting the process. The optimum pH for microorganisms involved in composting lies between 6.5 and 7.5. The pH of most animal manures is approximately 6.8 to 7.4. Composting itself leads to major changes in materials and their pH, as decomposition occurs. For example, release of organic acids may, temporarily or locally, lower the pH (increase acidity), and production of ammonia from nitrogenous compounds may raise the pH (increase alkalinity) during early stages of composting. Irrespective of the pH of the starting materials, composting always yields an end product with a stable pH usually near neutral.

Aerobic composting requires large amounts of oxygen, particularly at the initial stage. Aeration is the source of oxygen, and, thus, indispensable for aerobic composting. Rapid aerobic decomposition can only occur in the presence of sufficient oxygen. Moreover, aeration removes excessive heat, water vapour and other gases trapped in the pile. It may be achieved by controlling the physical quality of the materials (particle size and moisture content), pile size and ventilation and by ensuring adequate frequency of turning. The changes in oxygen concentration in the compost atmosphere between turnings represent net result of O_2 utilized by microorganisms and that replenished by convection and diffusion through compost. During the composting process oxygen is used up quickly by the microbes as they metabolize the organic matter. The change in O_2 concentration is related with temperature. In the center of compost pile, where the temperature is highest, anaerobic conditions exist.

Porosity refers to the space between particles in the compost pile, and is calculated by taking the volume of spaces or pores, and dividing it by the total volume of the pile. If the material is not saturated with water, these spaces are partially filled with air that can supply oxygen to decomposers and provide a path for air circulation. As the material becomes water saturated, the space available for air decreases. Compacting the compost pile reduces its porosity. Excessive shredding can also impede air circulation by creating smaller particles and pores. Turning fluffs up the material and increases its porosity. Adding coarse materials, such as straw or woodchips, can increase the pile porosity, although some coarse materials will be slow to decompose. As the compost process proceeds, the porosity decreases, restricting aeration (Rawat and Johri, 2013).

Moisture plays an essential role in the metabolism of microorganisms and indirectly in the supply of oxygen (Margesin *et al*., 2006). Microorganisms can utilize only those organic molecules that are dissolved in water. A moisture content of 40- 60% provides adequate moisture without limiting aeration. A moisture content of 50-60% is ideal for composting and microbial activity. Composting occurs more slowly if moisture content is less as it will not favour the growth of microorganisms. When the moisture content exceeds 60%, nutrients are leached, air volume is reduced, odours are produced (due to anaerobic conditions), and decomposition is slowed. If the pile becomes too wet, it should be turned and restacked. This allows air to circulate back into it and loosens the materials for better draining and air drying. Adding dry material, such as straw, sawdust or finished compost can also be a remedy to the excess moisture problem. Water content depends on the properties of the organic components within the composting mixtures (Li and Jang, 1999). In general, 50% moisture is the minimum requirement for maintaining high microbial activity (Liang *et al*., 2003). The change in moisture content affects change in temperature during composting.

DIVERSITY OF THERMOPHILIC FUNGI IN COMPOST

Composting is a highly dynamic process involving changing microbial communities that are very efficient in organic matter decomposition. Compost is a rich reservoir of mesophilic and thermophilic bacteria, fungi and actinomycetes. Bacteria are the most common of all the microorganisms found in the compost but the predominant component is fungi. Protozoa help consume bacteria and fungi, balancing out the composting cycle. The mesophilic microflora forms the pioneer community which rapidly breaks down soluble, readily degradable compounds resulting in production of heat which raises the temperature of compost and thus paves the way for thermophilic microflora at or above 45 ^{0}C; latter forms the climax community of compost. During the thermophilic phase, high temperature accelerates the breakdown of proteins, fats, and complex carbohydrates like cellulose and hemicellulose, the major structural molecules in plants. As the supply of these high-energy compounds becomes exhausted, the compost temperature gradually decreases and mesophilic microorganisms once again take over for the final phase of "curing" or maturation of the remaining organic matter. The structural divergence and species distribution is probably most significantly affected by temperature distribution. Thermophilic forms exhibit less structural divergence compared to mesophilic counterparts (Takaku *et al*., 2006).

The microbial community succession during composting is a classical example of how the growth and activity of one group of organisms can create conditions necessary for the growth of others. Microbiological parameters can serve as indicators of compost maturity (Eiland *et al*., 2001;

Benito *et al*., 2003). The study of community structure and diversity by various workers (Straatsma *et al*., 1994a; Beffa *et al*., 1996; Peters *et al*., 2000; Rawat *et al*., 2005) has been instrumental in manipulating the compost environment in order to quicken the composting process and to improve the compost quality.

a. Structural diversity

Fungi are the most predominant component of compost. *Aspergillus*, *Chaetomium*, *Humicola*, *Mucor*, *Penicillium* and *Thermomyces* are the dominant fungi of compost ecosystems. Species of *Aspergillus* and *Mucor* are predominant in composting of biowaste (Ryckeboer *et al*., 2003). *Aspergillus fumigatus* and *Humicola grisea* var. *thermoidea* have been reported to be the dominant member of the spent mushroom compost. Other fungi reported from spent mushroom compost are, *Aspergillus flavus, Aspergillus nidulans, Aspergillus terreus, Aspergillus versicolor, Chrysosporium luteum, Mucor* spp., *Nigorospora* spp., *Oidiodendron* spp., *Paecilomyces* spp., *Penicillium chromogenum, Penicillium expansum, Trichoderma viride* and *Trichurus* spp. (Kleyn and Wetzler, 1981).

Chaetomium thermophile, *Humicola* spp. and *Sporotrichum thermophile* have been reported to be abundantly present in paddy straw compost (Satyanarayana, 1978). Antagonism appears to play a significant role in determining the population structure. The volatiles of *Chaetomium thermophile* and *Sporotrichum thermophile* can inhibit conidial germination of *Humicola lanuginosa* by impairing essential metabolic processes whereas *Chaetomium thermophile* suppresses mycelial growth of *Humicola lanuginosa* and *Torula thermophila.* However, effect of the fungistatic volatile factors in compost ecosystem is only marginal in view of the high temperature at which they grow (Johri and Rajni, 1999).

Satyanarayana and Grajek (1999) observed that the colonizing ability of thermophilic fungi on paddy straw was directly proportional to the inoculum concentration. For example, colonization by *Humicola lanuginosa, Sporotrichum thermophile* and *Torula thermophila* increased with higher inoculum dose. During peak heating period, only a few thermophilic fungal propagules were present exhibiting high rate of respiration. However, Johri and Rajni (1999) reported that thermophilic fungi were not present at peak high temperature in wheat and broadbean straw composts; however, when it cooled down to 51.5 ^{0}C *Myriococcum albomyces, Penicillium dupontii* and *Sporotrichum thermophile* were found in abundance.

Thermophilic fungi grow extensively during the last phase of composting in mushroom compost from the spores that survive the pasteurization temperature (Straatsma *et al*., 1989). Thus, they contribute significantly towards the quality of compost. However, their presence throughout the course of composting is largely responsible for the maintenance of biological equillibrium that ultimately leads to unique selectivity wherein *Agaricus bisporus* multiplies without competition. These fungi influence growth of *A. bisporus* at three distinct levels (Weigant, 1992): First, they decrease concentration of ammonia in compost which otherwise would counteract the growth of the mycelium. Second, they immobilize nutrients in a form, which improves apparent availability to the mushroom mycelium. Third, they exert direct growth promotory influence on the mushroom mycelium viz., *Scytalidium thermophilum*. The course of fungal succession is partially dependent on the ecophysiological conditions in compost (Satyanarayana *et al*., 1992).

The pioneer thermophilic mycoflora of mushroom compost comprises of fast growing and rapidly sporulating fungi such as *Aspergillus fumigatus* and *Rhizomucor* spp. with a pH optima below 7.0 and temperature optima of about 40 ^{0}C. When self-heating and ammonification starts and pH reaches 9.0, the pioneer flora disappears and paves way for *Talaromyces thermophilus* and *Thermomyces lanuginosus*; during massive heat production these fungi possess moderate growth rate, as they exhibit high thermal death point and pH tolerance, but do not degrade cellulose. At the end of the composting process, about 50-70% of the compost biomass is constituted by thermophilic fungi (Sparling *et al*., 1982; Weigant, 1992). While most of the species are eliminated, *Scytalidium thermophilum* appears as near exclusive species after phase II composting and constitutes a climax species in the mushroom compost along with thermophilic actinomycetes (Straatsma *et al*., 1994b). The number of CFU of *S. thermophilum* in fresh matter of phase II is about 10^6 g^{-1} compost (Bilai, 1984), however, actinomycetes and bacteria appear to play a decisive role in successful colonization by this thermophile. The presence of *S. thermophilum* throughout the composting period i.e., from zero day, dominance during phase II and at the end of phase II is supported by its relative abundance (0.68) as observed by Rawat (2004) and the earlier observations of Straatsma *et al*. (1994a) on the subject.

In mushroom compost maximal diversity amongst thermophilic fungal morphotypes is observed in fourth turning of phase I compost (H'=2.14; E1=0.89 and D=8.88); and least in peak heat stage morphotypes (H'=0.75 and D=1.80). End of Phase I is most rich in species make up (R1=4.07 and R2=3.37). Fourth turning of phase I compost is maximally diverse for thermophilic community. The least fungal diversity is observed at peak-heat stage (Rawat, 2004). This is not unusual since only limited fungal species spectrum has been reported from this stage of composting (Straatsma *et al*., 1994a).

In the beginning of phase II of mushroom compost, thermophilic fungi and actinomycetes extensively colonize the plant matter until temperature reaches 60 ^{0}C; this is an outcome of slow peak-heating for about two days (Straatsma *et al*., 1994a). The high temperature of the first indoor period of phase II kills most of the pathogenic and

non-pathogenic microorganisms, except the spores of actinomycetes and thermophilic fungi such as *S. thermophilum* (Straatsma *et al*., 1991); the latter was most abundant at the end of phase II compost (abundance = 0.68) (Rawat, 2004). Klamer *et al*. (1998) reported *Aspergillus fumigatus* and *Rhizomucor pusillus* as predominant species before peak heating and *Paecilomyces variotii*, *Scytalidium thermophilum* and *Thermomyces lanuginosus* as dominant forms after peak heating. Tewari (2000) reported the presence of *Humicola lanuginosa* and *Scytalidium thermophilum* during peak heat stage of phase II composting.

Thermophilic fungi of the *Torula-Humicola* complex are necessary and dominant component of the community in mushroom compost during Phase II. *Scytalidium thermophilum* is a natural inhabitant of compost ingredients including drainage from compost, and has been documented to be present throughout composting. Dominance of *S. thermophilum* has been reported by several workers (Straatsma *et al*., 1991; Vijay, 1996; Klamer *et al*., 1998; Rajni *et al*., 1998) while *Humicola grisea* var. *thermoidea* and *H. insolens* have been described by others (Fergus, 1964). They are inherently close partners in the degradation processes in compost and provide selectivity to compost (Straatsma *et al*., 1989; Opden Camp *et al*., 1990). Rajni *et al*. (1998) and Rawat (2004) observed nearly similar microbial distribution pattern in compost as reported by Straatsma *et al*. (1991) with predominance of *Scytalidium thermophilum* although inputs in the European and Indian composts are substantially different. In twenty day schedule of compost preparation, *S. thermophilum* was detected from the first turning (i.e., after 5th day of composting) till fifth turning (i.e. 17th day) whereas species of *Paecilomyces* were present only during the last turning i.e., 20th day. Two isolates of *Malbranchea cinnamomea* were recorded between the second and fifth turning stage (8th to 17th day). The presence of *M. cinnamomea* and *Paecilomyces* sp., which are normally slow growers, provided an opportunity to evaluate their influence on *in situ* mycelial extension of *Agaricus bisporus*. At 24th day of composting the population of *Scytalidium thermophilum* was 10^8 propagules g^{-1} of compost.

The genetic variation exhibited by *Torula-Humicola* complex has drawn wide attention. Azevedo *et al*. (1999) distinguished homokaryotic strains of *Humicola grisea* var. *thermoidea* in two groups displaying uniform DNA profile reflecting heterogeneity in the wild genome using RAPD analysis. Straatsma and Samson (1993) studied the genetic diversity among *Scytalidium thermophilum* isolates using RAPD analysis. These strains exhibited a distinct pattern of amplified DNA bands. Rajni (1999) studied the genetic diversity between the black and white strains of *S. thermophilum* by RAPD analysis. A distinct band pattern was exhibited by these isolates. The protein profiling of *Torula-Humicola* complex exhibited that *Scytalidium thermophilum* was more closely related to *Humicola grisea* var. *thermoidea* while the latter was similar to *H. insolens* than *H. lanuginosa*; *H. insolens* and *H. lanuginosa* were more distantly related. The RAPD analysis and sequence analysis of ITS region of rDNA exhibited wide genetic variation in *Torula- Humicola* complex (Lyons *et al*., 2000). RAPD analysis of 34 geographically diverse isolates revealed two distinct groups showing differences in the banding pattern. An examination of the genetic distance matrix indicated differences between isolates belonging to *Scytalidium thermophilum* cultural types 1 and 2. The sequence analysis of ITS 1, 5.8 S and ITS 2 region of rDNA suggested high homology between the isolates with minor sequence variation. Genetic distance values, among type 1 and 2 varied by a value of 0.005%. The RAPD groupings mirror closely the morphological and thermogravimetric data for *S. thermophilum* isolates and provides further evidence of the variation, which exists between the species complex (Straatsma and Samson, 1993; Lyons and Sharma, 1998). Isolates of *S. thermophilum* bear close similarity to those of *Humicola grisea* var. *thermoidea* and *H. insolens*. RAPD analysis showed that a majority of structurally or functionally dominant fungal species recovered from different stages of composting belonged to *Torula - Humicola* complex (Rawat, 2004). During composting of sugar cane bagasse and coast-cross straw compost, prepared for the production of *Agaricus brasiliense*, the filamentous fungi exhibited much lower population densities and were less diverse than other micro-organisms, although *Aspergillus fumigatus* was present during the whole composting process and after pasteurisation (Silva *et al*., 2009).

The fungal diversity has been found to be high and phylotypes similar to yeasts were abundantly observed in the full-scale drum and tunnel processes (Hultman, 2009). In addition to phylotypes similar to *Candida*, *Geotrichum* and *Pichia*, moulds from genus *Penicillium* and *Thermomyces* were also observed in tunnel stages of composting. *Zygomycetes* were detected in the pilot-scale composting processes and in the compost piles members of Basidiomycota became abundant in the cooling and maturation phase of the compost.

Ghaly *et al.* (2012) tested the effectiveness of inoculating the compost with three thermophilic-cellulolytic microorganisms (*Thermomonospora curvata*, *T. fusca* and *Thermoascus aurantiacus*) in degradation of phenols in creosote-treated wood waste. Used cooking oil was added to the composting system as a bio-available carbon source. The temperature profiles showed that the thermophilic phase (>45 °C) was achieved and successfully maintained due to the addition of used cooking oil. The moisture content decreased because the water produced by microbial respiration did not compensate for the water vapour lost with the exhaust gases. The breakdown of organic nitrogen to ammonium caused an initial increase

in the pH which was decreased due to the formation of organic acids from the decomposition of fats and the loss of ammonia with the exhaust gases. The inoculated experiments achieved higher reductions in volatile solids, total carbon, TKN, phenols, cellulose and lignin compared to the control. Different degradation rates were observed in the psychrophilic, mesophilic and thermophilic stages of composting. The product from the inoculated experiment had improved stability and phytotoxicity compared to that of the control (uninocualted). The inoculation of thermophilic-cellulolytic microorganisms (*T. curvata*, *T. aurantiacus* and *T. fusca*) accelerated the composting process and resulted in higher degradation of phenolic compounds, lignocellulose and lignin.

b. Thermophilic fungal community succession

The genetic profiling techniques which are cultivation independent have great potential in identifying the population structure and community succession. These techniques utilize DNA or RNA extracted directly from environmental samples and amplification of signature genes by PCR or reverse transcription-PCR (RT-PCR) with primers, bind to conserved regions and produce homologous gene fragments. The products can be subsequently analyzed to determine their nucleotide differences by techniques such as, denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE), terminal restriction fragment length polymorphism (t-RFLP) and single-stranded conformation polymorphism (SSCP) (Muyzer *et al*., 1993; Lee *et al*., 1996; Liu *et al*., 1997; Schwieger and Tebbe, 1998). SSCP has the potential to be more easily applied in contrast to DGGE and TGGE, as no GC clamps or construction of gradient gel is required (Lee *et al*., 1996). However, it is quite essential to compare diversity results obtained by both cultivation-dependent and cultivation-independent methods for better understanding and for eliminating the biases associated with genetic profiling.

Kowalchuk *et al*. (1999) detected β-subgroup proteobacterial ammonia oxidizer-like sequences in commercial mushroom compost by separating the products of PCR and RT- PCR by DGGE and identifying them by hybridization with a hierarchial set of oligonucleotide probes designed to detect ammonia oxidizer-like sequence clusters. However, there are known examples of thermophilic ammonia oxidizing bacteria; cell activity is probably impaired or destroyed by high temperatures (Focht and Verstraete, 1977), thus cell survival at high temperatures may be facilitated by the formation of microniches (Derikx *et al*., 1990) and the moisture content may contribute towards cell survival (Gromov and Pavlenko, 1989).

Poulsen *et al*. (2005) observed that the genetic and functional diversity of the indigenous microbial communities in compost samples could be linked using DGGE and simultaneously measuring the activity of chitinase. Two types of composts, a garden compost and a household waste compost showed different genetic diversity as measured by PCR-DGGE of total extracted DNA; this also had different chitinase levels, viz., 0.46 and 3.97 µmole 4MU/hour x g dry matter, respectively. The addition of chitin in the composts induced a change in both the bacterial and fungal genetic diversity when compared to the non-amended compost samples. The N-mineralization in the household waste compost was apparently increased by the addition of chitin, while such an effect was not observed in the garden compost.

DGGE profiles and clone library analysis revealed that, the microbial community drastically changed during the garbage composting process from the thermophilic to the maturation stages. SSCP profiles of fungal community of different stages of mushroom compost revealed a total of 95 distinct bands (Rawat, 2004). The banding pattern of community underwent a rapid change with the onset of composting; this corroborated well with the reports of Peters *et al*. (2000). Zero day compost samples consisted of many faint bands which disappeared with the onset of composting; during peak-heat stage only prominent bands were present. Principal Component Analysis (PCA) of SSCP profiles revealed that the two samples of zero day compost (PWI and PWII) were quite similar while different stages of phase I compost shared nearly identical profile pattern; patterns of cropping sequences were quite similar to each other (Rawat, 2004). The community profiles of Phase I compost were found to be identical to each other; maximum diversity was observed during end of phase I compost, casing soil and cropping stage (H'= 0.37) and least at peak-heat stage (H'= 0.22).

Klamer *et al.* (1998) studied the succession of mycoflora during eight months of composting of *Miscanthus* straw and pig slurry in well insulated containers. Before peak heating, *Aspergillus fumigatus* and *Rhizomucor pusillus* were dominant. Forms developing after peak heating could be divided into two groups: those appearing from day 15 to 27, and others developing from day 50 to 225. The first group was dominated by, *Paecilomyces variotii, Scytalidium thermophilum* and *Thermomcyces lanuginosus,* and the second by, *Acremonuim* spp. and *Thermomcyces lanuginosus.* The Brillouin diversity index changed with temperature; diversity was high before peak heating, low during elevated temperature, and increased again during the third phase of composting. Temperature was the main controlling parameter that changed fungal community during the first month of composting. Based on PCA, it was concluded that when the temperature reached an ambient level, only minor change in fungal community was detected.

The study of physiological diversity of compost by techniques like phospholipid fatty acids analysis (PLFA) and metabolic fingerprinting has been instrumental in tracking changes in microbial communities and thus

understanding the *in situ* community structure (Garland and Mills, 1991; Petersen *et al*., 1991; Kennedy and Busacca, 1995; Inssam *et al*., 1996; Boggs *et al*., 1998; Campbell and Cooper, 1999; Cahayani *et al*., 2002). Community analysis of composting of dairy manure and pine shave beddings based on carbon source utilization as tool revealed that microbial utilization of γ-aminobutyric acid was increased over time while histidine was utilized at similar level at all sampling times. The ability to utilize sucrose, galactose and fructose increased while trehalose utilization decreased during composting (Boggs *et al*., 1998).

The fungal phylotypes, revealed by cloning and sequencing of fungal internal transcribed spacer (ITS) region of samples of different stages of composting in a full-scale and a pilot-scale composting reactors, could be grouped into those that dominated the mesophilic low pH initial phases (sequences similar to genera *Candida, Dipodascaceae* and *Pichia*) and those found mostly or exclusively in the thermophilic phase (sequences clustering to *Candida*, *Rhizomucor* and *Thermomyces*), but a few were also present throughout the whole process (Hultman *et al*., 2010).

THERMOPHILIC MYCOFLORA IN COMPOST MANAGEMENT

Composting operations are a rich source for prospection of biomass degradation enzymes. Many enzymes released by microorganisms, such as cellulases, hemicellulases, proteases, phosphatases, arlylsulphatases, and lipases, play key roles in the composting process (Johri *et al*., 1999; Rawat and Johri, 2002; Rawat *et al*., 2005). Therefore, most enzymes have thermostable characteristics. Compost harbours a number of guilds. The *in situ* functionality of each microbial component especially the extracellular enzymatic machinery viz., polysaccharases, proteases and lipases, plays an important decisive role in successful colonization and succession in mushroom compost.

The wide exploration of enzymatic machinery of the individual microbial component and their *in situ* enzymatic action in their own niche would help in greater understanding of the role played by them in compost management. However, the available information appears largely biased towards the functional role of thermophilic mycoflora of compost probably because they are a dominant component of the functional niche occupied by compost microbiota. The wide enzymatic potential exhibited by these fungi along with enzyme multiplicity with different physico-chemical characteristics helps in functioning of each component under different biophysical conditions. The increase in cellulolytic and amylolytic activity during composting is a reflection of change in the population and community structure of the resident microflora. The enzymatic diversity of compost microbiota is known to result in specificity and successional change besides providing a niche to various species to survive in the absence of simple sugars (Rawat and Johri, 2002).

In compost the pioneer microflora, in general, can utilize simple sugars but such biota disappears early and only those organisms with wide polysaccharolytic ability persist. The cellulolytic and hemicellulolytic ability of thermotolerant *Aspergillus fumigatus* allows it to persist in the wheat straw compost whereas thermophilic *Mucor pusillus* does not recur even after temperature becomes suitable for growth due to lack of polysaccharolytic ability. *Humicola lanuginosa* persists throughout composting due to its ability to lead commensal life with others along with its cellulolytic and hemicellulolytic ability. *Chaetomium thermophile, Humicola insolens, Humicola lanuginosa* and *Talaromyces dupontii* develop abundantly in the 'plateau' period and rapidly utilize cellulose and hemicellulose. When compost temperature drops, thermophilic *Sporotrichum thermophile* and mesophilic *Coprinus cinereus* and *Clitopilus pinsitus* appear which can utilize cellulose and hemicellulose in wheat straw at a slower rate (Chang and Hudson, 1967).

Thermostable enzymes and thermophilic cell factories may afford economic advantages in the production of chemicals and biomass-based fuels. Genome analyses and experimental data of two thermophilic fungi, *Myceliophthora thermophila* and *Thielavia terrestris* by Berka *et al.* (2011) suggests that both these thermophiles are capable of hydrolyzing all major polysaccharides found in biomass. Examination of transcriptome data and secreted proteins suggested that they shared approaches in the hydrolysis of cellulose and xylan but distinct mechanisms existed for pectin degradation. Characterization of the biomass-hydrolyzing activity of recombinant enzymes suggested that these two thermophiles are highly efficient in biomass decomposition at both moderate and high temperatures.

Pang *et al.* (2009) cloned and identified genes encoding three glycoside hydrolase family (GHF) 9 endoglucanases and one GHF 5 endoglucanase from metagenome of compost soil. The predicted amino acid sequence of these genes and their closest homologues in the database were less than 70% identical. The recombinant protein, Umcel9B, showed activity against carboxymethyl cellulose, indicating that Umcel9B is an endoactive enzyme.

The xylanase gene xyn10J cloned from a compost metagenomic library was predicted to encode a protein of 378 amino acid residues with a putative signal peptide of 27 amino acid residues (Jeong *et al.,* 2012). The molecular mass of the mature Xyn10J was calculated to be 39,882 Da with a pI of 6.09. Xyn10J had a motif GVKVHFTEMDI characteristic of most members of glycosyl hydrolase family 10. Site-directed mutagenesis of the expected active site based on the sequence analysis indicated that an aspartic acid residue (Asp207), in addition to the identified catalytic residues

Glu165 and Glu270, plays a crucial role for the catalytic activity. The purified Xyn10J had a mass of about 40 kDa and was optimally active at pH 7.0 and 40 °C. Xyn10J hydrolyzed beechwood xylan > birchwood xylan > oat spelt xylan > arabinoxylan. Xyn10J hydrolyzed xylotetraose and xylohexaose exclusively to xylobiose, xylopentaose, and xylotriose mainly to xylobiose with transglycosylation activity. The saccharification of reed (*Phragmites communis*) powder by commercial enzymes was significantly increased by the addition of a small amount of Xyn10J to the commercial preparation. Xyn10J is the first xylanase screened directly from a compost metagenomic library, and the enzyme has the potential to convert biomass to fermentable sugars for biofuel production.

Two cellulase-positive and five xylanase-positive clones were selected from metagenomic library constructed from compost made with pig manure and mushroom cultural waste using fosmid vector (Kwon *et al*., 2010). Cellulase of clone C1 showed maximal activity at 50 °C and pH 6.0, and retained its original activity after 30 min of heat treatment at 60 °C. Optimum temperature for xylanases of clones X1, X2, X3, and X4 was 50 °C, and that of clone X5 was 55 °C. Thermostabilities of xylanases were in the order of X4>X5>X1, X2, and X3. Optimum pH of xylanases of X1, X2 and X3 was 6.0, pH of X4 was 5.5 and that of X5 was 5.5 ~ 8.0. Xylanase positive clones could be divided into three groups, X1/X2/X3, X4, and X5, based on the influence of temperature and pH on enzyme activity. Sequence analysis of positive subclone of clone C1 suggested that cellulase and xylanase from metagenomic library were novel enzymes.

Kang *et al.* (2011) constructed a metagenomic library from compost for the screening of novel lipolytic enzymes. Clone of estCS2 was selected for lipolytic properties on a tributyrin-containing medium. The estCS2 sequence encodes a protein of 570 amino acid residues, with a predicted molecular mass of 63 kDa; based on amino acid identity it most closely matched (45%) the carboxylesterase from *Haliangium ochraceum* DSM 14365. EstCS2 belongs to family VII and it retains the catalytic triad Ser245-Glu363-His466 that is typical of an a/b hydrolase. The Ser245 residue in the catalytic triad of EstCS2 is located in the consensus active site motif GXSXG. The EstCS2 exhibits strong activity toward p-nitrophenyl caproate (C6), and it is stable up to 60°C with an optimal enzymatic activity at 55 °C. The maximal activity is observed at pH 9, and it remains active between pH 6-10. EstCS2 shows remarkable stability in up to 50% (v/v) dimethyl sulfoxide (DMSO) or dimethylformamide (DMF). The enzyme has the ability to cleave sterically hindered esters of tertiary alcohol, as well as to degrade polyurethanes, which are widely used in various industries. The high stability of EstCS2 in organic solvents and its activity towards esters of ketoprofen and tertiary alcohols, and in polyurethane suggests that it has potential uses for many applications in biotransformation and bioremediation.

Metagenomic approaches have provided access to environmental genetic diversity for biotechnology applications, enabling the discovery of new enzymes and pathways for numerous catalytic processes. Discovery of new glycoside hydrolases with improved biocatalytic properties for the efficient conversion of lignocellulosic material to biofuels is a critical challenge in the development of economically viable routes from biomass to fuels and chemicals. Twenty-two putative ORFs (open reading frames) were identified from a switchgrass-adapted compost community based on sequence homology to related gene families (Dougherty *et al*., 2012). These ORFs were expressed in *Escherichia coli* and assayed for predicted activities. Seven of the ORFs were demonstrated to encode active enzymes, encompassing five classes of hemicellulases. Four enzymes were overexpressed *in vivo*, purified to homogeneity and subjected to detailed biochemical characterization. Their pH optima ranged between 5.5 to 7.5 and they exhibited moderate thermostability up to ~60-70 °C. These may serve as the starting points for future protein engineering towards the goal of developing efficient enzyme cocktails for biomass degradation under diverse process conditions.

The success of microflora in competitive saprophytic colonization (CSC) such as that operative in plant residues and soil depends upon its intrinsic ability to decompose that substrate and the ability to succeed in the competition. The strongly cellulolytic and hemicellulolytic *Aspergillus fumigatus, Sporotrichum thermophile* and *Torula thermophila* exhibit greater colonization ability than the weakly cellulolytic, *Humicola lanuginosa* (Johri and Satyanarayana, 1984). The dominance of *Scytalidium thermophilum* has been attributed to the presence of complete complement of polysaccharolytic enzyme machinery (Rawat, 1998; Tewari, 2000; Rawat *et al.*, 2005). The hydrolytic potential of such thermophilic fungi is responsible for solubilization of complex ingredients of compost and making the nutrient availabile to *Agaricus bisporus*. The stimulation of growth of mushroom mycelium by cellulose decomposing mycoflora has been well-documented (Stanek, 1969; Straatsma *et al.*, 1994a; Johri and Rajni, 1999).

Mushroom compost that harbours high population of thermophilic flora yields more mushroom produce (Shandilya, 1982; Vijay, 1996). The selectivity of compost is brought about by the static population of thermophilic flora, which becomes inactive at the time of spawning. The thermophilic microbial biomass is a concentrated source of nutrients required for the growth of *A. bisporus*. Thermophilic fungi appear to use up all the readily available nutrients during the process of composting and thus a major portion of the available nutrients is locked up inside their cells (Betterely, 1993). *A. bisporus* possesses the complement of enzymes viz., β-N-acetyl galactosaminidase, laminarinase, protease, etc. by which it can degrade thermophilic bacteria, fungal and

actinomycete mycelium for its own growth (Fermor and Grant, 1985; Rawat *et al.*, 2005). Sparling *et al.* (1982) reported that microbial biomass contributed less than 10% to mushroom biomass and therefore *A. bisporus* probably obtained bulk of its carbon nutrition from straw. However, the microbial biomass can act as a concentrated source of nitrogen and minerals. The selectivity of compost is lost if dormant thermophilic biomass is destroyed by heat or chemicals (Ross and Harris, 1983), and growth rate of *A. bisporus* mycelium is reduced on sterilized compost (Wood and Matchman, 1980). Rawat (2004) found that dominant functional forms in mushroom compost were representatives of T3 stage.

The role of *Scytalidium thermophilum*, predominant component of compost, in compost management has been well documented by various workers (Ross and Harris, 1983; Straatsma *et al.*, 1989; Johri and Rajni, 1999; Rawat, 2004; Rawat and Johri, 2013). Among various groups actively engaged in studying the ecology of mushroom compost, Straatsma's group in Holland had laid considerable emphasis on population dynamics of *S. thermophilum* for improved compost management. The disappearance of ammonia and selectivity of compost for the growth of *Agaricus bisporus* mycelium that occurs in Phase II at temperature 45-55 ^{0}C is linked to the presence of *Scytalidium thermophilum* (Ross and Harris, 1983). The density of *S. thermophilum* was found to be positively correlated with mushroom yield (Straatsma *et al.*, 1989). The causal relationship between the presence of *S. thermophilum* and the crop yield of mushroom remains still obscure.

Straatsma *et al.* (1991) observed that this fungal species merely affects the radial extension rate rather than having a positive influence on the surface growth rate of *Agaricus bisporus* mycelium. It reduces the growth of pathogenic microorganisms by virtue of inhibitory influence. Respiratory CO_2 of this species may play a stimulatory role (Weigant *et al.*, 1992) but under different experimental conditions, neither volatiles nor CO_2 were stimulatory (Straatsma *et al.*, 1994a). The mere presence of *Scytalidium thermophilum* is, however, quite essential. Ross and Harris (1983) suggested that visible but dormant biomass of this species in compost fills an otherwise biological vacuum, which in turn allows growth of *Agaricus bisporus* mycelium. The disappearance of ammonia and selectivity of compost for the growth of *A. bisporus* mycelium that occurs in Phase II at temperature 45-55 ^{0}C are linked to the presence of *Scytalidium thermophilum* (Ross and Harris, 1983). Other thermophilic fungal species such as, *Chaetomium thermophilum*, *Malbranchea sulfurea*, *Myriococcum thermophilum*, *Stilbella thermophila*, *Thielavia terrestris* and two unidentified Basidiomycetes were also found to be promotory for mycelial growth of *Agaricus bisporus* on sterilized compost along with *Scytalidium thermophilum* (Straatsma *et al.*, 1994a).

Considering the fact that culturable microbial populations are limited on account of our poor understanding of their nutritional requirements, detailed, *in situ* enzymatic investigations are likely to provide a better understanding of the relationship between structural and functional diversity of thermophilic fungal community. Iiyama *et al.* (1996) observed that the loss of cellulose and lignocellulose and increase in protein content during the composting period was a result of increased polysaccharolytic activity of the fungal biomass resulted in increased level of reducing sugars. In mushroom compost the level of enzymes was found to increase from zero day to the end of phase I compost; thereafter it decreased continuously and the results were well corroborated with the population structure (Rawat, 2004).

It has been observed that *in situ* change in lignocellulose, cellulose and loss in weight during composting is corroborated with the activity of thermophilic fungi. The analysis of plant residues after decomposition by pure thermophilic fungal cultures resulted in biochemical changes that were similar to those observed during composting of organic materials by natural mixed microflora (Satyanarayana, 1978). During the 24 day composting sequence of button mushroom, Rajni (1999) reported that the level of organic carbon decreased from 18.12 to 10.57% while that of cellulose and lignocellulose from 32.3-23.0% and 52.4 – 43.1%, respectively. An increase of 77.5% and 86% in protein and reducing sugar level was observed. The level of amylase, endo- cellulase and exo-cellulase also changed. Based on the regression analysis between the population of *S. thermophilum* and chemical parameters, a 92.5% change in lignocellulose was found as a result of rise in population of *S. thermophilum* in compost. The levels of dehydrogenase activity decreased by 72%; protease by 32%; xylanase and cellulase by 50 % during peak heat while during phase II and post peak heat stage of mushroom compost, levels again increased by 71%, 23% and 33.3%, respectively. These changes were found to be well corroborated with the change in population structure of compost microflora (Tewari, 2000).

Yu *et al.* (2007) reported that hemicellulose and cellulose were partially degraded during initial stage of composting of agricultural wastes; thereafter, the degradation ratio was almost unaltered due to high temperature followed by large decomposition during the temperature falling phase (12-20d) and initial stage of the second fermentation (21-40d of composting). Lignin was slightly decomposed during the initial stage of composting. When the temperature was lower than the maximum value during thermophilic phase, lignin was greatly degraded until the temperature began to fall. The microbes containing Q-9 or Q-10(H2) as major quinone were found to be the most important hemicellulose and cellulose degrading microorganisms during composting while those containing Q-9(H2) as major quinone and several thermophilic

actinobacteria were believed to be responsible for lignin degradation during the composting of agricultural wastes.

Miyatake and Iwabuchi (2005) observed that the highest level of enzyme activity of thermophilic bacteria was observed at 54 ^{0}C. The highest levels of superoxide dismutase (SOD) and catalase activity in thermophilic bacteria were observed at 54 ^{0}C and decreased sharply after 60 ^{0}C. The decline in activity did not coincide with microbial extinction but with a decrease in metabolic activity. Extracellular lactate dehydrogenase (LDH) activity and the species diversity index value at 60 ^{0}C were almost the same as those at 54 ^{0}C. At 63 ^{0}C, extracellular LDH activity reached the highest level, and the species diversity index value was the lowest, indicating that bacterial diversity was reduced and certain bacteria died at 63 ^{0}C. An increase in SOD activity was observed at 70 ^{0}C without a corresponding increase in catalase activity. Dehydrogenase activity is the most suitable indicator of compost stability and maturity (Tiquia, 2005).

Stanek (1969) observed stimulation of growth of mushroom mycelium by cellulase decomposing microflora mainly, actinomycetes and fungi. It is, however, difficult to draw a conclusive relationship between restricted cellulolysis and growth promotion of *Agaricus bisporus* since species such as *Aspergillus fumigatus* and *Corynascus thermophilum* are cellulolytic but not growth promotory whereas the reverse is true for *Chaetomium thermophilum* and *Sporotrichum thermophile*. Thus, growth promotory species can be cellulolytic but not necessary pioneer colonizers of the compost biota. Such an influence is exerted by the climax species of mushroom compost, *Scytalidium thermophilum*, perhaps due to production of a complete complement of enzyme machinery (Rawat, 1998; Tewari, 2000; Rawat, 2004).

An increase in cellulase activity and decrease in laccase activity was observed after the addition of casing soil to the surface of compost colonized by *Agaricus bisporus* (Gillman *et al*., 1994). The increase in cellulolytic and amylolytic activity during composting is a reflection of change in the population and community structure of the resident microflora. The enzymatic diversity of compost microbiota is known to result in specificity and successional change besides providing a niche to various species to survive in the absence of simple sugars.

The importance of polysaccharolytic enzymes in mushroom compost has led researchers to stimulate composting by supplementing the substrate with commercial enzymes. Savoie and Libmond (1994) observed that microbial enzyme activities, number of bacteria, and solubilization of carbon and nitrogen were greater in compost treated with polysaccharidases. However, this had no positive effect on mushroom yield. Libmond *et al*. (1995) observed that supplementation of wheat straw with Express (trade name of poysaccharidase complex) reduced the time of mushroom composting besides, releasing low quantities of readily available sugars, increased enzyme activities and number of microrganisms, particularly aerobic bacterial population in the substrate.

Some workers have exploited the enzymatic potential of thermophilic fungi for preinoculation of compost with them. Salar and Aneja (2007) observed the growth of *A. bisporus* on sterile compost pre-colonized with four thermophilic fungi *viz*., *Chaetomium thermophile, Malbranchea sulfurea, Thermomyces lanuginosus* and *Torula thermophila*, either singly or in different combinations. A mixed inoculum of *Malbranchea sulfurea* and *Torula thermophila* was found to be the best amongst the various treatments that promoted growth of *Agaricus bisporus*. The effect of *Thermomyces lanuginosus* when inoculated singly or in combination with other thermophilic fungus/ fungi in compost was insignificant resulting in lower growth rates. This study revealed that thermophilic fungi provide for compost selectivity and protection against negative effects of compost bacteria on mycelial growth of *Agaricus bisporus.* Improved growth of *A. bisporus* mycelium in compost treated with *Scytalidium thermophilum* has been extensively reported in literature (Ross and Harris, 1983; Weigant, 1992; Straatsma and Samson, 1993; Straatsma *et al*., 1994 a; b; Rawat, 2004; Salar and Aneja, 2007). Straatsma *et al*. (1994a) reported that nine thermophilic fungi viz., *Chaetomium thermophilum*, an unidentified *Chaetomium* sp., *Malbranchea sulfurea*, *Myriococcum thermophilum*, *S. thermophilum*, *Stilbella thermophila*, *Thielavia terrestris*, and two unidentified basidiomycetes, promoted mycelial growth of *Agaricus bisporus* on sterilized compost.

The wide enzymatic potential exhibited by thermophilic fungi with different physico-chemical characteristics helps in the fuctioning of each component under different biophysical conditions. They also exhibit enzyme multiplicity which helps them function efficiently under different eco-physiological conditions. Multiplicity of hemicellulolytic enzymes has been widely reported in *Chaetomium thermophile* var. *coprophile*, *Humicola grisea* var. *thermoidea*, *Melanocarpus albomyces*, *Talaromyces emersonii*, *Thermoascus aurantiacus* and *Scytalidium thermophilum* (Thakur *et al*., 1992; Tuohy *et al*., 1993; Johri and Rajni, 1999; Rawat, 2004; Rawat *et al*., 2005).

CONCLUSIONS

Compost is a unique man-made ecosystem which harbours a complete spectrum of microbial diversity. The microbial abundance, composition and activity change substantially during the composting process and this is correlated with high microbial diversity and low activity in matured compost. It is a complex ecosystem which harbours a number of guilds. Thus it necessitates the study of structural diversity complemented with functional diversity to have a fair picture of microbial diversity of the

ecosystem. The recent metagenomic approaches have given us new insights into this ecosystem. The survival strategies and competitive behaviour of thermophilic mycoflora in this interesting niche requires to be understood. The biopotentiality of thermophilic fungi of compost ecosystem can be exploited in industrial applications beside hastening the composting process and improving the quality of compost.

ACKNOWLEDGEMENTS

Thermophilic fungal research of BNJ cited in this chapter has been supported by INSA, DST, ICAR and CSIR whereas more recent molecular microbial ecology component has been supported by the Department of Biotechnology and Ministry of Environment and Forests. Efforts of a series of doctoral and postdoctoral research students especially Dr. T. Satyanarayana is greatfully acknowledged since these early researches laid the foundation of nearly three decades of "Thermophilic saga".

REFERENCES

Antizar-Ladislao, B., Lopez-Real, J., and Beck, A.J. 2005. Laboratory studies of the remediation of polycyclic aromatic hydrocarbon contaminated soil by in-vessel composting. *Waste Mgmt.* **25**: 281–289.

Azevedo, M.O., Felipe, M.S.S. and Satyanarayana, T. 1999. Molecular and general genetics. In: *Thermophilic moulds in biotechnology* (Eds.: Johri, B.N., Satyanarayana, T. and Olsen, J.). Kluwer Academic Publishers, Netherlands, 265-288.

Beffa, T., Blanc, M. and Aragno, M. 1996. Obligately and facultatively autotrophic, sulfur and hydrogen-oxidizing thermophilic bacteria isolated from hot composts. *Arch. Microbiol.* **165**: 34-40.

Benito, M., Masaguer, A., Moliner, A., Arrigo, N. and Palma, R.M. 2003. Chemical and microbiological parameters for the characterisation of the stability and maturity of pruning waste compost. *Biol. Fertil. Soils.* **37**: 184–189.

Berka, R.M., Grigoriey, I.V., Otillar, R., Grimwood, J., Reid, I., Ishmael, N., John, T., Darmond, C., Moisan, M.C., Henrissat, B., Coutinho, P.M., Lombard, V., Natyig, D.O., Lindquist, E., Schmutz, J., Lucas, S., harris, P., Powlowski, J., Bellemare, A., Taylor, D., Butler, G., de Vries, R.P., Allijn, I.E. and van den Brink, J. 2011. Comparative genomic analysis of the thermophilic biomass-degrading fungi *Myceliophthora thermophila* and *Thielavia terrestris. Nature Biotech.* **29:** 922–927.

Betterley, D.A. 1993. Supplements, composting, mushroom nutrition and future direction. *Mushroom News.* **41**: 8-12.

Bilai, V.T. 1984. Thermophilic micromycete species from mushroom composts. *Mikrobiol. Zh. (Kiev).* **46**: 35-38.

Boggs, L.C., Kennedy, A.C. and Reganold, J.P. 1998. Use of phospholipids fatty acids and carbon source utilization patterns to track microbial community succession in developing compost. *Appl. Environ. Microbiol.* **64**: 4062-4064.

Cahayani, V.R., Watanabe, A., Matsuya, K., Asakawa, S. and Kimura, M. 2002. Succession of microbiota estimated by phospholipids fatty acid analysis and changes in organic constituents during the composting process of rice straw. *Soil Sci. Plant Nut.* **48**: 735-743.

Campbell, C.D. and Cooper, J.N. 1999. Community succession and decomposition of microbial biomass during the composting of pot ale liquor. In: *Microbial process during composting. Proc. 8th Int. Symp. on Microbial. Ecol.* (Eds.: Bell, C.R., Brylinsky, M. and Johnson, P.G.). Halifax, Canada.

Chang, Y. and Hudson, H.J. 1967. The fungi of wheat straw compost. I. Ecological studies. *Trans. Br. Mycol. Soc.* **50**: 649-666.

Cowan, D., Meyer, Q., Stafford, W., Muyanga, S., Cameron, R. and Wittwer, P. 2005. Metagenomic gene discovery: past, present and future. *Trends Biotechnol.* **23**:321-329.

Derikx, P.J.L., OpDen Camp, H.J.M., Vander Drift, C., van Griensnen, L.J.L.D. and Vogels, G.D. 1990. Biomass and biological activity during the production of compost used as a substrate in mushroom cultivation. *Appl. Environ. Microbiol.* **56**: 3029-3034.

Dougherty, M.J., D'haeseleer, P., Hazen, T.C., Simmons, B.A., Adams, P.D. and Hadi, M.Z. 2012. Glycoside hydrolases from a targeted compost metagenome, activity-screening and functional characterization. *BMC Biotechnol.* **12**: 38-43.

Eiland, F., Klamer, M., Lind, A., Leth, M. and Baath, E. 2001. Influence of initial C/N ratio on chemical and microbial composition during long term composting of straw. *Microb. Ecol.* **41**: 272–280.

Eklind, Y., Salomonsson, L., Wivstad, M. and Ramert, B. 1998. Use of herbage compost as horticultural substrate and source of plant nutrients. *Biol. Agric. Hortic.* **16**: 269–290.

Fergus, C.L. 1964. Thermophilic and thermotolerant molds and actinomycetes of mushroom compost during peak heating. *Mycol.* **56**: 267-284.

Fermor, T.R. and Grant, W.D. 1985. Degradation of fungal and actinomycetes mycelia by *Agaricus bisporus*. *J. Gen. Microbiol.* **131**: 1729-1734.

Focht, D.D. and Verstraete, W. 1977. Biochemical ecology of nitrification and denitrification. In: *Advances in Microbial Ecology* (Ed.: Alexander, M.). Plenuan Press, New York, 135-214.

Garland, J.L. and Mills, A.L. 1991. Classification and characterization of heterotrophic microbial communities on the basis of community-level sole carbon source utilization. *Appl. Environ. Microbiol.* **57**: 2351-2354.

Ghaly, A.E., Dave, D. and Zhang, B. 2012. Augmenting composting microbial community with thermophilic cellulolytic organisms for enhanced degradation of phenolic compounds in creosote treated wood waste. *J. Bioremed. Biodegrad.* **3**: 3-6.

Gillman, L., Lebeault, J.M. and Cochet, N. 1994. Influence of casings on the microflora of compost colonized by *Agaricus bisporus. Acta Biotechnol.* **14**: 275-282.

Gromov, B.V. and Pavlenko, G.V. 1989. Autoecology of bacteria: water activity. In: *Bacterial Ecology*. Leningrad University Press, Leningrad, USSR, 78pp.

Herrmann, R.F. and Shann, J.F. 1997. Microbial community changes during composting of municipal solid waste. *Microb. Ecol.* **33**: 78-85.

Hiraishi, A., Narihiro, T. and Yamanaka, Y. 2003. Microbial community dynamics during start-up operation of flowerpot-using fed-batch reactors for composting of household biowaste. *Environ. Microbiol.* **5**: 765–776.

Hoitink, H.A.J. and Boehm, M.J. 1999. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. *Ann. Rev. Phytopathol.* **37**: 427–446.

Hultman, J. 2009. *Microbial diversity in the municipal composting process and development of detection methods*. Ph.D. Thesis, University of Helsinki.

Hultman, J., Vasara, T., Partanen, P., Kurola, J., Kontro, M., Paulin, L., Auvinen, P. and Romantschuk, M. 2010. Determination of fungal succession during municipal solid waste composting using a cloning-based analysis. *J. Appl. Microbiol.* **108**: 472–487.

Iiyama, K., Stone, B.A. and Macauley, B.J. 1996. Changes in the concentration of soluble anions in compost during composting and mushroom growth. *J. Food Sci. Agric.* **72**: 243-249.

Inssam, H., Amor, K., Renner, M. and Crepaz, C. 1996. Changes in functional abilities of the microbial community during composting of manure. *Microb. Ecol.* **37**: 77-87.

Jeong, Y.S., Na, H.B., Kim, S.K., Kim, Y.H., Kwon, E.J., Kim, J., Yun, H.D., Lee, J.K. and Kim, H. 2012. Characterization of xyn10J, a novl family 10 xylanase from a compost metagenomic library. *Appl. Biochem. Biotechnol.* **166**:1328-39.

Johri, B.N. and Rajni. 1999. Mushroom compost: Microbiology and applications. In: *Modern Approaches and Innovations in Soil Management* (Eds.: Bagyaraj, D.J., Verma, A., Khanna, K.K. and Kheri, H.K.). Rastogi Publ., Meerut, India, 345-358.

Johri, B.N. and Satyanarayana, T. 1984. Ecology of thermophilic fungi. In: *Progress in Microbial Ecology* (Eds.: Mukherji, K.G., Agnihotri, V.P. and Singh, R.P.). Print House, Lucknow, 349-361.

Johri, B.N., Satyanarayana, T. and Olsen, J. 1999. Thermophilic moulds in biotechnology. Kluwer Academic Publishers, Netherlands, 354pp.

Kang, C.H., Oh, K.H., Lee, M.H., Oh, T.K., Kim, B.H. and Yoon, J.H. 2011. A novel family VII esterase with industrial potential from compost metagenomic library. *Microbial Cell Factories*. **10**: 41-45.

Kennedy, A.C. and Busacca, A.J. 1995. Microbial analysis to identify source of PM-10 material. In: *Proc. Air and Waste Manag. Assoc*., Pittsburgh, 670-675.

Klamer, M. and Baath, E. 1998. Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. *FEMS Microbiol. Ecol.* **27**: 9-20.

Klamer, M., Sochting, U. and Szmidt, R.A.K. 1998. Fungi in a controlled compost system with special emphasis on the thermophilic fungi. In: *Proc. Int. Symp. on composting and use of Composted materials for horticulture*, Ayr, U.K., 5-11 Apr. 1997, No. 469, 405-412.

Kleyn, J.G. and Wetzler, T.F. 1981. The microbiology of spent mushroom compost and its dust. *Can. J. Microbiol.* **27**: 748-753.

Kowalchuk, G.A., Naovmenko, Z.S., Derikx, P.J.L., Felske, A., Stephen, J.R. and Arkhipchenko, I.A. 1999. Molecular analysis of ammonia-oxidizing bacteria of the β-subdivision of the class proteobacteria in compost and composted materials. *Appl. Environ. Microbiol.* **65**: 396-403.

Kwon, E.J., Jeong, Y.S., Kim, Y.H., Kim, S.K., Na, H.B., Kim, J., Yun, H.D. and Kim, H. 2010. Construction of a metagenomic library from compost and screening of cellulase- and xylanase-positive clones. *J. Korean Soc. Appl. Biol. Chem.* **53:** 702-708.

Lee, D.H., Zo, Y.G. and Kim, S.J. 1996. Non-radioactive method to study genetic profiles of natural bacterial communities by PCR-single stranded conformation polymorphisms. *Appl. Environ. Microbiol.* **62**: 3112-3120.

Li, G. S., and Jang, F. S. 1999. The solid waste composting and the organic compound fertilizer is produced. Chemical industry publishing Co., China. 76pp.

Liang, C., Das, K.C., and McClendon, R.W. 2003. The influence of temperature and moisture content regimes on the aerobic microbial activity of a biosolids composting blend. *Bioresour. Technol.* **86**: 131–137.

Libmond, S., Savoie, J.M. and Elliott, T.J. 1995. Stimulation of mushroom composting by a polysaccharidase complex induction of cellulase production in *Bacillus subtilis*. In: *Proc. 14th Int. Cong. Sci Cult. Edible fungi,* UK, 195-202.

Liu, W.T., March, T.L., Cheng, H. and Forney, L.J. 1997. Characterization of microbial diversity by determining terminal restriction fragment polymorphisms of genes

encoding 16S rRNA. *Appl. Environ. Microbiol.* **63**: 4516-4522.

Lorenz, P., Liebeton, K., Niehaus, F. and Eck, J. 2002. Screening for novel enzymes for biocatalytic processes: accessing the metagenome as a resource of novel functional sequence space. *Curr. Opin. Biotechnol.* **13**: 572-577.

Lyons, G.A. and Sharma, H.S.S. 1998. Differentiation of *Scytalidium thermophilum* isolates by thermogravimetric analysis of their biomass. *Mycol. Res.* **102**: 843-849.

Lyons, G.A., McKay, G.J. and Sharma, S.H. 2000. Molecular comparison of *Scytalidium thermophilum* isolates using RAPD and ITS nucleotide sequence analysis. *Mycol. Res.* **104**: 1431-1438.

Mantel, E.F.K., Agarwala, R.K. and Seth, P.K. 1972. *A guide to mushroom cultivation*. Ministry of Agriculture. Farm Information Unit, Directorate of Extension, New Delhi, Farm Bull, No. 2.

Margesin, R., Cimadom, J., and Schinner, F. 2006. Biological activity during composting of sewage sludge at low temperatures. *Int. Biodeterior. Biodegrad.* **57**: 88–92.

Mathur, S.P., Owen, G., Dinel, H. and Schnitzer, M. 1993. Determination of compost biomaturity. *Biol. Agric. Hortic.* **10**: 65–85.

Miller, F.C. 1994. Conventional composting system. In: *Agaricus compost* (Ed.: Nair, N.G.). Australian mushroom growers association, Windsor, Australia, 1-18.

Miyatake, F. and Iwabuchi, K. 2005. Effect of high compost temperature on enzymatic activity and species diversity of culturable bacteria in cattle manure compost. *Bioresource Technol.* **96**: 1821-1825.

Muyzer, G., deWaal, E.C. and Uitterlinden, A.G. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction- amplified genes coding for 16S rRNA. *Appl. Environ. Microbiol.* **59**: 695-700.

Namkoong, W., Hwang, E.Y., Park, J. S., and Choi, J.Y. 2002. Bioremediation of diesel contaminated soil with composting. *Environ. Pollut.* **119**: 23–31.

Odlare, M. 2005. *Organic residues – a resource for arable soils*. Ph.D. Thesis, Swedish University of Agricultural Sciences, Uppsala. 51pp.

Opden Camp, H.J.M., Stumm, C.K., Straatsma, G., Derikx, PJ.L. and van Griensven, L.J.L.D. 1990. Hyphal and mycelial interactions between *Agaricus bisporus* and *Scytalidium thermophilum* on agar medium. *Microb. Ecol.* **19**: 303-309.

Pang, H., Zhang, P., Duan, C.J., Mo, X.C., Tang, J.L. and Feng, J.X. 2009. Identification of cellulase genes from the metagenomes of compost soils and functional characterization of one novel endoglucanase. *Curr. Microbiol.* **58**: 404-408.

Peters, S., Koschinsky, S., Schwieger, F. and Tebbe, C.C. 2000. Succession of microbial communities during hot composting as detected by PCR-Single Strand-Conformation Polymorphism-based genetic profiles of small subunit rRNA gene. *Appl. Environ. Microbiol.* **66**: 930-936.

Petersen, S.O., Henriksen, K., Blackburn, T.H. and King, G.M. 1991. A comparison of phospholipid and chloroform fumigation analyses for biomass in soil: Potentials and limitations. *FEMS Microbiol. Ecol.* **85**: 257-268.

Poulsen, P.H.B., Møller, J. and Magid, J. 2005. Linking microbial genetic- and functional diversity in compost: DGGE-profiling separated different types of compost regarding chitinase activity. http://orgprints.org/5856.

Purohit, H.J., Kapley, A. and Raje, D.V. 2005. Dynamics of microbial community: an environmental perspective. In: *Microbial Diversity: Current Perspectives and Potential Applications* (Eds.: Satyanarayana, T. and Johri, B.N.). I.K. International Pvt. Ltd., New Delhi, 559-572.

Rajni, Rastogi, S., Johri, B.N. and Singh, R.P. 1998. Microbial dynamics and its influence in cultivation cycle of *Agaricus bisporus*. *Mushroom Res.* **7**: 63-70.

Rajni. 1999. *Molecular ecology of Scytalidium thermophilum.* Ph.D. Thesis, G.B. Pant Univ. of Agril. and Technol., Pantnagar. 88pp.

Rawat, S. 1998. *Production and characterization of amylases from Scytalidium thermophilum*. M.Sc. Thesis, G.B. Pant Univ. of Agril. and Technol. Pantnagar. 86pp.

Rawat, S. 2004. *Microbial diversity of mushroom compost and xylanase of Scytalidium thermophilum*. Ph.D. Thesis, G.B. Pant Univ. of Agril. & Technology, Pantnagar. 199pp.

Rawat, S., Agarwal, P.K., Chaudhary, D.K. and Johri, B.N. 2005. Microbial diversity and community dynamics of compost ecosystem. In: *Microbial Diversity: Current perspectives and applications* (Eds.: Satyanarayana, T. and Johri, B.N.). I.K. International Pvt. Ltd., New Delhi, 181-206.

Rawat, S. and Johri, B.N. 2002. Ecology and structural diversity of thermophilic fungi. In: *Frontiers of fungal diversity in India* (Eds.: Rao, G.P., Manoharachari, C., Bhat, D.J., Rajak, R.C. and Lakhanpal, T.N.). Int. Book Distributing Co., Lucknow, 205-232.

Rawat, S. and Johri, B.N. 2013. Role of thermophilic microflora in composting. In: *Thermophilic microbes in environmental and industrial biotechnology: Biotechnology of thermophiles* (Eds.: Satyanarayana,

T., Jennifer, L. and Yutaka, K.). Springer Science & Business Media, Dordrecht, 137-169.

Ross, R.C. and Harris, P.J. 1983. An investigation into the selective nature of mushroom compost preparation. *Scientia Horti*. **20**: 61-70.

Ryckeboer, J., Megaert, J., Coosemans, J., Deprins, K. and Swings J. 2003. Microbiological aspects of biowaste during composting in a monitored compost bin. *J. Appl. Microbio*l. **94**: 127-137.

Salar, R.K. and Aneja, K.R. 2007. Significance of thermophilic fungi in mushroom compost preparation: effect on growth and yield of *Agaricus bisporus* (Lange) Sing. *J. Agril. Technol.* **3**: 241-253.

Satyanarayana, T. 1978. *Thermophilic microorganism and their role in composting process*. Ph.D. Thesis. Sagar University, Sagar. 212 pp.

Satyanarayana, T. and Grajek, W. 1999. Composting and solid state fermentation. In: *Thermophilic moulds in biotechnology* (Eds.: Johri, B.N., Satyanarayana, T. and Olsen, J.). Kluwer Academic Publishers, Netherlands, 265-288.

Satyanarayana, T., Johri, B.N. and Klein, H. 1992. Biotechnological potential of thermophilic fungi. In: *Handbook of Applied Mycology* (Eds.: Arora, D.K., Flander, R.P. and Mukherjee, K.G.). Marcel Dekker Inc., New York, 729-761.

Savoie, J.M. and Libmond, S. 1994. Stimulation of environmentally controlled mushroom composting by polysaccharides. *World J. Microbiol. Biotechnol.* **10**: 313-311.

Schloss, P. D., Hay, A. G., Wilson, D. B. and Walker, L. P. 2003. Tracking temporal changes of bacterial community fingerprints during the initial stages of composting. *FEMS Microbiol. Ecol*. **46**: 1–9.

Schwieger, F. and Tebbe, C.C. 1998. A new approach to utilize PCR-single strand conformation polymorphisms for 16S rRNA gene-based microbial community analysis. *Appl. Enviorn. Microbiol.* **64**: 4870-4876.

Shandilya, T.R. 1982. Composting and casing research at mushroom research centre, during past seven years. *Ind. J. Mushroom.* **8**: 5-13.

Sinden, J.W. and Hauser, E. 1950. The short method of composting during fermentation. *Mushroom Sci.* **1**: 52-59.

Silva, C.F., Azevedo, R.S., Braga, C., Silva, Rda, Dias, E.S. and Freitas, S. 2009. Microbial diversity in a bagasse-based compost prepared for the production of *Agaricus brasiliensis*. *Brazil J. Microbiol.* **40**. doi: 10.1590/S1517-83822009000300023.

Schmeisser, C., Steele, H. and Streit, W.R. 2007. Metagenomics, biotechnology with non-culturable microbes. *Appl. Microbiol. Biotechnol.* **75**: 955-962.

Sparling, G.P., Fermor, T.R. and Wood, D.A. 1982. Measurement of the microbial biomass in composted wheat straw and the possible contribution of the biomass to the nutrition of *Agaricus bisporus*. *Soil. Biol. Biochem.* **14**: 609-611.

Stanek, M. 1969. Diewitkung der zelulose setzenden mikroorganismen auf das waehstun des champignons. *Mushroom Sci.* **7**: 161-172.

Steger, K., Jarvis, A., Vasara, T., Romantschuk, M. and Sundh, I. 2007. Effects of differing temperature management on development of Actinobacteria populations during composting. *Res. Microbiol.* **158**: 617-624.

Straatsma, G., Gerrits, J.P.G., Augustijn, M.P.A.M., OpDenCamp, H.J.M., Vogels, G.D. and Van Griensven, L.J.L.D. 1989. Population dynamics of *Scytalidium thermophilum* in mushroom compost and stimulatory effects on growth and yield of *Agaricus bisporus*. *J. Gen. Microbiol.* **135**: 751-789.

Straatsma, G., Gerrits, J.P.G., Gerrits, T.M., Op Dencamp, H.J.M. and van Griensven, L.J.L.D. 1991. Growth kinetics of *Agaricus bisporus* mycelium on solid substrate (mushroom compost). *J. Gen. Microbiol.* **137**: 1471-1477.

Straatsma, G., Olijnsma, T.W., Gerrits, J.P.G., Amsing, J.G.M., OpDen Camp, H.J.M. and Van Griensven, L.J.L.D. 1994b . Inoculation of *Scytalidium thermophilum* in button mushroom compost and its effect on yield. *Appl. Environ. Microbiol.* **60**: 3049-3054.

Straatsma, G. and Samson, R.A. 1993. Taxonomy of *Scytalidium thermophilum,* an important thermophilic fungus in mushroom compost. *Mycol. Res.* **97**: 321-328.

Straatsma, G., Samson, R.A., Olijnsma, T.W., Opden Camp, H.J.M., Gerrits, J.P.G., Griensven, L.J.L.D. Van and van Griensven, L.J.L.D. 1994a. Ecology of thermophilic fungi in mushroom compost with emphasis on *Scytalidium thermophilum* and growth stimulation of *Agaricus bisporus* mycelium. *Appl. Environ. Microbiol.* **60**: 454-458.

Takaku, H., Kodaira, S., Kimoto, A., Nashimoto, M. and Takagi, M. 2006. Microbial communities in the garbage composting with rice hull as an amendment revealed by culture-dependent and -independent approaches. *J. Biosci. Bioengg*. **101**: 42-50.

Tewari, P. 2000. *Thermophilic microorganisms from mushroom compost and their polysaccharolytic activities*. M.Sc. Thesis, G.B. Pant Univ. of Agril. and Technol., Pantnagar. 99pp.

Thakur, I. S., Rana, B. K. and Johri, B. N. 1992. Multiplicity of xylanase in *Humicola grisea* var. *thermoidea.* In: *Xylan and Xylanases* (Eds.: Visser, J., Beldman, M.A., van Someren, K. and Voragen, A.G.J.). Elsevier, Amsterdam, 511-514.

Tiquia, S.M. 2005. Microbiological parameters as indicators of compost maturity. *J. Appl. Microbiol.* **99**: 816-828.

Tiquia, S.M., Wan, J.H.C. and Tam, N.F.Y. 2002. Microbial population dynamics and enzyme activities during composting. *Compost Sci. Util.* **10**: 150–161.

Tognetti, C., Laos, F., Mazzarino, M.J., Hernandez, M.T. 2005. Composting vs.vermicomposting: a comparison of end product quality. *Compost Sci. Util.* **13**: 6-13.

Tuohy, M.G., Puls, J., Claeyssens, M., Vrsanska, M. and Coughlan, M.P. 1993. The xylan degrading enzyme system of *Talaromyces emersonii* novel enzymes with activity against β-D- xylosides and unsubstituted xylans. *Biochem. J.* **290**: 515-523.

Vijay, B. 1996. *Investigations on compost mycoflora and crop improvement in Agaricus bisporus (Lange) Imbach*. Ph. D. Thesis, H.P. Univ., Shimla.

Vivas, A., Moreno, B., Garcia-Rodriguez, S. and Benitez, E. 2009. Assessing the impact of composting and vermicomposting on bacterial community size and structure, and microbial functional diversity of an olive-mill waste. *Bioresour. Technol.* **100**: 1319-1326.

Weigant, W.M. 1992. Growth characteristics of thermophilic fungus *Scytalidium thermophilum* in relation to production of mushroom compost. *Appl. Environ. Microbiol.* **58**: 1301-1307.

Weigant, W.M., Wery, J., Buitenhuis, E.T. and de Bont, J.A.M. 1992. Growth-promoting effect of thermophilic fungi on the mycelium of the edible mushroom of *Agaricus bisporus*. *Appl. Environ. Microbiol.*, **58**: 2654-2659.

Wood, D.A. and Matchman, S.E. 1980. Growth of *Agaricus bisporus* in composted straw. Evidence for microbial interaction. In: *Second Int. Symp. on Microbial Ecol.*, Warwick. 233pp.

Yu, H., Zeng, G., Huang, H., Xi, X., Wang, R., Huang, D., Huang, G. and Li, J. 2007. Microbial community succession and lignocellulose degradation during agricultural waste composting. *Biodegrad.* **18**: 793-802.