Chapter 29
Thermophilic Fungi in Composts: Their Role in Composting and Industrial Processes

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29.1 Introduction

Composting is a natural biological process, carried out under controlled aerobic conditions (requires oxygen) (Tiquia and Tam 1998a, 2002; Tiquia et al. 2000; Zhang et al. 2016; Wei et al. 2018). In this process, the organic matter is transformed into a more stable organic matter, with a final product sufficiently stable for storage and use in agriculture as fertilizer, in gardening, or in landscaping (Richard and Tiquia 1999; Tiquia et al. 2002a; Krause et al. 2003; Pampuro et al. 2017). A typical aerobic composting is a self-heating process in which microbial metabolism drives the temperature above 50 °C, followed by sustained high temperatures between 60 and 80 °C, and then followed by gradual cooling of the compost pile (Tiquia et al. 1996, 1997a; Tiquia 2005a, b; Kumar 2011). The high temperature (50–80 °C) oxidizes phytotoxins and destroys animal and plant pathogens (Senesi 1989; Tam and Tiquia 1994; Tiquia 2000, 2010a; Tiquia and Tam 1998b; Tiquia et al. 1998a). The composting process represented a combined activity of a wide succession of environments, as one enzyme/microbial group overlapped the other and each emerged gradually due to the continual change in temperature and progressive breakdown of complex compounds to simpler ones (Tiquia et al. 1997b, 2001; Tiquia 2002a; Yu et al. 2018). Composting has been suggested as a potential strategy to eliminate antibiotic residues (Gou et al. 2018; Liu et al. 2018a).

Microbes play a key role as degraders during the composting process; the mesophilic microorganisms constitute the pioneer microflora, while thermophilic microorganisms are the dominant microflora that contribute significantly to the quality of compost (Tiquia et al. 1998c; Tiquia 2003, 2005b; Liu et al. 2018b). These mesophilic and thermophilic microbial consortia have distinct physiological requirements

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S. M. Tiquia-Arashiro, M. Grube (eds.), *Fungi in Extreme Environments: Ecological Role and Biotechnological Significance*,
https://doi.org/10.1007/978-3-030-19030-9_29

and tolerances, consistent with the continuously changing environment throughout composting (Tiquia et al. 2002b; Tiquia 2005a; Federici et al. 2011; Jurado et al. 2014; Waqas et al. 2018). Bacteria including those that belong to the groups *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* are by far the most important decomposers during the most active stages of composting (Partanen et al. 2010; Neher et al. 2013; Zhang et al. 2016), partly because of their availability to grow rapidly on soluble proteins, and other readily available substrates, and partly because they are the most tolerant of high temperatures (Kuok et al. 2012). Most fungi are eliminated above 50 °C; only a few have been recovered that can grow at all up to 62 °C (Tiquia 2005a; Langarica-Fuentes et al. 2014), which suggests that their degradative activities during the thermophilic stages of composting are minor compared to those of bacteria (Martins et al. 2013; Langarica-Fuentes et al. 2014). As peak heats are attained in composts, fungi tend to disappear from the central zone of the compost. In grass and straw composts where a peak heat of 70 °C was recorded, the thermophilic fungi disappear from the compost core for a period of 3 days (Chang and Hudson 1967). As compost temperatures fall below 60 °C, thermophilic fungi reappear in the middle of the compost (Chang and Hudson 1967). While current understanding tells us that bacteria are the dominant degraders in thermophilic composting processes, there is much to be said about the minority of thermophilic fungi during the composting process.

Composting is a promising source of new organisms and thermostable enzymes (Dougherty et al. 2012; D'Haeseleer et al. 2013; Nguyen et al. 2013; Tiquia-Arashiro 2014; Habbeche et al. 2014; Pomaranski and Tiquia-Arashiro 2016) that may be helpful in environmental management and industrial processes (Tiquia and Mormile 2010; Tiquia-Arashiro and Mormile 2013; Salar 2018). Fungi are known to have an important role in the composting process as degraders of recalcitrant materials such as cellulose and lignin and thermophilic fungi have been suggested as the main contributors to lignocellulose degradation. Despite the relevance of fungi in composting, especially the thermophilic fraction, most of the research on the diversity, composition, and succession of these microorganisms had been conducted several decades ago using classical culture-based methods (Chang and Hudson 1967; Kane and Mullins 1973; Klamer and Sochting 1998).

This chapter covers the diversity of thermophilic fungi during composting, their role, and potential applications in biotechnology. Readers may find that the available information on several aspects of compost ecosystem is scanty which is due to horizontal advancements in some areas and because compost represents a complex ecological system from the viewpoint of microbial distribution and activity.

29.2 Fungal Communities in Composts

Culture-independent methods, including denaturing gradient gel electrophoresis (DGGE) of PCR-amplified DNA fragments, terminal restriction fragment length polymorphism analysis (T-RFLP), clone library analysis, and more recently

high-throughput sequencing, have been used extensively to investigate microbial successions in composts (Ishii and Takii 2003; Tiquia 2005a, 2010b; Tiquia and Michel Jr. 2002; Tiquia et al. 2005; Szekely et al. 2009; De Gannes et al. 2013). However, few investigations have focused on fungal populations of large-scale composting processes using molecular techniques. Bonito et al. (2010) used DGGE to study windrow-type systems; Hultman et al. (2009) and Hansgate et al. (2005) utilized a clone library approach to study fungi in a rotating drum system and a reactor-type system, respectively; and Langarica-Fuentes et al. (2014) took advantage of high-throughput sequencing to monitor fungal succession in an in-vessel composting system. Gu et al. (2017) used the Dirichlet multinomial mixtures mode to analyze Illumina sequencing data to reveal both temporal and spatial variations of the fungi community present in the aerobic composting.

Bonito et al. (2010) identified fungi microflora associated with composting organic municipal wastes to gain a better understanding of the diversity of fungi at different stages of composting. A disproportionate number of yeast sequences have been detected from day-0 clone libraries, including the human pathogens *Candida tropicalis* and *Candida krusei* (*Saccharomycetales*). *Basidiomycetes* account for over half of the clones from the day-210 compost sample while *Cercophora* and *Neurospora* species account for most of the fungal clones from day-410 sample. Surprisingly, no *Zygomycetes* or *Aspergillus* species were detected.

Hansgate et al. (2005) employed F-ARISA (fungal-automated rRNA intergenic spacer analysis) and 18S rRNA gene cloning and sequencing to examine changes in fungal community structure during composting. Sequencing of the 18S rRNA portion of cloned F-ARISA products revealed the presence of four distinct fungal genera including *Backusella* sp., *Mucoraceae*, *Geotrichum* sp., and the yeast *Pichia* sp. Clone libraries constructed using fungus-specific 18S rRNA primers contained sequences similar to several other fungal genera including *Penicillium* sp., *Aspergillus* sp., *Hamigera* sp., *Neurospora* sp., and the yeast *Candida* sp.

Langarica-Fuentes et al. (2014) characterized the fungal community composition at different stages of in-vessel composting process. A complex succession of fungi is revealed, with 251 fungal OTUs identified throughout the monitoring period. The *Ascomycota* are the dominant phylum (82.5% of all sequences recovered), followed by the *Basidiomycota* (10.4%) and the subphylum *Mucoromycotina* (4.9%). In the early stages of the composting process, yeast species from the order *Saccharomycetales* are abundant, while in later stages and in the high-temperature regions of the pile, fungi from the orders *Eurotiales*, *Sordariales*, *Mucorales*, *Agaricales*, and *Microascales* are the most prominent. This study presents an in-depth view on the succession of fungi during the composting of municipal solid waste and provides a guide to those species that drive an in-vessel composting process towards a satisfactory product. Similar communities are likely to be observed in other composting plants where municipal solid waste is processed; however, differences in the process nature, length of composting, and conditions achieved (temperature, pH, water content, etc.) are likely to determine the exact succession and communities present.

Gu et al. (2017) characterized fungal diversity in the aerobic composting with Illumina sequencing. A total of 670 operational taxonomic units (OTUs) were detected, and the dominant phylum was *Ascomycota*. There were four types of samples of fungi communities during the composting process. Samples from the early composting stage (type I) were dominated by *Saccharomycetales* sp. Fungi in the medium composting stage (types II and III) were dominated by *Sordariales* spp. and *Acremonium alcalophilum*, *Saccharomycetales* sp., and *Scedosporium minutisporum*. Samples from the late composting stage (IV) were dominated by *Scedosporium minutisporum*. The results of their study indicate that time and depth influence fungal distribution and variation in the waste during static aerobic composting.

29.3 Thermophilic Fungi in Composts

Cooney and Emerson (1964) define thermophilic fungi as fungi with a maximum growth temperature of 50 °C or higher and a minimum growth temperature of 20 °C or higher. Thermotolerant species have a maximum growth temperature of about 50 °C and a minimum well below 20 °C (Cooney and Emerson 1964; Awasthi et al. 2014). Crisan (1973), however, defines thermophilic fungi as fungi with a temperature optimum of 40 °C or higher. Most thermophiles are isolated from composts (Tansey and Brock 1978; Awasthi et al. 2014; López-González et al. 2015; Sebők et al. 2016; Ahirwar et al. 2017; Wang et al. 2018); their prevalence in composts can be explained by the high temperatures, humidity, and aerobic conditions within the composts. Moreover, the supply of carbohydrates and nitrogen in composts favors the development of thermophilic microflora (Cooney and Emerson 1964). During the composting process, various organic materials are converted into simpler units of organic carbon and nitrogen (Tiquia 2002a, b, 2003; Tam and Tiquia 1999; Tiquia and Tam 2000; Tiquia et al. 1998b, 2002c). The overall efficiency of organic material degradation depends on the microbes and their activities (Tiquia et al. 2002b, c). Thermophilic fungi promote the degradation of organic materials by secreting various types of cellulolytic and xylanolytic enzymes. These fungi might have enzymes that maintain their activities at high temperatures. *Aspergillus*, *Chaetomium*, *Humicola*, *Mucor*, *Penicillium*, and *Thermomyces* spp. 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 members of the spent mushroom compost. Other fungi reported from spent mushroom compost are *Aspergillus flavus*, *Aspergillus nidulans*, *Aspergillus terreus*, *Aspergillus versicolor*, *Chrysosporium luteum*, *Malbranchea cinnamomea* NFCCI 3724, *Melanocarpus albomyces*, *Mucor* spp., *Myceliophthora thermophila, Nigrospora* spp., *Oidiodendron* spp., *Paecilomyces* spp., *Penicillium chrysogenum*, *Penicillium expansum*, *Trichoderma viride*, and *Trichuris* spp. (Kleyn and Wetzler 1981; Ahirwar et al. 2017; Kertesz and Thai 2018).

Several known thermophilic fungi have been found in mushroom composting. Mushroom composting represents an interesting example of thermogenic solid-state fermentation process that results from succession of microbial communities. The composting process consists of two phases. Phase I is an outdoor fermentation process during which the raw materials are mixed, wetted, and stacked with considerable dry mass losses. Phase II is an indoor process of pasteurization to produce a selective and pathogen-free substrate (Noble and Gaze 1994). During phase I, fungal and bacterial activities produce large quantities of heat. Temperature ranges between ambient and 80 °C in distinct zones within the cross sections of the compost stack and ammonia disappear most rapidly in the range of 40–45 °C. 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 actinomycetes. In phase I, the pioneer thermophilic mycoflora of mushroom compost comprises fast-growing and rapidly sporulating fungi such as *Aspergillus fumigatus* and *Rhizomucor* spp. with a pH optimum below 7.0 and temperature optima of about 40 °C. When self-heating and ammonification start 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 1991). While most of the species are eliminated, *Sporotrichum 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. 1994). 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. In the beginning of phase II of mushroom composting, thermophilic fungi and actinomycetes extensively colonize the plant matter until temperature reaches 60 °C, as an outcome of slow peak heating for about 2 days (Straatsma et al. 1994). The high temperature of the first indoor period of phase II kills most of the pathogenic and nonpathogenic microorganisms, except the spores of actinomycetes and thermophilic fungi such as *Scytalidium thermophilum* (Straatsma et al. 1991). Klamer et al. (1998) reported *A. fumigatus* and *Rhizomucor pusillus* as predominant species before peak heating and *P. variotii*, *S. thermophilum*, and *T. lanuginosus* as dominant forms after peak heating. Tewari (2000) reported the presence of *H. lanuginosa* and *S. thermophilum* during peak-heat stage of phase II composting. *S. 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 1999), while *H. 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 (1999) and Rawat (2004)

observed nearly similar microbial distribution patterns in compost as reported by Straatsma et al. (1991), with predominance of *S. thermophilum* (Kertesz and Thai 2018). In mushroom compost, thermophilic fungi are responsible for the degradation of lignocellulose, which is a prerequisite for the growth of the edible fungus (Sharma 1989; Kertesz and Thai 2018). 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.

29.3.1 Thermophilic Fungi in Straw Compost

Thermophilic fungi of wheat straw compost were studied in detail by Chang and Hudson (1967). Initial high population of mesophilic fungi results in peak heating in the central region of the pile wherein temperature rises rapidly and reaches a plateau around 50 °C. Thermophilic fungi rapidly develop replacing the mesophilic population and persist until the compost cools down. In wheat and broad bean straw composts, thermophilic fungi are not present at peak high temperature. However, when the composts cool down to 51.5 °C, *Penicillium dupontii*, *Myriococcum albomyces*, *Thermomyces lanugionsus*, and *Sporotrichum thermophile* are found in abundance (Moubasher et al. 1982; Zhang et al. 2015). Several critical factors reported to influence the colonization by thermophilic fungi include (1) existence of suitably high temperature to promote germination and growth arid multiplication of propagules; (2) ability of thermophilic fungi to break down complex carbon substances; and (3) absence of repressive activity among the compost-inhabiting organisms. In a complex of microbial interactions such as above, succession of individual species is governed by their traditional requirements and availability of suitable temperature and pH conditions. For example, due to simple nutritional requirements thermophilic mucoraceous members appear early in the composting process. *Humicola lanuginosa* develops early but exists throughout the composting process as it lives as a commensal with other thermophilic organisms (Hedger and Hudson 1974; Salar 2018). Besides, this organism can tolerate a wide range of temperatures on either side of optima and elaborates a variety of hydrolytic enzymes that help in continuous presence.

29.3.2 Thermophilic Fungi in Municipal Waste Composts

Municipal wastes generally contain, among other things, substrates rich in lignohemicellulose. Thermophilic fungi play a significant role in the conversion of these materials. Some species are unique in their ability to degrade plastic substances and hence special interest has been envisioned in their study from municipal waste compost. Thermophilic fungi isolated from municipal waste composts include

Thermomucor (Subrahmanyam et al. 1977; Singh et al. 2016), *Thermoascus aurantiacus* (Cooney and Emerson 1964; Sebők et al. 2016), and *Myceliophthora thermophila* (Sen et al. 1980; Sebők et al. 2016).

29.3.3 Thermophilic Fungi in Paddy Straw Composts

Paddy straw is an excellent substrate for the colonization of thermophilic fungi. In an extensive controlled study of this substrate, Satyanarayana and Johri (1984) observed that 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* (*Scytalidium thermophilum*) increased with higher inoculum concentration. *Aspergillus fumigatus* showed a strong competitive ability both in pure and mixed cultures. Decomposing ability of these organisms varied with C:N ratio and the length of paddy straw pieces. During peak heating period, only a few thermophilic fungal propagules were present but these exhibited high rate of respiration as suggested by the evolution of carbon dioxide.

29.4 Industrial Applications

The biotechnological applications of thermophilic fungi are numerous. Pure culture studies of thermophilic fungi have provided clear evidence that they possess a variety of extracellular enzymes capable of hydrolyzing polymers such as starch, protein, pectin, hemicellulose, cellulose, and lignin. They have also been reported to produce, among others, many antibacterial and antifungal substances, extracellular phenolic compounds, and organic acids. Some thermophilic fungi have already been used in industries involving food processing, bioconversion of organic materials, biodegradation of plastics, biosorption of metals/radionuclides, cancer treatment, and synthesis of nanoparticles (Bengtsson et al. 1995; Zafar et al. 2013; Aydi Ben Abdallah et al. 2015; Tiquia-Arashiro and Rodrigues 2016a; Salar 2018).

29.4.1 Production of Thermostable Enzymes

Thermostable enzymes have become the focus of biotechnological interest because they are more tolerant to the conditions in industrial processes and storage. The production of thermostable enzymes has grown through advances in isolating many thermophilic microorganisms. The advantage of the use of thermostable enzymes is the possibility of conducting biotechnological processes at elevated temperatures and thus reducing the risk of contamination by mesophilic microorganisms,

decreasing the viscosity of the reaction medium, increasing the bioavailability and solubility of organic compounds, and increasing the diffusion coefficient of substrates and products resulting in higher reaction rates (Kumar and Nussinov 2001).

Cellulose is one of the main components of plant cell wall material and is the most abundant and renewable nonfossil carbon source on earth. Degradation of cellulose to its constituent monosaccharides has attracted considerable attention to produce food and biofuels. Cellulose can be hydrolyzed to glucose and other soluble sugars by using cellulase enzymes of bacteria and fungi (Plecha et al. 2013). Thermophilic cellulases are key enzymes for efficient biomass degradation. Their importance stems from the fact that cellulose swells at higher temperatures, thereby becoming easier to break down. In industrial processes, cellulolytic enzymes have been employed in the extraction of pigments and flavor compounds in fruit juice and wine production; as additive of detergents for washing jeans; in the pretreatment of biomass to improve the nutritional quality of forage for animal feed; in the textile industry in the polishing process of cotton fibers; and for saccharification of lignocellulosic residues to obtain reducing sugar (Ando et al. 2002; Baffi et al. 2013). The interest in the use of cellulases to produce fermentable sugars from cellulosic wastes at present is focusing on biofuel production such as biogas, bioethanol, biodiesel, and fuel cells. The use of whole biomass to obtain alcohol-based fuels requires an efficient conversion of lignocellulosic material into fermentable pentose and hexose sugars. Thermal stability of several commercial cellulase preparations is an important parameter for the success of the process. Thus, the industries have been developing cellulases with higher thermal stability and especially stable at industrially relevant conditions. Many thermophilic fungi from composts (*Myriococcum thermophilum*, *Sporotrichum thermophile*, *Thermoascus aurantiacus*, and *Thermomyces lanuginosus*) have been isolated in recent years and the cellulases produced by these eukaryotic microorganisms have been purified and characterized at both structural and functional level (Lee et al. 2014; de Cassia Pereira et al. 2015; Mehta et al. 2016; Jain et al. 2017).

Several studies have reported the production of thermostable xylanase from thermophilic and hyperthermophilic organisms, prokaryotes, and eukaryotes. Among thermophilic compost fungi, *Mycothermus thermophilus* (Lee et al. 2014; Ma et al. 2017), *Talaromyces thermophilus* (Maalej et al. 2009), *Thermomyces lanuginosus* (Jiang et al. 2005; Lee at al. 2014), *Thermoascus aurantiacus* (Lee at al. 2014), and *Rhizomucor miehei* (Zhou et al. 2014) produce thermostable xylanases with action from 50 °C up to 80 °C. A large variety of xylanases produced by these thermophilic fungi have become a major group of industrial enzymes that are capable of degrading xylan to renewable fuels and chemicals, in addition to their use in food, paper, and pulp industries.

Pectinases are a group of enzymes that catalyze the degradation of pectic substances by depolymerization reaction and by de-esterification reactions. One of the most common applications of pectinases is in fruit processing for various purposes like musts, juices, pastes, and purées. These extraction processes are carried out at temperatures greater than 65 °C and subsequently cooled to 50 °C (Lea 1995); thus, the use of thermostable pectinases avoids the cooling step and so it could reduce the time and cost of processes (Zhang et al. 2011). Thermostable pectinases are also

very useful in the degradation of pectin waste from processing plant material industry, reducing BOD and COD (Kapoor et al. 2000). Pectinase from *Penicillium echinulatum* is associated with a cellulolytic enzyme complex and has improved sugarcane bagasse saccharification, suggesting a new application for these enzymes (Delabona et al. 2013). Several pectinolytic thermophilic fungi have been isolated so far including those belonging to the genera *Thermomyces*, *Aspergillus*, *Monascus*, *Chaetomium*, *Neosartoria*, *Scopulariopsis*, and *Thermomucor* (Martin et al. 2010). The thermophilic *Thermoascus aurantiacus* produces considerable amounts of pectinase in media based on citrus peel (Martins et al. 2002), which showed optimal activity at 70 °C and stability at 60 °C for 2 h.

In nature, lignocellulose accounts for the major part of biomass and, consequently, its degradation is essential for the operation of the global carbon cycle (Sánchez 2009). Lignocellulose, such as wood, is mainly composed of a mixture of cellulose (ca. 40%), hemicellulose (ca. 20 ± 30%), and lignin (ca. 20 ± 30%) (Bajpai 2016). Lignin is an integral cell wall constituent, which provides plant strength and resistance to microbial degradation (Ochoa-Villarreal et al. 2012). The ligninolytic capacity of most thermophilic fungi is largely known. However most of them are known to be able to degrade wood or other lignocelluloses, celluloses, or hemicelluloses (Sharma 1989; Kuhad et al. 1997; Dashtban et al. 2009). The thermophilic fungus *Thermoascus aurantiacus* has a high ligninolytic capacity (McClendon et al. 2012), and it has been isolated from composts.

29.4.2 Plastic Biodegradation

Polyurethanes (PUs) are synthetic plastics with a wide range of applications in the medical, automotive, construction, furnishing, and industrial sectors (Krasowska et al. 2012). They are known to be vulnerable to microbial attack as they contain ester linkages within the backbone of the polymer that are naturally vulnerable to esterases (Zafar et al. 2013). In contrast, polyether PUs, which contain ether linkages within the polymer backbone, are reported to be far more recalcitrant (Darby and Kaplan 1968). It has been reported that a number of fungal isolates are able to degrade impranil (liquid dispersion of PU) including thermotolerant and thermophilic fungi (Zafar et al. 2013), and a number of fungal species that are capable of degrading PU have been isolated and identified (Darby and Kaplan 1968; Pathirana and Seal 1984; Cosgrove et al. 2007; Mathur and Prasad 2012). Zafar et al. (2013) demonstrated that polyester PU is susceptible to fungal biodegradation in compost under both thermophilic (thermophilic stage) and mesophilic (maturation phase) conditions and that positive selection for rare taxa from the existing compost community on the PU surface occurs. The most dominant fungi identified from the surfaces of PU coupons by pyrosequencing was *Fusarium solani* at 25 °C (mesophilic phase), while at both 45 °C and 50 °C (thermophilic phase) *Candida ethanolica* was the dominant species. The diversity in the fungal community recovered from polyester PU coupons buried at the surface of compost pile was dependent on the incubation temperature (Zafar et al. 2014). At 37 °C, *Acremonium flavum* and

Candida rugosa are consistent mesophilic species with dominant *Arthrographis kalrae* on day 28. At 45 °C on day 2, the biomass obtained from the surface of buried polyester PU coupons are dominated by *Aspergillus* spp. and on day 28 a mixed community of *Lichtheimia* sp. and *Aspergillus fumigatus* with occasional isolates of *Malbranchea cinnamomea* and *Emericella nidulans* are found. *A. fumigatus* and *E. nidulans* have previously been isolated as potential polyester PU degraders (Barratt et al. 2003). *M. cinnamomea* and *A. fumigatus* have also been recovered in the compost at 50 °C. The major population at 50 and 55 °C is *Thermomyces lanuginosus*, a PU degrader (Zafar et al. 2014).

29.4.3 Remediation of Metals and Radionuclides

The use of biological materials for metal removal and recovery technologies has gained important credibility during the past decade, because of the good performance and low cost of this complexing material (Wu et al. 2005; Cho et al. 2012; Lakherwal 2014; Bowman et al. 2018). The natural affinity of biological compounds for metallic elements could contribute to economically purifying heavily metal-loaded wastewater. Among the various resources in biological wastes, dead biomass of microorganisms (bacteria, yeasts, fungi, algae) exhibits particularly interesting metal-binding capacities (Cho et al. 2010). For instance, *Rhizopus arrhizus*, a Mucorale filamentous fungus, can accumulate lead or uranium, up to I (1% and 16% of its own dry mass, respectively) (Tobin et al. 1984). These properties are attributed to the high content of complexing functional groups in their cellular wall (e.g., amino, amide, hydroxyl, carboxyl, sulfhydryl, phosphate radicals) (Tiquia-Arashiro 2018). Residual biomass, produced by the thermophilic fungus, *Talaromyces emersonii* CBS 814.70, following growth on glucose-containing media, was examined for its ability to take up uranium from aqueous solution (Bengtsson et al. 1995). It was found that the biomass had a relatively high observed biosorption capacity for the uranium (280 mg/g dry weight biomass). The calculated maximum biosorption capacity obtained by fitting the data to a Langmuir model was calculated to be 323 mg uranium/g dry weight biomass. Some of the critical biosorption parameters have already been identified, and pH was shown to influence to a large extent the formation of metal-biosorbent complexes. pH variation can modify the speciation and the availability of the metallic elements in solution and also the chemical state of the chemical functional groups responsible for metal binding in the biomass.

29.4.4 Cancer Treatment

Aspergillus terreus, a thermophilic fungus abundant in composts (Aydi Ben Abdallah et al. 2015), produces asperjinone, a nor-neolignan, and terrein, a suppressor of ABCG2-expressing breast cancer cells, which can restore drug sensitivity and

could be the key to improve breast cancer therapeutics. Terrein displayed strong cytotoxicity against breast cancer MCF-7 cells. Treatment with terrein significantly suppressed the growth of ABCG2-expressing breast cancer cells. This suppressive effect was achieved by inducing apoptosis via activating the caspase-7 pathway and inhibiting the Akt signaling pathway, which led to a decrease in ABCG2-expressing cells and a reduction in the side-population phenotype (Liao et al. 2012).

29.4.5 Nanoparticle Synthesis

Some microorganisms have developed the ability to resort to specific defense mechanisms to quell stresses like toxicity of heavy metal ions or metals (Tiquia-Arashiro 2018; Bowman et al. 2018; Tiquia-Arashiro and Rodrigues 2016a). The microorganisms can survive and grow even at high metal ion concentrations and are capable of binding large quantities of metallic cations (Tiquia-Arashiro and Rodrigues 2016a, b). The remarkable ability of these group of microbes to reduce heavy metal ions makes them one of the best candidates for nanoparticle synthesis (Tiquia-Arashiro and Rodrigues 2016b, c, d, e, f). Syed et al. (2013) elucidated the biosynthesis of silver nanoparticles (AgNPs) by the thermophilic fungus *Humicola* sp., a dominant fungus in compost ecosystems. The fungus when reacted with Ag^+ ions reduces the precursor solution and leads to the formation of extracellular nanoparticles. The uniqueness of this study is that the investigators achieved superior control over the size of these nanoparticles, focusing upon them to be in the size range of 5–25 nm, so that these AgNPs when employed in biomedical applications will not block the glomerulus of the kidneys and will easily pass through urine within a short period of time. The AgNPs synthesized are nontoxic to cancer and normal cells up to concentrations of 50 μg/mL and thus will find various applications in drug and targeted drug delivery systems (Syed et al. 2013).

Gadolinium oxide nanoparticles are very important as nuclear, electronic, laser, optical, catalyst, and phosphor materials (Tiquia-Arashiro and Rodrigues 2016a, b). Many organic compounds use Gd_2O_3 for their dimerization (Gündüz and Uslu 1996). It is also used in imaging plate neutron detectors, as neutron reactors (Gündüz and Uslu 1996), and as an additive in ZnO_2 to enhance its toughness. Gd_2O_3 has several potential applications in biomedicine, too. For example, it is used in magnetic resonance imaging, since it exhibits superparamagnetism and involves T1 relaxation, and can be useful as a multimodal contrast agent for in vivo imaging (Bridot et al. 2007). It can also be easily doped with other lanthanides and exploited as a fluorescent tag, thus replacing other fluorescent organic mrolecules. Khan et al. (2014) showed that the thermophilic fungus *Humicola* sp. can be used for the synthesis of Gd_2O_3 nanoparticles at 50 °C. $AsGdCl_3$ is dissolved in water along with fungal biomass, and $GdCl_3$ ionizes to Gd^{3+} and $3Cl^-$. The Gd^{3+} ions are then attracted towards anionic proteins, which are secreted by *Humicola* sp. in solution. Reductase enzymes present in the anionic protein fraction act on Gd^{3+} and convert it to Gd^{2+}. Oxidase enzymes, which are also secreted by the fungus in the solution mixture, act

on these Gd^{2+} ions resulting in the formation of Gd_2O_3 nanoparticles. The $GdCl_3$ NPs are irregular in shape, presenting an overall quasi-spherical morphology. Particle size distribution analysis of Gd_2O_3 nanoparticles confirmed that the nanoparticles are in the range of 3–8 nm with an average size of 6 nm. Since Gd_2O_3 nanoparticles have proved their value in site-specific drug delivery systems for cancer therapy, Khan et al. (2014) extended the work of biosynthesis of Gd_2O_3 nanoparticles to bioconjugation with taxol. Bioconjugation of taxol with gold and iron oxide nanoparticles has also been reported (Gibson et al. 2007; Hwu et al. 2009). Taxol is one of the most important anticancer drugs used for breast, ovarian, and lung cancers. The potent anticancer effect of taxol is mainly attributed to its mechanism of action. It stabilizes microtubules by preventing their depolymerization Khan et al. (2014). However, taxol is a hydrophobic drug and less specific to certain tumors due to its low solubility in water. To counter these problems, we carried out the bioconjugation of chemically modified taxol with biocompatible Gd_2O_3 nanoparticles, which may result in an enhancement of the hydrophilicity of taxol and may render it more potent in killing tumor/cancer cells (Khan et al. (2014).

29.5 Conclusions

Thermophilic fungi occur widely in composts, manures, and decomposing plant materials. They play an important role in the decomposition of organic matter due to their avidity for degrading various components of organic matter such as starch, pectin, hemicellulose, cellulose, and, to a lesser extent, lignin. While thermophilic fungi have long been known to be involved in composting and humification, the mechanisms involved in the accelerated decomposition of biomass are not well understood. This literature survey shows that although several thermophilic fungi have been isolated and identified, little knowledge about the physiology of this group is available. The role of thermophilic fungi in decomposition during composting suggests that thermophilic fungi may be good sources of thermostability of enzymes that can be applied in many industrial processes.

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