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A COMPARATIVE STUDY OF COW DUNG COMPOST, GOAT PELLETS, POULTRY WASTE MANURE AND PLANT DEBRIS FOR THERMOPHILIC, THERMOTOLERANT AND MESOPHILIC MICROFLORA WITH SOME NEW REPORTS FROM PAKISTAN

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Abstract

Isolations from the cow dung, goat pellets, poultry waste manure and plant debris samples yielded 44 fungal and 15 bacterial species. The isolated fungi included 13 mesophilic, 11 thermotolerant and 20 thermophilic species. Similarly, six mesophilic, five thermotolerant, and four thermophilic bacteria were isolated. Of these, three species of fungi *viz.*, *Annelophora africana*, *Conidiobolus thermophilus* and *Haplotrichum croceum* appeared to be new records from Pakistan, not hitherto reported. The highest percentage of thermophilic fungi and bacteria occurred in cow dung followed by goat pellet, poultry waste and plant debris in descending order. Similarly, highest percentage of thermotolerant fungi and bacteria occurred in goat pellet followed by poultry waste, cow dung and plant debris, respectively. The highest percentage of mesophilic fungi and bacteria occurred in plant debris followed by poultry waste, goat pellet and cow dung. The growth of thermophilic fungi was better on yeast starch agar (YSA) as compared to potato sucrose agar (PSA) medium. However, the thermotolerant fungi showed better growth on PSA medium. Among the mesophilic fungi, *Aspergillus flavus, Penicillium dipodomyis, Stachybotrys chartarum* and *Syncephalastrum racemosum* showed better growth on YSA medium, whereas, PSA was more suitable for the remaining mesophilic fungi. The optimal temperature for growth of the mesophilic microorganisms was 28°C whereas thermotolerant and thermophilic microorganisms grew best at 40 and 50°C, respectively.

Key words: Plant debris, Manure, compost, Thermophilic, Thermotolerant, Mesophilic, Bacteria, Fungi.

Introduction

Composting is the major process used for recycling agricultural and livestock wastes through the degradation of biodegradable components by microbial communities mainly constituted by bacteria and fungi (Sun *et al*., 2016; Yamamoto *et al*., 2011). It results in manure stabilization and reduction in mass, moisture and populations of pathogens (Changa *et al*., 2002). Composting is a management tool that results in various environmental benefits, safe disposal of poultry wastes coupled with satisfactory crop yields (Wood *et al*., 2010). Since the composting is carried out by the microorganisms and the process of composting involves a gradual increase in temperature and followed by a gradual decline, the compost may contain mesophilic, thermotolerant and thermophilic microorganisms. It has been reported that fly larvae, pathogenic bacteria and viruses are destroyed during composting and the compost also results in suppression of plant diseases (Noble & Coventry, 2005; Postma *et al*., 2003).

Microorganisms involved in composting process have variable capabilities of decomposing organic matter. Since different types of composts originate from different sources and differ in nutrient composition, the microorganism involved in degradation of different organic matter also vary with the source of the organic matter. These groups of microorganisms may play an important role in soil ecology, plant disease management and may be a good source of industrially important enzymes. The present study was therefore conducted to investigate the mesophilic, thermotolerant and thermophilic microbial populations in different composts samples collected from agricultural areas around Karachi.

Materials and Methods

Isolation of bacteria and fungi from compost: Ten compost samples of each of cow dung, goat pellet, poultry manure and plant debris were collected and bacteria and fungi were isolated using serial dilution (Waksman & Fred, 1922) and soil plate (Warcup, 1950) methods.

a) Serial dilution method (Waksman & Fred, 1922): One g powdered compost was suspended in 10 ml sterilized water in a test tube to make a standard suspension. Using a sterilized pipette, a dilution of 10^2 was obtained by transferring one ml suspension from standard to another test tube containing 9 ml sterilized water. This process was repeated to get a serial dilution of 10^3, 10^4, 10^5 and 10^6. For the isolation of mesophilic, thermotolerant and thermophilic fungi, one ml suspension from 10^1, 10^2 and 10^3 dilutions were transferred to Petri plates containing either potato sucrose agar (PSA: potato 200g, sucrose 20g, Agar 20g, distilled water 1L) or Yeast starch agar (YSA: yeast extract Difco powder, 4g; K_2HPO_4 1g; $MgSO_4.7H_2O$ 0.5g; soluble starch, 15g; Agar, 20g; distilled water 1L) (Cooney & Emerson, 1964). Penicillin @ 10,000 units L^{-1} and Streptomycin @ 0.02 g L^{-1} were used to suppress bacterial growth during isolation of fungi. For the isolation of bacteria one ml of suspension from 10^4, 10^5 and 10^6 dilutions were transferred on nutrient agar (NA) medium (Peptone 5g, beef extract 3g, NaCl 5g, Agar 20g, distilled water 1L) plates without antibiotics.

b) Soil plate method (Warcup, 1950): About 0. 2 g of a compost sample was suspended in one ml of sterilized water in a Petri dish and mixed into 10ml of molten PSA, YSA (with antibiotics) and NA (without antibiotics) media by gently rotating the dishes.

Three replicate plates of each sample on each medium were incubated for five days at 28°C for mesophilic, 40°C for thermotolerant and 50°C for thermophilic microorganisms. After five days incubation, incidence percentage of bacteria and fungi were calculated by using following formula:

$$\text{Incidence\%} = \frac{\text{No. of colonies of the species in all plates}}{\text{Total no. of colonies of all the species in all plates}} \times 100$$

The fungi were identified after reference to Barnett & Hunter (2010), Domsch *et al*., (1980), Ellis (1971, 1976) and Samson *et al*., (2014). The bacterial identification was done after two to three days incubation based on morphological as well as biochemical tests, using standard procedures as given in Bergey's manual of determinative bacteriology (Holt *et al*., 1994).:

Confirmation of mesophilic, thermotolerant, and thermophilic nature: The isolated fungi were inoculated on PSA and YSA, whereas, the bacteria were inoculated on NA medium. The plates were incubated for 5 days at 28, 40, and 50°C to see the temperature required for best growth of the isolated microorganisms. Organisms showing best growth at 28°C were classified as mesophilic, whereas, those showing best growth at 40 or 50°C were classified as thermotolerant and thermophilic, respectively.

Results

Incidence of thermophilic, thermotolerant and mesophilic bacteria and fungi in compost samples: Isolation from compost samples yielded a total number of 44 fungal and 15 bacterial species. The isolated fungi include 13 mesophilic, 11 thermotolerant, and 20 thermophilic species whereas bacteria include six mesophilic, five thermotolerant and four thermophilic species.

Cow dung and goat pellet were most suitable sources for the isolation of all the four thermophilic bacteria whereas poultry waste manure and plant debris yielded only *Bacillus licheniformis* and *B. stearothermophilus* (Table 1). The highest number of thermophilic fungi *i.e.*, all the 20 species of fungi were isolated from cow dung whereas goat pellets, poultry waste manure and plant debris showed 14, 9 and 5 species, respectively. *Aspergillus fumigatus*, *Humicola fuscoatra*, *H. grisea* var. *thermoidea*, *Rhizomucor pusillus* and *Thermomyces lanuginosus* were most abundant in all the composts. The highest incidence of thermophilic fungi was observed in cow dung followed by goat pellet, poultry waste manure and plant debris in descending order (Table 2).

All the five thermotolerant bacteria were isolated form all the compost samples with highest incidence in goat pellet followed by poultry waste manure, cow dung and plant debris in descending order (Table 3). Of the thermotolerant fungi, 11 species were isolated from goat pellet whereas 10 species isolated from poultry waste manure, nine from cow dung, and only six from plant debris (Table 4).

All the 6 species of mesophilic bacteria were isolated from plant debris in the highest incidence whereas poultry waste manure, goat pellet and cow dung showed 5 and 2 species of mesophilic bacteria, respectively (Table 6). Plant debris had 13 mesophilic fungi whereas, goat pellet and poultry waste manure and cow dung showed the occurrence of 7 and 2 species, respectively. Plant debris showed the presence of all mesophilic fungi. The highest incidence of mesophilic bacteria and fungi were observed in plant debris followed by poultry waste manure, goat pellet and cow dung in descending order (Table 5).

Thermophilic bacteria *viz*., *Bacillus licheniformis* and *B. stearothermophilus,* and fungi *viz*., *Aspergillus fumigatus*, *Humicola fuscoatra*, *H. grisea* var. *thermoidea*, *Rhizomucor pusillus* and *Thermomyces lanuginosus* were present in all the types of composts.

Similarly, thermotolerant bacteria *viz*., *Bacillus cereus, B. megaterium, B. pumilus, B. subtilis* and *Micrococcus varians,* and fungi *viz*., *Acrophialophora fusispora*, *Aspergillus nidulans* (Anamorph), *A. niger, A. rugulosus, Paecilomyces variotii* and *Penicillium citrinum* were present in all the composts. Furthermore, mesophilic bacterium *Staphylococcus aureus* and fungus *Syncephalastrum racemosum* were present in all types of the composts.

Table 1. Incidence (%) of thermophilic bacteria in composts samples.

S. No.	Organism	Cow dung	Goat pellets	Poultry manure	Plant debris
01.	*Bacillus licheniformis*	80	70	50	20
02.	*Bacillus stearothermophilus*	70	60	40	10
03.	*Geobacillus toebii*	70	50	0	0
04.	*Thermus thermophilus*	60	40	0	0

Table 2. Incidence (%) of thermophilic fungi in composts samples.

S. No.	Organism	Cow dung	Goat pellets	Poultry manure	Plant debris
01.	*Acremonium thermophilum*	80	70	60	0
02.	*Annelophora africana*	60	0	0	0
03.	*Aspergillus fumigatus*	10	80	50	70
04.	*Aureobasidium pullulans*	700	50	20	0
05.	*Chaetomium thermophilum* var. *coprophile*	60	50	10	0
06.	*C. thermophilum* var. *dissitum*	70	60	0	0
07.	*Conidiobolus thermophilus*	60	0	0	0
08.	*Gilmaniella humicola*	70	0	0	0
09.	*Haplotrichum croceum*	70	20	0	0
10.	*Humicola fuscoatra*	80	70	40	10
11.	*H. grisea* var. *thermoidea*	10	70	50	30
12.	*Isaria fumosorosea*	70	40	0	0
13.	*Mucor fragilis*	60	30	0	0
14.	*Myriococcum thermophilum*	40	0	0	0
15.	*Paraconiothyrium minitans*	60	0	0	0
16.	*Penicillium dupontii*	60	20	0	0
17.	*Rhizomucor miehei*	60	0	0	0
18.	*Rhizomucor pusillus*	90	70	60	30
19.	*Scytalidium thermophilum*	70	50	40	0
20.	*Thermomyces lanuginosus*	100	80	80	30

Table 3. Incidence (%) of thermotolerant bacteria in compost samples.

S. No.	Organism	Cow dung	Goat pellets	Poultry	Plant debris
01.	*Bacillus cereus*	60	80	70	30
02.	*Bacillus megaterium*	50	80	80	30
03.	*Bacillus pumilus*	20	60	40	10
04.	*Bacillus subtilis*	60	90	70	40
05.	*Micrococcus varians*	30	60	60	10

Table 4. Incidence (%) of thermotolerant fungi in compost samples.

S. No.	Organism	Cow dung	Goat pellets	Poultry manure	Plant debris
01.	*Acrophialophora fusispora*	30	60	50	10
02.	*Aspergillus floccosus*	0	20	0	0
03.	*A. nidulans* (Anamorph)	70	90	80	50
04.	*A. nidulans* (Teleomorph)	30	70	60	0
05.	*A. niger*	70	90	70	50
06.	*A. neoniveus*	40	60	60	0
07.	*Aspergillus rugulosus*	50	80	70	20
08.	*Myceliophthora sepedonium*	0	70	60	0
09.	*Paecilomyces variotii*	60	90	80	50
10.	*Penicillium citrinum*	20	70	60	10
11.	*Scytalidium lignicola*	40	50	40	0

Table 5. Incidence (%) of mesophilic fungi in compost samples.

S. No.	Organism	Cow dung	Goat pellets	Poultry	Plant debris
01.	*Alternaria alternata*	0	10	40	70
02.	*A. flavus*	0	20	0	70
03.	*A. terreus*	30	0	40	90
04.	*Botryotrichum piluliferum*	0	0	0	60
05.	*Botrytis cinerea*	0	0	0	60
06.	*Chaetomium globosum*	0	30	40	70
07.	*Curvularia hawaiiensis*	0	20	10	70
08.	*Myrothecium roridum*	0	0	0	70
09.	*Penicillium dipodomyis*	0	20	30	80
10.	*Stachybotrys chartarum*	0	30	30	80
11.	*Syncephalastrum racemosum*	40	60	60	80
12.	*Trichoderma harzianum*	0	0	0	80
13.	*T. virens*	0	0	0	70

Table 6. Incidence (%) of mesophilic bacteria in compost samples.

S. No.	Organism	Cow dung	Goat pellets	Poultry manure	Plant debris
01.	*Aeromonas* sp.	0	40	50	70
02.	*Enterococcus* sp.	0	20	40	70
03.	*Escherichia coli*	20	0	0	20
04.	*Klebsiella* sp.	0	30	30	40
05.	*Pseudomonas flourescens*	0	50	70	80
06.	*Staphylococcus aureus*	30	20	20	40

Table 7. Growth of thermophilic fungi on two different media.

S. No.	Fungi	PSA medium	YSA medium
01.	*Acremonium thermophilum*	-	+++
02.	*Annelophora africana*	-	+++
03.	*Aspergillus fumigatus*	+++	+++
04.	*Aureobasidium pullulans*	+	+++
05.	*Chaetomium thermophilum* var. *coprophile*	+	+++
06.	*Chaetomium thermophilum* var. *dissitum*	+	+++
07.	*Conidiobolus thermophilus*	-	+++
08.	*Gilmaniella humicola*	+	+++
09.	*Haplotrichum croceum*	-	+++
10.	*Humicola fuscoatra*	+	+++
11.	*Humicola grisea* var. *thermoidea*	+	+++
12.	*Isaria fumosorosea*	+	+++
13.	*Mucor fragilis*	+	+++
14.	*Myriococcum thermophilum*	-	+++
15.	*Paraconiothyrium minitans*	+	+++
16.	*Penicillium dupontii*	-	+++
17.	*Rhizomucor miehei*	+	+++
18.	*Rhizomucor pusillus*	+	+++
19.	*Scytalidium thermophilum*	+++	+++
20.	*Thermomyces lanuginosus*	+	+++

+++= Best growth, ++= Better growth, += Good growth, -= No growth

Table 8. Growth of thermotolerant fungi on two different media.

S. No.	Fungi	PSA medium	YSA medium
01.	*Acrophialophora fusispora*	+++	++
02.	*Aspergillus floccosus*	++	+++
03.	*Aspergillus nidulans* (Anamorph)	+++	++
04.	*Aspergillus nidulans* (Teleomorph)	+++	++
05.	*Aspergillus niger*	+++	++
06.	*Aspergillus neoniveus*	+++	++
07.	*Aspergillus rugulosus*	+++	++
08.	*Myceliophthora sepedonium*	+++	++
09.	*Paecilomyces variotii*	++	+++
10.	*Penicillium citrinum*	+++	++
11.	*Scytalidium lignicola*	+++	++

+++= Best growth, ++= Better growth, += Good growth

Table 9. Growth of mesophilic fungi on two different media.

S. No.	Fungi	PSA medium	YSA medium
01.	*Alternaria alternata*	+++	+
02.	*Aspergillus flavus*	+++	++
03.	*Aspergillus terreus*	+++	+
04.	*Botryotrichum piluliferum*	+++	+
05.	*Botrytis cinerea*	+++	+
06.	*Chaetomium globosum*	+++	+
07.	*Curvularia hawaiiensis*	+++	+
08.	*Myrothecium roridum*	+++	+
09.	*Penicillium dipodomyis*	+++	++
10.	*Stachybotrys chartarum*	+++	++
11.	*Syncephalastrum racemosum*	+++	++
12.	*Trichoderma harzianum*	+++	+
13.	*Trichoderma virens*	+++	+

+++= Best growth, ++= Better growth, += Good growth

Growth of fungi and bacteria on YSA and PSA: YSA medium was more suitable for all thermophilic fungi as compared to PSA medium except for *Aspergillus fumigatus* and *Scytalidium thermophilum* which showed similar growth on both the media. In contrast, PSA medium was more suitable for 11 thermotolerant fungi, whereas, two thermotolerant fungi *viz.*, *Aspergillus floccosus* and *Paecilomyces variotii* showed better growth on YSA as compared to PSA. YSA was more suitable medium for mesophilic fungi viz., *Aspergillus flavus, Penicillium dipodomyis, Stachybotrys chartarum* and *Syncephalastrum racemosum,* whereas, PSA was more suitable for the remaining nine mesophilic fungi (Table 7-9). The best growth of the mesophilic, thermotolerant and thermophilic fungi and bacteria was observed at 28°C, 40°C and 50°C, respectively.

Discussion

Composted organic materials such as plant debris and animal manure have been used for more than 2,000 years to improve soil fertility. Composting provides an inexpensive alternate method for waste management of animal dung and plant debris. The temperatures achieved during properly managed compost greatly reduce the number of most pathogens (Ritz & Worley, 2009) thus minimizing the chance of spreading disease. Properly composted material is environmentally safe and a valuable soil amendment for growing certain crops (Bonhotal *et al*., 2008).

During the present investigation cow dung showed highest diversity and frequency of occurrence of thermophilic fungi. Similar results have been reported by Rajavaram *et al*., (2010) where 14 species of thermophilic fungi belonging to six genera were isolated from cattle dung while other substrates gave less number of species. They also found that *A. fumigatus* was the abundant species in most of the sources; *Humicola grisea*, *Mucor* species, and *Scytalidium thermophilum* were isolated from cattle dung, and *Thermomyces lanuginosus* was isolated from cattle dung and poultry manure. The results of the present study also supported the findings of Parkash *et al*., (2011) who obtained the highest number of thermophilic species from cow dung while donkey dung and goat pellet yielded comparatively less number of species. In our studies, five thermophilic fungi viz., *A. fumigatus*, *H. fuscoatra*, *H. grisea* var. *thermoidea*, *R. pusillus* and *T. lanuginosus* were the dominant fungi associated with cow dung, goat pellet, poultry manure and plant debris.

Sreelatha *et al*., (2013) isolated *Aspergillus fumigatus*, *Rhizomucor pusillus* and *Scytalidium thermophilum* from cow dung, *Humicola grisea* and *Thermomyces lanuginosus* from cow dung and poultry manure. Similarly, Perveen & Shahzad (2013) isolated *Isaria fumosorosea* from compost. No previous report on the occurrence of thermophilic fungi *viz.*, *Annelophora africana*, *Haplotrichum croceum* and *Conidiobolus thermophilus* are available from Pakistan so these fungi have been reported for the first time from Pakistan.

Our results show that some species were of restricted occurrence on certain types of dung, while some others such as *C. globosum* were of common occurrence in almost all types of dung. Such similar observations on the species-substrate relationship among coprophilous fungi were also made by Richardson (2001).

In the present investigation, 10 different compost samples gave 15 bacteria, belonging to 10 genera *viz., Aeromonas, Bacillus, Enterococcus, Escherichia, Geobacillus, Klebsiella, Micrococcus, Pseudomonas, Staphylococcus,* and *Thermus.* Similar reports have been made where different compost samples yielded species belonging to *Pseudomonas, Klebsiella, Bacillus,* and *Thermus* (Tuomela *et al*., 2000), *B. megaterium* (Takaku *et al*., 2006), *Micrococcus varians* and *B. pumilus* (Adegunloye *et al*., 2007), *Bacillus cereus, Bacillus subtilis* and *Bacillus licheniformis* (Radha & Rao, 2014), *Geobacillus toebii* (Sung *et al*., 2002) and *B. stearothermophilus* (Sakai *et al*., 1994; Wang *et al*., 2007). Choudhary & Johri (2009) and Ubani *et al*., (2016) reported that as much as 87% of the randomly selected colonies during the thermophilic phase of composting belonged to the genus *Bacillus*. Similar results have been observed during the present study. Abundance of thermotolerant and thermophilic *Bacillus* species in compost samples was attributed to the thick spore wall in *Bacillus* species that increases resistance to extreme conditions.

A variety of standard mycological media can be used for the isolation of thermophilic fungi (Cooney & Emerson, 1964). We observed that the growth of *A. fumigatus* and *S. thermophilum* was similar on PSA and YSA media. Our results are in confirmation with the report made by Satyenarayana & Johri (1984) indicating that simple nitrogen sources like nitrates of sodium and potassium, asparagines and yeast extract support good growth of thermophilic fungi.

Our results also corroborated well with Cordova *et al*., (2003) who suggested that the optimal temperature was 5-35°C for the growth of mesophiles, at or above 50°C for growth of thermophiles whereas microorganisms with maximum temperature near 50°C were classified as thermotolerant. According to Mohammad *et al*., (2017) thermophilic microorganisms require a maximum temperature for growth at or above 50°C and a minimum temperature for growth at or above 20°C. Ahmed *et al*., (2012) also reported that the optimal temperature of thermophilic/thermotolerant fungi is 40-50°C. Similarly, the majority of mesophiles grew between 5°C and 37°C, with an optimum temperature of 25-30°C (Dix & Webster, 1995).

Results of the present study suggest that the thermophilic, thermotolerant and mesophilic groups of microorganisms prefer different substrates and media. The highest incidence of thermophilic, thermotolerant and mesophilic bacteria and fungi was observed in cow dung, goat pellet, and plant debris, respectively. Yeast starch agar was more suitable for thermophilic fungi while thermotolerant and mesophilic fungi showed good growth on Potato starch agar. Thermophilic bacteria and fungi showed best growth at 50°C, thermotolerant and mesophilic bacteria and fungi grew best at 40°C and 28°C respectively. Selected substrate and medium can therefore be used in combination of temperature for the selective isolation of desired group of microorganisms.

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