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ASSESSING THE ENZYMATIC ACTIVITIES OF COMPOST ASSOCIATED MESOPHILIC, THERMOTOLERANT AND THERMOPHILIC BACTERIA AND FUNGI

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ABSTRACT

Microorganisms including bacteria and fungi produce enzymes that are used in various industrial processes. In this work, we set out to identify the enzymes produced by compost associated bacterial and fungal species and then assessed their enzymatic activities. Several bacterial and fungus species were isolated from compost samples. We found that 33 fungi and 7 bacterial species exhibited amylase activity. We have identified *Aspergillus floccosus, Aspergillus rugulosus, Emericella nidulans* and *Mucor fragilis* as the novel source for amylase activity. Similarly, 40 fungi and 6 bacteria showed cellulase activity. In addition, we have identified *Annelophora africana, Aspergillus floccosus, Haplotrichum croceum, Trichoderma virens* and *Micrococcus varians* as the novel source for cellulase enzymes. Our analysis also identified 17 fungal and 7 bacterial species that produce chitinase whereas 20 fungal and 5 bacterial species produce pectinase. Here, *Aspergillus rugulosus* and *Micrococcus varians* are identified as novel source of pectinase enzymes. Furthermore, 27 fungal and 6 bacterial species have shown protease activity. We suggest that enzymes produced by indigenous microorganisms identified in this study have great commercial potential.

Key words: Amylase, Cellulase, Chitinase, Pectinase, Proteinase, Mesophilic, Thermotolerant, Thermophilic fungi and bacteria, Compost.

INTRODUCTION

Composting is a microbial decomposition of organic matter that converts organic waste into stable humus like product known as compost (Agarwal *et al.*, 2005). A large varieties of mesophilic, thermotolerant and thermophilic aerobic microorganisms including bacteria, yeasts, and fungi take part in the process of composting (Ishii *et al*., 2000).The composting materials contain complex compounds. Degradation of these compounds requires the use of extra cellular enzymes (Miyatake and Iwabuchi, 2005). Microorganisms release various enzymes during composting, these enzymes breakdown numerous complex organic matter into simple water soluble compounds (Castaldi *et al*., 2008).

Amylase is an enzyme that breaks down starch into sugar. Amylase was the first enzyme to be discovered and isolated (Hill and Needham, 1970). In fermentation of starch many bacteria and fungi produce extracellular amylases (Adeniran and Abiose, 2009). The microbes produced amylase enzyme convert the starch into the oligosaccharides with quick reduction in blue colour and appearance of clear zone (Najafi *et al.*, 2005).Agricultural waste such as Potato peels, which presently constitutes a menace to solid waste management, may be a rich source of amylolytic bacteria (Ali *et al.*, 1998), the utilization of agriculture waste materials reduced the pollution and upgrade material. These fungal and bacterial amylases have potential industrial applications (Sun *et al*., 2010).

Cellulose is the main component of plant biomass and is found in many waste streams, as well as agricultural and food industry wastes, brewery water wastes and sledges wastes (Kapdan and Kargi, 2006). The decay of organic wastes during the composting process is carried out by a succession of microbial communities, which is critical for the utilization of complex substrates such as cellulose, hemicellulose and lignin (Takaku *et al*., 2006). The cellulolytic bacteria *Bacillus*, *Pseudomonas*, *Streptomyces* and *Staphylococcus* from compost (Eida *et al*., 2012) decaying vegetables (Sakthivel *et al.*, 2010) and animal wastes slurry (Kim *et al*., 2012) have been shown to have cellulose degrading abilities (Gautam *et al.*, 2010). In addition, *Aspergillus*, *Penicillium* and *Trichoderma* also produce cellulolytic enzymes during organic degradation (Wilson, 2011).

Bacteria and fungi are important decomposers of chitin in soil and thus contribute to the recycling of carbon and nitrogen resources in soil ecosystems. A vast number of chitinolytic bacteria have been isolated from garden and park waste compost (Poulsen *et al*., 2008), shellfish waste (Wang and Hwang, 2001), shrimp shell-enriched soil (Zhu *et al*., 2007).

The enzymes that hydrolyse pectic substances are generally known as pectinolytic enzymes or pectinases, which consist of polygalacturonase (PGase), pectin esterase, pectin lyase and pectate lyase on the basis of their mode of action (Alkorta *et al*., 1998). Pectinase is an extracellular enzyme, which is produced by various organisms including bacteria, *Pseudomonas* sp. and *Bacillus* sp. fungi, *Aspergillus niger*, *Aspergillus flavus* and *Penicillium chrysogenum* and some actinomycetes (Geetha *et al*., 2012).

Proteolytic enzymes, the proteases are important group of commercial enzymes and are largely used in the food, pharmaceutical, leather and textile industries as well as used in detergents, baking, debittering of protein hydrolysate, and manufacture of soy products (Mozersky *et al*., 2002). Few thermophilic *Bacillus* species that produce proteases with optimum activity at 60°C have been isolated; the first isolate being *Bacillus stearothermophilus* (Razak *et al*., 1993).

MATERIALS AND METHODS

Enzymatic activity assays

We isolated 44 species of mesophilic, thermotolerant and thermophilic fungi and 15 species of bacteria from ten compost samples (cow dung, goat pellet, poultry manure and plant debris) and then tested for their amylase, cellulase, chitinase, pectinase, and protease activities. To determine the amylase, cellulase, pectinase and protease activity in both bacterial and fungi species, we performed in *vitro* assays as described by Aneja (1996).

a) Amylolytic activity: A 5mm inoculation disc from pure culture of either a mesophilic, thermotolerant or thermophilic fungus was transferred in the center of a Petri plates containing Starch Peptone Agar (SPA) medium (Starch 5g, Peptone 10g, Agar 20g , distilled water 1L) amended with Penicillin and Streptomycin. The bacterial cultures were maintained on SPA medium but without antibiotics. There were three replicates for each microorganism. The Petri plates were incubated for 2-3 days at an optimum growth temperature suitable for each microorganism (28°C for mesophilic, 40°C for thermotolerant and 50°C for thermophilic). The Petri plates were exposed to iodine vapours. Formation of a clear zone around the colonies was indicated of a positive amylolytic activity. Medium where starch was not utilized turned blue when exposed to iodine vapours.

b) Cellulolytic activity: Cellulose agar (CA) (Cellulose 10 g, agar 20g, distilled water 1L),(method described in above paragraph). Formation of clear zone around the colonies showed the cellulolytic activity and indicated a positive reaction. Medium where cellulose was not utilized turned pinkish blue when exposed to iodine vapours.

c) Chitinase activity: Colloidal chitin was prepared from chitin flakes as described by Mathivanan *et al*. (1997). In brief, the chitin flakes were grinded into powder form, then this powder was slowly added to freshly prepared 10N HCl in a beaker with vigorous stirring. The suspension thus prepared was transferred to 4°C refrigerator. After overnight incubation, then suspension was added to a cold 50% ethanol with rapid stirring, incubated at 25°C overnight. The precipitate was collected by centrifugation at 10,000 rpm for 20 min and washed with sterile distilled water until the colloidal chitin became neutral (pH 7.0). Chitin thus obtained was frozen dry to powder form and stored at 4°C until further use.

Qualitative screening for chitinolytic activity

A screen for chitinolytic activity was performed using the protocol by Maria *et al*., (2005). In brief, a 5mm inoculation disc from pure culture of a mesophilic, thermotolerant and thermophilic fungus was inoculated in the center of a Petri plate containing colloidal chitin agar Penicillin and Streptomycin amended medium (colloidal chitin, 2 g; agar, 20 g; distilled water, 1 L). The bacterial colony was streaked on colloidal chitin agar medium but without antibiotics. There were three replicates of each species of bacterial or fungal culture plates. The Petri plates were incubated at their optimum growth temperature suitable for each microorganism (described in above paragraph). After approximately 5 days of incubation bacterial or fungi culture plates were observed for chitinase activity. We identified the clear zone surrounding the microbes' colonies as positive for chitinase activity. Medium where chitin was not utilized showed no clear zone.

d) Pectinase activity: A 5mm inoculation disc from pure culture of a mesophilic, thermotolerant and thermophilic fungi was inoculated in the center of Petri plates containing Hanskin's medium (mineral salt solution containing $(NH_4)_2SO_4$ 2g, K_2HPO_4 4g, Na_2HPO_4 6g, $FeSO_4.7H_2O$ 0.2g, $CaCl_2$ 0.001g, H_3BO_3 0.00001g, MnSO4 0.00001g, ZnSO4 0.00007g, MoO_3 0.00001g, was dissolved in 100mL of distilled water and the total volume was made to 500ml. Next, 1g yeast extract, 5g pectin, and 20g of agar, were added to 500mL of distilled water, then both

solutions were combined to a final volume of one liter of medium, amended with Penicillin and Streptomycin. The bacteria were streaked on Hanskin's medium without antibiotics. There were three replicates of each species of bacterial or fungal culture plates. The Petri plates were incubated at their optimum growth temperature suitable for each microorganism (described in above paragraph). After two to three days of incubation, the plates were flooded with1% aqueous solution of hexa-decyle-tri-methyl ammonium bromide. A clear zone around the colonies indicated positive pectinase activity. There was no color change around the colonies indicated negative pectinase activity.

e) Proteolytic activity: A nutrient gelatin medium (peptone 5g, beef extract 3g, gelatin 120g, distilled water 1L) was prepared, and autoclaved, after cooling it down to 45-50 °C the medium was poured into test tubes. Using inoculation loop, a stab inoculation of mesophilic, thermotolerant and thermophilic bacterial and fungal species were made. Uninoculated test tubes served as control. There were three replicates of each species of bacterial or fungal test tubes. The test tubes were incubated at their optimum growth temperature suitable for each microorganism (described in above paragraph), after two to three days of incubation, test tubes were placed in a refrigerator at 4°C for 15 minutes. After 15 minutes all test tubes were observed for their solid or liquid states. Gelatin inoculated test tubes that remained in liquid state indicated of a positive reaction for gelatin hydrolysis whereas test tubes that remained in solid state were marked as negative reaction.

RESULTS

Assay to determine enzymatic Activity

a) Amylolytic activity:

As shown in Table 1 and Fig. 1, a total number of 33 fungi and seven bacteria showed amylolytic activity. It includes 13 thermophilic, 10 thermotolerant, 10 mesophilic fungi and two thermophilic, four thermotolerant and one mesophilic bacterial species showing the highest amylase activity.

b) Cellulolytic activity:

As shown in Table 1 and Fig. 2, 40 fungi and 6 bacteria showed cellulolytic activity. It includes 17 thermophilic, 10 thermotolerant and 13 mesophilic fungi, and one thermophilic, four thermotolerant and one mesophilic bacteria showed extensive cellulase activity.

c) Chitinase activity:

As shown in Table 1, 17 fungi and 7 bacteria showed chitinolytic activity. These includes 7 thermophilic, 3 thermotolerant and 7 mesophilic fungi and 2 thermophilic, two thermotolerant and 3 mesophilic bacteria. We found that these microorganisms produced very high chitinase activity.

d) Pectinase activity:

As shown in Table 1 and Fig. 3 a total number of 20 fungi and five bacteria showed pectinolytic activity. It includes eight thermophilic, six thermotolerant and six mesophilic fungi and one thermophilic, three thermotolerant and one mesophilic bacteria were the highest pectinase producers.

e) Proteolytic activity:

As shown in Table 1 and Fig. 4 and 5 a total number of 27 fungi and six bacteria showed proteolytic activity. It included 11 thermophilic, six thermotolerant and 10 mesophilic fungi, and two thermophilic, two thermotolerant and two mesophilic bacteria showed medium to high proteinase activity.

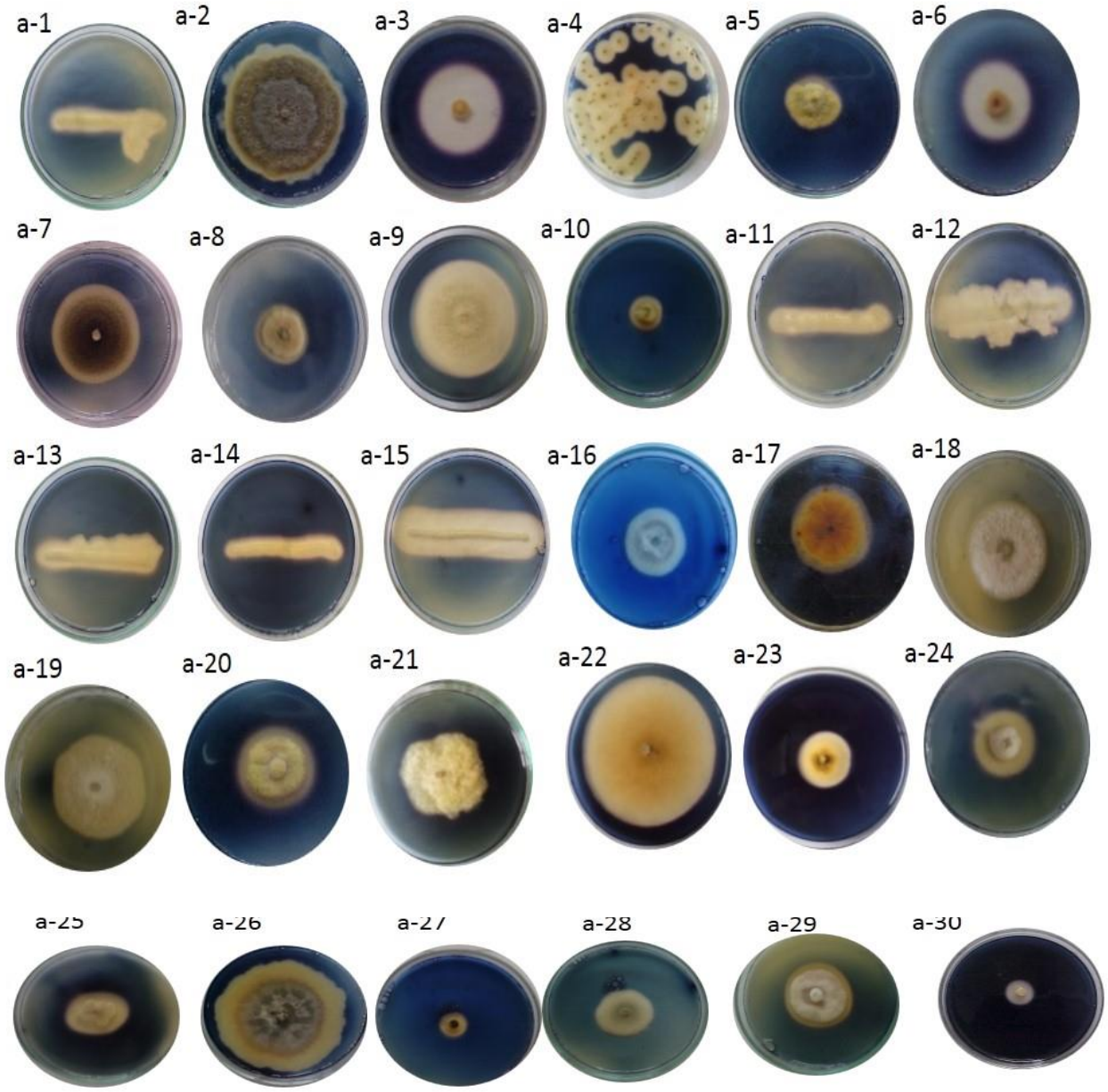

Fig. 1. Amylolytic activity of mesophilic, thermotolerant and thermophilic bacteria and fungi.
a-1= *Aeromonas* sp., a-2= *Aureobasidium pullulans*, a-3= *Aspergillus floccosus*, a-4= *A. fumigatus*, a-5= *A. nidulans*, a-6= *A. neoniveus*, a-7= *A. niger*, a-8= *A. rugulosus*, a-9= *A. flavus*, a-10= *Alternaria alternata*, a-11= *Bacillus cereus*, a-12= *B. licheniformis*, a-13= *B. stearothermophilus*, a-14= *B. subtilis*, a-15= *B. megaterium*, a-16= *Chaetomium globosum*, a-17=*Emericella nidulans* a-18= *Humicola fuscoatra*, a-19= *H. grisea* var. *thermoidea*, a-20= *Isaria fumosorosea*, a-21= *Mucor fragilis*, a-22= *Paecilomyces variotii*, a-23= *Penicillium dipodomyis*, a-24= *P. dupontii*, a-25= *Rhizomucor pusillus*, a-26= *Scytalidium thermophilum*, a-27= *Stachybotrys chartarum* a-28= *Syncephalastrum racemosum*, a-29= *Thermomyces lanuginosus*, a-30= *Trichoderma virens.*

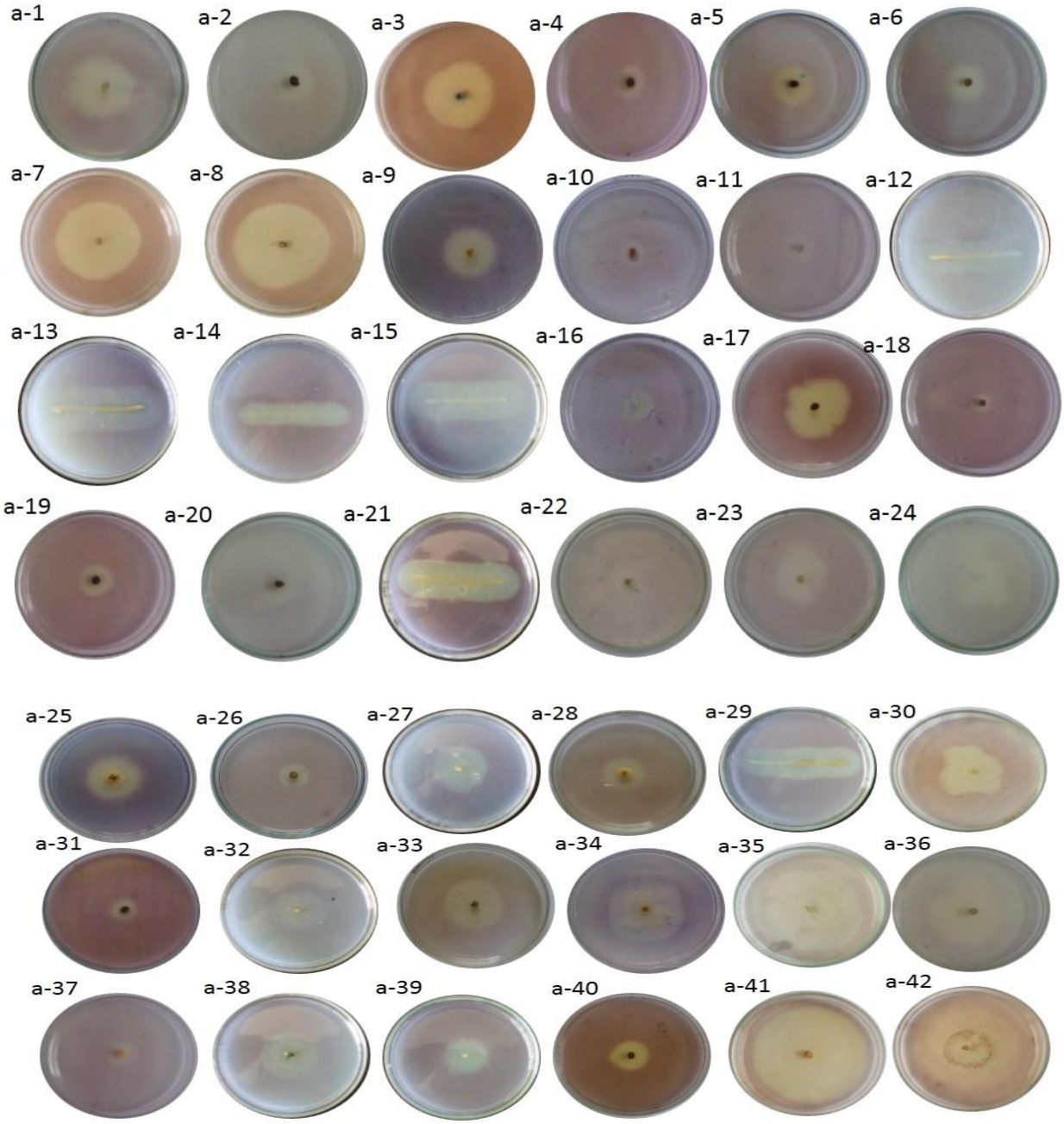

Fig. 2. Cellulolytic activity of mesophilic, thermotolerant and thermophilic bacteria and fungi.
a-1= *Aureobasidium pullulans*, a-2=*Annelophora africana*, a-3= *Acremonium thermophilum*, a-4= *Alternaria alternata*, a-5= *Aspergillus nidulans*, a-6= *A. neoniveus*, a-7= *A. niger*, a-8= *A. flavus*, a-9= *A. floccosus*, a-10= *A. rugulosus*, a-11= *Botryotrichum piluliferum*, a-12= *Bacillus cereus*, a-13= *B. licheniformis*, a-14= *B. pumilus*, a-15= *B. subtilis*, 16=*Chaetomium thermophilum* var. *coprophile*, a-17= *C. thermophilum* var. *dissitum*, a-18= *C. globosum*, a-19=*Curvularia hawaiiensis*, ,a-20= *Emericella nidulans*, a-21=*Enterococcus* sp., a-22= *Gilmaniella humicola*, a-23= *Humicola fuscoatra*, a-24= *H. grisea* var. *thermoidea*, a-25= *Haplotrichum croceum*, a-26= *Isaria fumosorosea*, a-27= *Mucor fragilis*, a-28= *Myceliophthora sepedonium*, a-29= *Micrococcus varians*, a-30= *Myriococcum thermophilum*, a-31= *P. variotii*, a-32= *Penicillium dipodomyis*, a-33= *P. citrinum*, a-34= *Rhizomucor pusillus*, a-35= *R. miehei*, a-36= *Scytalidium thermophilum*, a-37=*S. lignicola*, a-38= *Stachybotrys chartarum* a-39= *Syncephalastrum racemosum*, a-40= *Thermomyces lanuginosus*, a-41= *Trichoderma virens*, a-42= *T. harzianum.*

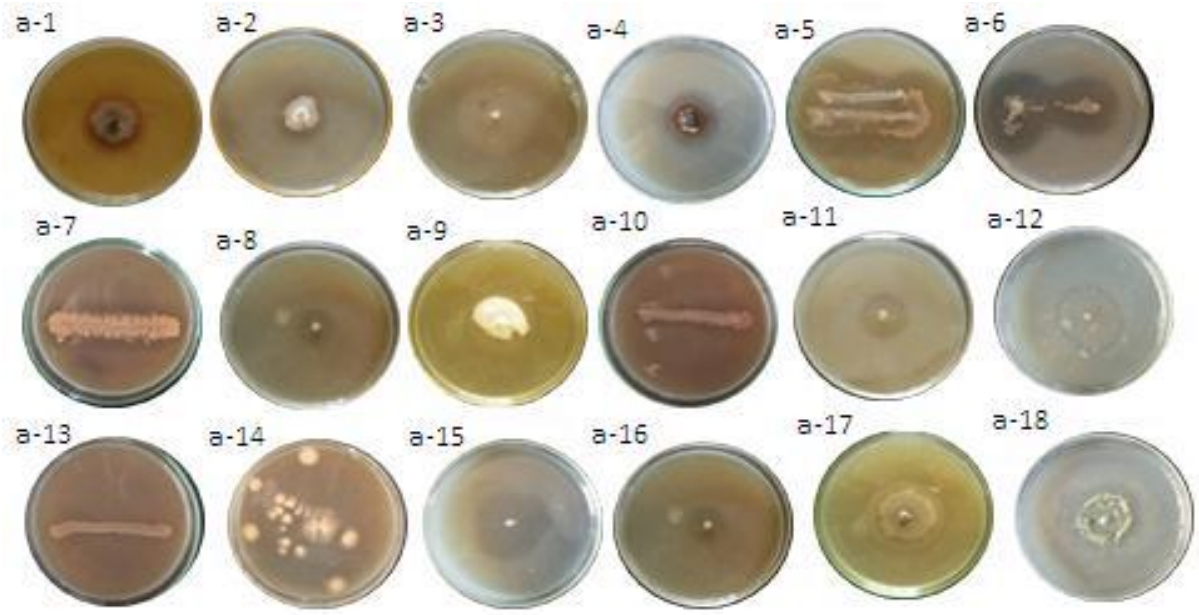

Fig. 3. Pectinolytic activity of mesophilic, thermotolerant and thermophilic bacteria and fungi: a-1= *Aspergillus nidulans*, a-2= *A. rugulosus*, a-3= *Aureobasidium pullulans,* a-4= *Alternaria alternata*, a-5= *Bacillus pumilus*, a-6= *B. stearothermophilus*, a-7= *B. subtilis*, a-8= *Botrytis cinerea*, a-9= *Isaria fumosorosea*, a-10= *Micrococcus varians*, a-11= *Myriococcum thermophilum* a-12= *Mucor fragilis*, a-13= *Pseudomonas flourescens*, a-14= *Paecilomyces variotii*, a-15= *Penicillium citrinum*, a-16= *Rhizomucor miehei*, a-17= *Thermomyces lanuginosus*, a- 18= *Trichoderma harzianum*

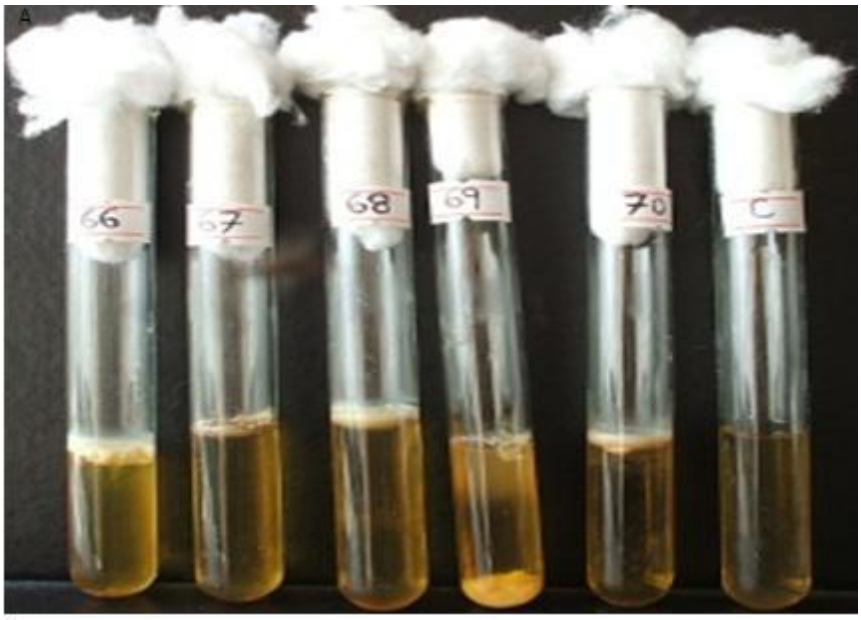

Fig. 4. Proteolytic activity of mesophilic, thermotolerant and thermophilic bacteria.
C= Control. 66= *Aeromonas* sp., 67= *B. licheniformis*, 68= *B. stearothermophilus*, 69= *B. subtilis*, 70= *Staphylococcus aureus.*

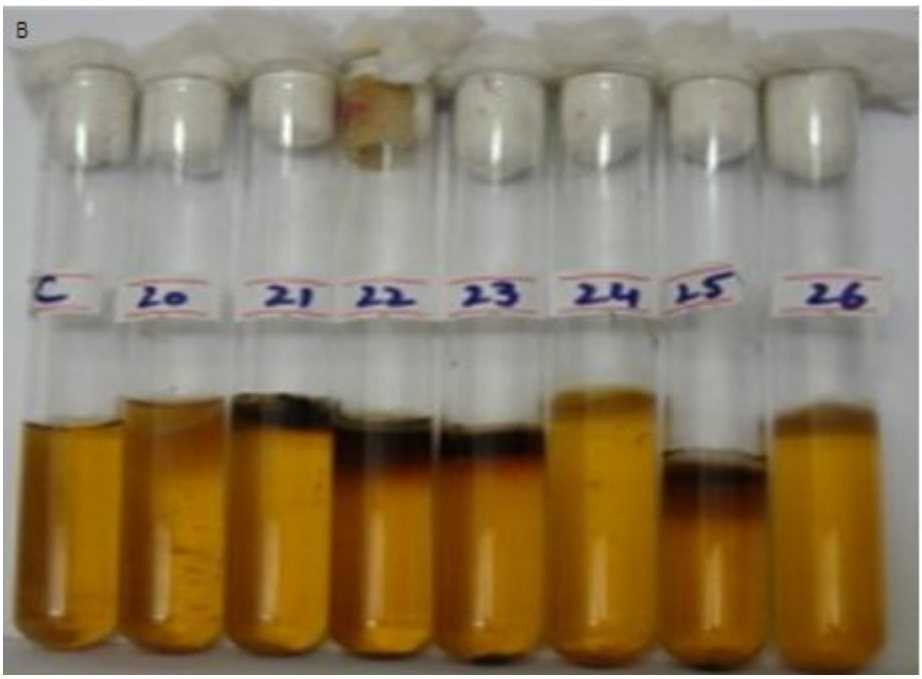

Fig. 5. Proteolytic activity of mesophilic, thermotolerant and thermophilic fungi.
C= Control, 20= *Conidiobolus thermophilus*, 21= *Humicola grisea* var. *thermoidea*, 22= *Alternaria alternate*, 23= *Botrytis cinerea*, 24= *Myriococcum thermophilum*, 25= *Aureobasidium pullulans*, 26= *Rhizomucor pusillus*.

Table 1. Enzymatic activity of fungi and bacteria isolated from compost samples.

S. No.	Test organisms	Amylolytic activity	Cellulolytic activity	Chitinolytic activity	Pectinolytic activity	Proteolytic activity
1	*Acremonium thermophilum*	-	+	-	-	-
2	*Acrophialophora fusispora*	-	-	-	-	-
3	*Aeromonas* sp.	+	-	+	-	+
4	*Alternaria alternata*	+	+	+	+	+
5	*Annelophora africana*	-	+	-	-	-
6	*Aspergillus flavus*	+	+	+	+	+
7	*Aspergillus fumigatus*	+	+	+	+	+
8	*Aspergillus terreus*	+	+	-	-	+
9	*Aspergillus nidulans*	+	+	+	+	+
10	*Aspergillus niger*	+	+	-	+	+
11	*Aspergillus neoniveus*	+	+	-	-	+
12	*Aspergillus rugulosus*	+	+	+	+	-
13	*Aspergillus floccosus*	+	+	-	-	-
14	*Aureobasidium pullulans*	+	+	+	+	+
15	*Botryotrichum piluliferum*	-	+	-	-	+
16	*Botrytis cinerea*	-	+	-	+	+
17	*Bacillus cereus*	+	+	+	-	+
18	*Bacillus licheniformis*	+	+	+	-	+
19	*Bacillus megaterium*	+	-	-	-	-
20	*Bacillus pumilus*	-	+	-	+	-
21	*Bacillus stearothermophilus*	+	-	+	+	+

22	*Bacillus subtilis*	+	+	+	+	+
23	*Chaetomium globosum*	+	+	+	-	+
24	*Chaetomium thermophilum* var. *coprophile*	+	+	-	-	-
25	*Chaetomium thermophilum* var. *dissitum*	-	+	-	-	-
26	*Conidiobolus thermophilus*	-	-	+	-	+
27	*Curvularia hawaiiensis*	-	+	-	-	-
28	*Emericella nidulans*	+	+	-	+	-
29	*Enterococcus* sp.	-	+	-	-	-
30	*Escherichia coli*	-	-	-	-	-
31	*Geobacillus toebii*	-	-	-	-	-
32	*Gilmaniella humicola*	-	+	-	-	-
33	*Haplotrichum croceum*	-	+	-	-	-
34	*Humicola fuscoatra*	+	+	-	-	-
35	*Humicola grisea* var. *thermoidea*	+	+	-	-	+
36	*Isaria fumosorosea*	+	+	+	+	+
37	*Klebsiella* sp.	-	-	+	-	-
38	*Micrococcus varians*	+	+	-	+	-
39	*Mucor fragilis*	+	+	+	+	+
40	*Myrothecium roridum*	+	+	-	-	+
41	*Myriococcum thermophilum*	+	+	-	+	+
42	*Myceliophthora sepedonium*	+	+	-	-	+
43	*Pseudomonas flourescens*	-	-	+	+	-
44	*Paecilomyces variotii*	+	+	+	+	+
45	*Paraconiothyrium minitans*	-	-	+	-	-
46	*Penicillium dipodomyis*	+	+	+	+	+
47	*Penicillium dupontii*	+	-	-	-	-
48	*Penicillium citrinum*	+	+	-	+	+
49	*Rhizomucor pusillus*	+	+	-	+	+
50	*Rhizomucor miehei*	+	+	-	+	+
51	*Scytalidium lignicola*	+	+	-	-	-
52	*Scytalidium thermophilum*	+	+	-	-	+
53	*Stachybotrys chartarum*	+	+	-	-	-
54	*Syncephalastrum racemosum*	+	+	+	-	-
55	*Staphylococcus aureus*	-	-	-	-	+
56	*Thermus thermophilus*	-	-	-	-	-
57	*Thermomyces lanuginosus*	+	+	+	+	+
58	*Trichoderma harzianum*	+	+	+	+	+
59	*Trichoderma virens*	+	+	+	+	+

DISCUSSION

Determination of enzymes activity is important in understanding microbial metabolism during composting (Mondini *et al*., 2004). In nature, most fungi and bacteria produce enzymes that degrade the complex organic molecules into very simple forms (Orth *et al*., 1993). The microbes produced amylase enzyme convert the starch into the oligosaccharides with quick reduction in blue colour and appearance of clear zone (Najafi *et al.,* 2005). During present studies, amylase enzymes produced by several thermophilic, thermotolerant and mesophilic bacteria and fungi. Similar report made by *B. subtilis*, *B. stearothermophilus*, *B. licheniformis* and *B. amyloliquefaciens* (Sivaramakrishnan *et al*., 2006), *Paecilomyces variotii* (Michelin *et al*., 2010), *Scytalidium thermophilum* (Aquino *et al*., 2003), *Thermomyces lanuginosus* (Kunamneni *et al*., 2005), *Aspergillus niger, A. flavus* (Al-Hindi *et al*., 2011). In this study we report here our novel findings of one thermophilic fungus *viz*., *Mucor fragilis*, three thermotolerant fungi *viz*., *Aspergillus floccosus, A. rugulosus* and *Emericella nidulans* are as sources of amylolytic enzymes.

The cellulase producing fungi and bacteria were selected on the formation of clear zone around their colonies on carboxymethyl cellulose agar plates (Immanuel *et al.,* 2006). Enrichment of compost with cellulotytic

microorganisms is one factor that can improve nutrient status of the compost (Shinde *et al.*, 1985). In this studies, cellulase enzymes produced by several thermophilic, thermotolerant and mesophilic bacteria and fungi. *Bacillus subtilis* (Shafaat *et al.*, 2011), *B. cerreus* (Lah *et al*., 2012), *B. licheniformis* (Acharya and Chaudhary 2012), *Aspergillus fumigatus, A. terreus, Corynascus sepedonium, Paecilomyces variotii, Scytalidium thermophilum* and *Thermomyces lanuginosus* (Abdullah and Al-Bader 1990), *Aspergillus niger* (Kang *et al*., ₵2004) have been reported earlier to produce cellulases. In addition, we found, two thermophilic fungi *viz*., *Annelophora africana* and *Haplotrichum croceum*, one thermotolerant fungus, *Aspergillus floccosus*, and one mesophilic fungus *viz., T. virens* are the novel potential sources of cellulolytic enzymes. Here, we also report for the first time the presence of cellulolytic activity in a bacterium *Micrococcus varians*.

Formation of clear zone around the colonies showed the chitinolytic activity and indicated a positive reaction (Sharaf, 2005). Liu *et al.* (2008) suggest that Chitinases play a major role in the defensive strategies of plants against fungal pathogens. In the present study, we have shown the presence of chitinase activity in several thermophilic, thermotolerant and mesophilic fungal and bacterial species as also reported in earlier studies for *Trichoderma harzianum* (Nampoothiri *et al*., 2004), *Thermomyces lanuginosus* (Guo *et al*., 2005), *Aspergillus nidulans* (Shin *et al*., 2009) and *Bacillus subtilis* (Wang *et al.*, 2006).

Mrudula and Anitharaj (2011) suggested that pectinase production was detected on the formation of clear zone around the~~ir~~ colonies in opaque white back ground. Pectinases are abundantly produced by saprophytic fungi, and decaying plant tissue represents the most common substrate for pectinase-producing microorganisms (Gummadi and Panda, 2003). The major degrader of fruit wastes are the pectinolytic bacteria (Rolz*et al.*, 2011). Castilho *et al*., (2000) observed pectin production using agricultural wastes as substrate. During present studies, pectinase enzymes produced by several thermophilic, thermotolerant and mesophilic bacteria and fungi. Similar results have been reported in earlier studies for *Aspergillus niger* (Mrudula and Anitharaj, 2011), *A. fumigatus* (Phutela *et al*., 2005), *Alternaria alternata* (Isshiki *et al*., 2001) and *Emericella nidulans* (Yadav *et al*., 2009). In addition, we found pectinase activity in thermotolerant *Aspergillus rugulosus* and *Micrococcus varians*. These fungal and bacterial species appears to be the new addition to the group showing pectinase activity.

Protease enzymes are extensively used in the food, pharmaceutical, leather and textile industries (Mozersky *et al.*, 2002). We found that several thermophilic, thermotolerant and mesophilic bacteria and fungi showed proteolytic activity. Similar reports have been made for *B. cinerea* (Abidi *et al*., 2008), *Chaetomium globosum (*Muhsin and Salih, 2000), *Penicillium chrysogenum* (Ikram-ul-haq *et al*., 2006), *Aspergillus terreus, Paecilomyces varioti, Trichoderma harzianum, T. virens* (Sohail *et al*., 2009) and *Staphylococcus aureus* (Karlsson and Arvidson, 2002).

Enzymatic hydrolysis is the significant method for the conversion of agricultural wastes into valuable product known as compost. Composting is a way of transforming agriculture waste into fertilizer accomplished by microorganisms which secrete enzymes. Agro industrial wastes such as grass clipping, fruit waste, leave and wheat straw are cheapest natural carbon sources for the production of industrially important enzymes. Theses enzymes that have numerous applications in food, drug and textile industries have been produced from thermophilic, thermotolerant and mesophilic bacteria and fungi from agricultural wastes. Utilization of agricultural wastes offers great potential for reducing the production cost and increasing the use of enzymes for industrial purposes.

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