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Research Article

MICROBIAL ECOLOGY OF COMPOST ECOSYSTEM: WITH SPECIAL REFERENCE TO MUSHROOM COMPOST

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ABSTRACT

Among various man-made ecosystems, compost is an interesting example which presents a complete spectrum that harbor multitude of microbial diversity since it is a result of degradation of agro-residues. The microbial abundance, composition and activity change substantially during the composting process. The succession of microbial communities 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. Several generations of microorganisms succeed each other during composting where microbial flora utilizes the available material in the substrate as also the cellular components of its predecessors for growth. Community structure and diversity are instrumental in manipulating compost environment in order to increase compost process and to improve compost quality.

Keywords: Mushroom compost, *Agaricus bisporus*, Microbial ecology, functional diversity, structural diversity, Microbial Community

INTRODUCTION

Since ancient times, mushrooms have been regarded as the 'food of the God'! Mushrooms have been a food supplement in various cultures and they are cultivated and eaten for their edibility and delicacy. They are considered as source of proteins, vitamins, fats, carbohydrates, amino acids and minerals[1]. Mushroom is about 16.5 % dry matter out of which 7.4 % is crude fibre, 14.6 % is crude protein and 4.48 % is fat and oil[2]. Protein contents vary between 4-9 % in *Auricularia* spp. and between 24-44 % in *Agaricus* species. Among the various edible mushrooms, *Agaricus bisporus*, commonly known as button mushroom has wide acceptability world over and constitutes approx. 90 % of commercial production. Cultivation of *A. bisporus* requires a nutritionally rich substrate which is a produced through composting of agricultural wastes like wheat straw, paddy straw and sugarcane bagasse, supplemented with additives such as horse manure, poultry manure, gypsum and small amounts of other ingredients like maize cob, brewer grain, seed meal, cotton hulls, urea and low cost agricultural products[3]. Besides fulfilling the nutritional requirement of mushroom crop, the composted substrate provides suitable physical conditions for anchorage of mushroom and maintenance of proper aeration and water holding capacity[4]. The production of compost for the growth of the edible mushroom *Agaricus bisporus* consists of an uncontrolled outdoor composting process, which takes about 10 days, followed by an indoor, temperature-controlled process[5]. Horse manure, straw, chicken manure, gypsum and water are thoroughly mixed and put on static pile for 5 days in windrow. In this indoor phase, first a temperature of 56 to 58°C is maintained for 12 to 20 h and then a temperature of 46 to 48°C is maintained for 4 to 5 days. The first indoor period, which is generally referred to as pasteurization is used to kill microorganisms that are pathogenic to mushrooms. The second indoor period is believed to promote the selectivity of compost[6].

Composting

The biological decomposition and stabilization of organic substrates under conditions, which allow development of thermophilic temperatures as a result of biologically produced heat, with a final product sufficiently stable for storage and application to land without adverse environmental effects, is termed composting[7].

The Composting Process

Composting has frequently been regarded as more an art than science. Composting process depends upon the feed stocks and processing conditions[8] (Table 1). The biochemistry and microbiology of composting remains poorly understood to date. Despite extensive research over the past twenty years into engineering aspects and the benifits of using composts, composting is still essentially considered a 'black box' process. This stems, in part, from the inherent complexity of the composting process, which is heterogeneous in nature and is directly influenced by factors such as feedstock composition and structure, temperature, pH, moisture, oxygen and ammonia concentrations[9]. In many cases indirect methods, such as calorimetric analyses for example, have been used to measure microbial metabolic activity[10].

Table 1: Key Stages of the Composting Process

Stage	Key features	Stage Characteristics	Approximate duration
High rate composting	Micro-organisms consume forms of carbon they can easily break down, e.g. sugars and starches	High rate of biological activity characterised by high oxygen demand and of heat generation rates tendency for pH to initially drop below the optimum of 6—8, then rise above 8 as composting proceeds	4—40 days depending upon system type
Stabilization	Micro-organisms consume forms of carbon they can break down fairly readily, e.g. cellulose	Biological activity starts to decline. Oxygen demand gradually decreases. Declining heat generation Tendency for pH to remain above 8	20—60 days depending on system type
Maturation (Curing)	Amount of available Carbon is much reduced and microbial consumption slowed down. Re-colonization by soil microbes	Reduced biological activity. Medium to low oxygen demand Little heat generation; temperature should be below 50 °C Oxidisation of ammonium to nitrate ions Tendency for pH to fall towards neutral	Variable duration depending upon test method used and intended end use

Source: Gilbert *et al*., 2001

Composting relies upon the inter-related activities of a diverse range of micro-organisms to convert organic waste substrates into a stabilized material ('compost'), which is high in humic substances ('humus') and contains useful plant nutrients. In most feedstock, the principal source of carbon and energy is derived from lignocelluloses[11]. Cellulase activities in composting materials have been widely studied and correlated to decreases in cellulose content[12]. The degradation of recalcitrant lignin in composting systems has been less well characterized, although thermophilic micro fungi, and to a lesser extent actinomycetes, are thought to play key roles[13]. Humification (the process of forming humus) is complex and thought to involve a number of degradative and condensation reactions involving lignins, carbohydrates and nitrogenous compounds[14]. Nuclear magnetic resonance spectroscopy, gas chromatography, mass spectrometry and Fourier transform infrared spectroscopy have all been used to track changes in feedstock composition and the formation of humic substances[15]. The composting process can be split into three key stages based on changes in temperature:

- Phase 1 is characterized by an increase in temperature from ambient as a result of microbial metabolic activity and has been termed the 'high rate' composting phase. During this phase simple carbohydrates and proteins are readily degraded, firstly by mesophiles, which are then succeeded by thermotolerant and thermophilic species as the temperature rises above 45°C.
- Phase 2 has been termed the 'stabilization' phase and is characterized by the attainment of thermophilic temperatures (50°C), which selects for thermophilic bacteria[16]. However, this may be an over simplistic assumption, with the survival of isolates typically characterized as mesophiles[17]. The thermophilic stage plays a key role in the thermal destruction of pathogenic micro-organisms; weed seeds and propagules, although antagonism such as competition and the formation of secondary metabolites may be significant[18].

The thermophilic composting phase has received the greatest attention to date, especially composts produced for the cultivation of mushrooms on a commercial scale[19]. Thermophilic actinomycetes[20], *Bacillus* species[16] and *Thermus* species[21] have all been shown to dominate, whilst thermo tolerant fungi from the genera *Aspergillus* and *Penicillium* have been widely reported. However, species diversity is thought to decrease at high temperatures, whilst Gram-positive bacteria have been shown to predominate[22].

- Phase 3 is the 'maturation' phase and is typically characterized by a reduction in temperature towards ambient as a result of decrease in metabolic activity following oxidation of readily biodegradable substrates. Mesophilic actinomycetes and fungi begin to predominate during this stage, and are thought to be responsible for degradation and conversion of lignins, which occurs optimally at these lower temperatures[13].

Mushroom Composting

Mushroom composting represents an interesting example of thermogenic, solid state fermentation process that results from succession of microbial community. These micro organisms convert and degrade ingredients into suitable substrate for cultivation of mushroom[23]. The composted substrates reach some level of biological stability by using readily available substrate, achieve physical uniformity and acceptable bulk density, convert nitrogen into organic form available to the mushroom crop and, transform nutrients into suitable form for mushroom growth and nutrition[24]. Various coworkers described "short method" of preparing compost for the cultivation of mushroom where they defined two phases in composting process[25,26]. Phase I is outdoor fermentation process during which raw materials are mixed, wetted and stacked with considerable dry matter losses: phase II is an indoor process of pasteurization and conditioning treatment to produce a selective and pathogen free-substrate[27]. During phase I, fungal and bacterial activity produces large quantities of heat. Temperature ranges between ambient and 80°C in distinct zones within the cross section of the compost stacks and ammonia disappeared most rapidly in the range, 40 to 45°C. Temperature above 50°C however prolonged the disappearance of NH_3. At this higher temperature and also at 40°C and below, non selective compost was produced.

Microbial Ecology of Mushroom Compost Ecosystem

Mushroom compost is an interesting example of a complete spectrum of microbial diversity. It is a rich reservoir of microbial types, comprising of mesophilic and thermophilic bacteria, fungi and actionomycates. The mesophilic microflora from the pioneer community while thermophiles represent the climax community. Microbial biodiversity of compost is important because it takes part in breakdown of organic material. The fast growing *Pseudomonads* and *Arthrobacter* constitute the pioneer flora[28] that rapidly degrades high concentration of organic matter. *Bacilli* have been reported to be the dominant bacteria of not only mushroom compost[29] but also of other compost ecosystems[16].

Microbial community succession during composting is a classical example of how the growth and activity of one group of organisms create the condition necessary for the growth of others. Several generations of micro-organisms succeed each other during composting wherein each crop of microbial form utilizes the available material in the substrate as also the cellular component of its predecessors for growth, spread and sustenance. The microbial abundance, composition and activity changes substantially during composting and compost maturity could be correlated with high microbial diversity and low activity[30]. The study of community structure and diversity has been instrumental in manipulating the compost environment in order to quicken the composting process and to improve compost quality[31]. Composting is a complex microbial process, since the temperature of the pile is initially low, wherein mesophilic flora starts to develop and degrades the straw. The community succession pattern of mushroom compost changes along with the temperature gradient of two phases of composting. In phase I, the outer cool region of the stack harbors mesophilic micro flora while in the center, where temperature can reach up to 70°C or higher, anaerobic bacteria may occur. The intervening zone with temperature range of 50 to 65^0C favors growth of aerobic thermophilic bacteria, fungi and actinomycetes[4]. *Agaricus bisporus* (white button mushroom) grows on compost, a product of aerobic fermentation by various microorganisms. These microorganisms convert and degrade straw to lignin humus complex, which is later utilized by the mushroom mycelium and ultimately contributes toward the nutrition for *A. bisporus*[23]. Thermophilic fungi play crucial role in determining selectivity of compost growth of *A. bisporus*. Amongst these, *Scytalidium thermophilium*, *Humicola grisea* and *Humicola insolense* have been isolated as predominant thermophiles in various composts prepared by long and short methods using variety of agro-waste substrates[32,33].

Functional Diversity

Mushroom compost is not only structurally diverse but also functionally active. *In situ* functionality of each microbial component especially the extracellular enzymatic machinery viz., polysaccharases, proteases and lipase play an important decisive role in successful colonization and succession in mushroom compost[34]. The composting process represents combined activity of a wide succession of environments, as one enzyme/microbial group overlaps the other and each emerged gradually due to continual change in temperature and progressive breakdown of complex compounds to simpler ones[35]. Composting represents biological decomposition of organic matter by microorganisms[21]. During composting, the starting material is transformed through a variety of biological and biochemical processes in which enzymes play a key role[36,37]. Mineralization of organic nitrogen is mediated by enzymes such as amidohydrolases and dehydrogenases[38]. Microorganisms and their composition and magnitude are important components of composting process. In the initial heating phase, bacteria utilize simple, easily degradable organic substances in compost[16]. Bacteria may also attack more complex materials and / or may exploit substances released from less degradable substances due to extracellular enzymatic activity of other organisms[39]. Fungi also play an important role in the initial rise in compost temperature[40]. Most fungi were eliminated by high temperature but were commonly recovered at moderate temperatures; these remaining substances are predominantly cellulose or lignin**.** Actinomycetes grow during later stage of composting and have been shown to attack polymers such as hemicellulose, lignin and cellulose. The population of these three microbial groups was positively correlated with the number of enzymes[41]. Growth of mushroom mycelium stimulated by cellulase decomposing micro flora mainly of actinomycetes and fungi[42], A conclusive relationship between restricted cellulolysis and growth promotion of *Agaricus bisporus* could not be established since species such as *Aspergillus fumigatus* and *Corynascus thermophilum* are cellulolytic but not growth promoting whereas the reverse is true for *Chaetomium thermophilum* and *Sporotrichum thermophile*. Thus, growth promotory species can be cellulolytic but not necessarily pioneers colonizers of the compost biota. Such an influence is exerted by the climax species of mushroom compost, *Scytalidium thermophilum*, perhaps due to production of complete complement of enzymatic machinery[29]. 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*[43]. The increase in cellulolytic and amylolytic activity during composting is a reflection of change in the population and community structure of the resident micro flora. The enzymatic diversity of compost micro biota is known to result in specificity and succession change besides providing a niche to various species to survive in the absence of simple sugar. Considering the fact that culturable microbial population 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. Increase in protein content and loss of cellulose and lignocellulose during the composting period was a result of increased polysaccharolytic activity of the fungal biomass; this resulted in increased level of reducing sugars[44]. The level of enzyme was found to increase from zero days to the end of Phase I compost[45]; subsequently it decreased continuously but the data was well corroborated with population structure.

Structural Diversity

The community succession pattern of mushroom compost changes along with the temperature gradient of composting[4]. mesophilic micro flora have a significantly higher population count of than that of thermophilic microflora[45]. Amongst thermophiles, population recovered at 50°C was higher than that recovered at 65°C; no fungus was recovered at 65°C. In mushroom compost, bacteria comprise of much lower proportion of total biomass although it is difficult to define the relative distribution of dominating species[4]. However, it has been reported a biomass ratio of 1.0:1.8 of bacteria to fungi after Phase II[46] while the ratio was 1.0: 0.9 in conventional compost and 1.0: to 2.3 in the experimental compost[6]. *Bacillus* represents a dominant component[3]. Mesophilic bacteria show much greater morphological variation than thermophilic forms[45]. The overall effect of structural divergence results in maximum diversity and abundance among the mesophilic bacterial isolates recovered from first turning of Phase I compost (H`= 1.33, N1 = 3.69). Maximum species richness ($R_1$ = 3.56 and $R_2$ = 2.39) was observed at zero day, and least diversity and species was exhibited by isolates recovered from third turning of Phase I (H`= 0.18; R_1 = 2.16 and R_2 = 1.88). Amongst thermophilic

bacterial morphotypes recovered at 50°C, maximum diversity was found among the morphotype of the third turning of Phase I (H`= 0.083); least among those of peak heat stage of Phase II. However, thermophilic bacterial morphotypes isolated at 65°C were least diverse structurally[45]. Presence of large number of highly thermophilic, heterotrophic bacteria growing at temperature above 70°C, i.e., during thermogenic stage of compost process has been observed[21]. These bacteria relate to the genus *Thermus*, isolated from thermogenic composts at temperature 65°C and 82°C. Nutritional analysis, total protein profile by gel electrophoresis, DNA-DNA hybridization and restriction fragment length polymorphism profiles of 16S rDNA showed that *Thermus* strains isolated from hot region of compost were closely related to *T. thermophilus.* Continuously stirred anaerobic thermophilic batch digester, inoculated with cattle manure were studied on laboratory scale[47]. Bacterial and archaeal community as well as digester perfomancer, were analyzed during reactor start-up for 20 days. PCR-SSCP and 16S rDNA analysis of microbial community revealed that microbial diversity of thermophilic ecosystem is lower than that of mesophilic one and major bacterial and archaeal species were close to thermophilic species. Thus, the dominant species taking part in the thermophilic bioconversion of cattle manure to methane and carbon-dioxide were not adapted to mesophilic microorganisms but thermophilic microorganism present in cattle manure at a subdominant level and which quickly became dominant under thermophilic anaerobic conditions. Nature and population size of microorganism in compost heap for successful composting depends on a number of factors viz., raw material being composted, its nutrient composition, moisture content, temperature, acidity or alkalinity and aeration[48]. While large heaps lead to generation of high temperature, small heap generate low temperature. *Aspergillus* spp., *Rhizopus oryzae, Trichoderma viride, Chaetomium* spp., *Penicillium* spp. and *Alternaria* spp. were observed as dominant mycoflora in the initial stage of composting. On 6th day, *Aspergillus* spp., *Penicillium* spp., *Fusarium* spp., *A. alternata, Mycothecium* spp and *Cephalosporium* spp. were observed. *Acremonium* spp. and *Paecilomyces variotii* were the additional mesophilic fungi in the later period of composting[49]. *Cladosporium* spp. and *Humicola fuscoatra, Penicliium* spp. and some yeast were frequently isolated during Phase II composting[50]. Thermophilic fungi grow extensively during the last phase of composting from the spores that survive in pasteurization temperature[32]. Thus thermophilic mycoflora forms the climax community and contributes significantly towards the quality of compost. However, their presence throughout the course of composting is largely responsible for maintenance of biological equilibrium that provides selectivity to compost for successful colonization by *A. bisporus*[32]. These fungi influence growth of *A. bisporus* at three distinct levels[6]. First, they decrease concentration of ammonia in compost which otherwise counteracts 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 influence on the mushroom mycelium viz., *S. thermophilum.* The spent mushroom compost is a material that still harbours large microbial diversity. A total bacterial count of 7.01 $\log_{10}$ CFUg^{-1} has been reported. Two novel species within the genera *Microbacterium* and *Stenotrophomonas* were reported along with *Bacillus licheniformis*, *B. subtilis, Enterobacter* spp., *Klebsiella* spp., *Microbacterium* spp., *Paenibacillus lentimorbus, Pseudomonas mevalonii, Sphingobacterium multivorum* and *Stenotrophomonas* spp.[51]. *B. licheniformis* is the most common bacterial isolate of spent mushroom compost[52]. Actinomycetes constitute an important microbial component of composting process and contribute extensively by their ability to produce antibiotics and enzymes and the ability to degrade complex and recalcitrant molecules especially lignocellulosics. Predominant forms in zero day mushroom compost are represented by the genera, *Saccharopolyspora* and *Streptomyces.* Species of *Streptomyces* make up the pioneer microflora of mushroom compost. Thermophilic actinomycetes grow extensively during Phase II of mushroom compost as evident from white wefts of mycelium (‘fire fang’) in compost[52]. Actinomycete population in composts is often dominated by species of *Thermomonospora*[53]. The population count of mesophilic actinomycetes[46] varies from 5.49 to 5.48 log $_{10}$ CFU (zero days to drench) while that of thermophiles from 3.67 to 4.52. The community succession of actinomycetes in mushroom compost was also investigated by[31]. *In situ* change in lignocellulose and loss in weight during composting has been reported to corroborate with the activity of thermophilic fungi. During the twenty four day composting sequence of button mushroom reported a decrease in the level of organic carbon from 18.12 to 10.57 %, and that of cellulose and lignocellulose from 32.3 % to 23.0 % and 52.4 % to 43.1 %, respectively[54]. The wide enzymatic potential exhibited by thermophilic fungi with different physico-chemical characteristics helps in the functioning of each component under different biophysical condition. Thermophilic fungi also exhibit enzyme multiplicity which helps them function efficiently under different eco-physiological conditions. Multiplicity of hemicellulolytic enzyme has been widely reported in *Chaetomium thermophilum var. coprophile, Humicola grisea var. thermoidea, Melanocarpus albomyces, Talaromyces emersonii, Thermoascus aurantiacus* and *Scytalidium thermophilum*[45].

Genetic Diversity

The genetic diversity among the isolates recovered from mushroom compost has not been widely studied. The genetic divergence among *Bacillus* related bacteria and strains of *Thermus thermophilus* has however been studied based on restriction fragment length polymorphisms (RFLP) profiles[21]. Phylogenetic diversity of thermohilic actinomycetes and *Thermoactinomyces* spp. from mushroom compost employing 16S rRNA gene sequencing has reported[55]. Operational Taxonomic Units based on endonuclease restriction profiles of cloned 16S ribosomal DNA recombinants isolated from hot composts were characterized[56] and measured changes in population diversity in young and old composts. Similarly, various coworkers demonstrated changes in microbial communities at deferent stages of the composting process using PCR amplification of small-subunit ribosomal RNA genes[31]. To understand diversity and community composition of the compost micro flora, different approaches are now followed by taxonomists, in order to characterize and identify isolates up to species level. The genomic era has resulted in development of new molecular tools and techniques for study of culturable microbial diversity including the DNA base ratio (mole % G + C)[57], DNA-DNA hybridization[58], DNA microarray[59], reverse sample genome probing[59], 16S rDNA sequencing[51]

and amplified rDNA restriction analysis. In addition, non-culturable diversity which makes a larger proportion of the existing population, can be characterized employing tools such as denaturing gradient gel electrophoresis (DGGE), temperature gradient gel electrophoresis (TGGE)[60] and single stranded conformational polymorphisms (SSCP)[31]. Metabolic fingerprinting by BIOLOG tests[61] and fatty acid analysis[62] are other most widely accepted tools to group and identify the bacterial diversity.

CONCLUSION

Microbial communities have great potential for temporal or spatial change, and represent a powerful tool for understanding community dynamics in ecological context. Variation in microbial community structure can influence ecosystem process. This study defines the complexity of microbial community dynamics and how it affected ecosystem process. Application of phenotypic and genotypic component of bacterial diversity, coupled to utilization of diversity indices has permitted deeper insights at subtle changes that result in biological conditioning of compost to permit mushrooming as also to suggest where artificial inocula could be used to hasten the composting process and associated mushroom yield. Besides the above, other tools have been used to characterize the whole microbial community of mushroom compost ecosystem to gain microbial diversity and function. Although methods to study diversity (numerical, taxonomic, and structural) are improving for both bacteria and fungi, there is still not a clear association between diversity and function. It is generally thought that a diverse population of organisms will be more resident to stress and more capable of adapting to environment changes.

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