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Bioactive Potential Assessment of Thermophilic Bacteria Present in Different Types of Compost

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The present investigation was carried out to find out the thermophilic microflora of compost and to study their bioactive potential. A total of 21 bacterial isolates were isolated of which 61.9% are found to be gram positive rods and 38% are gram negative rods. Highest thermophilic microorganisms are found in cow dung compost followed by mushroom compost. Gram positive organisms were identified as *Bacillus spp.* and *Paenibacillus spp.* and gram negative organism were identified as *Vibrio spp., Proteus mirabilis, providencia spp., Aeromonas spp.* out of 21 bacterial isolates 95% were found to be producer of cellulase, 90.4% were producer of amylase, 76.1% gelatinase, 76% lipase, 43% were protease producer. The quantitative estimation of amylase study resulted in highest production of amylase by *Bacillus licheniformis.*

Key Words: Compost, Thermophilic Microorganism, Enzymes, pH and Salt tolerance.

Compost is the decomposition of organic matter i.e. manures, straw, green waste etc. and microbiological characteristics must be the most effective parameter in considering compost. Alternative method of aerobic treatment of food waste known as Composting, which converts waste materials into hygienic, humus-rich, relatively stable product that conditions soil and nourishes plants (Mathur, 1991). The process relies on the right combination of carbon (wood, straw) and nitrogen (pig, poultry manure), the right moisture content and ability to blend and aerate the materials. Composting also leads to reduction in odour and the removal of pathogens. Composting process proceeds through 3 phases: mesophilic stage, thermophilic stage, maturation phase. Thermophilic composting is a rapid biodigestion process where the ideal conditions for the rapid growth and colonization of bacteria are created and maintained. This facilitates the expedient destruction and breakdown of organic materials, giving off heat as part of the biological reaction. Thermophilic organism**: -** A Thermophile is an organism - a type of Extremophile - that thrives at relatively high temperatures, between 45 and 80 °C (113 and 176 °F). Many thermophiles are Archaea. Thermophiles are found in various geothermally heated regions of the Earth such as hot springs like those in Yellowstone National Park and deep sea hydrothermal vents, as well as decaying plant matter such as peat bogs and compost. As a pre-requisite for their survival, thermophiles contain enzymes that can function at high temperature. Some of these enzymes are used in molecular biology (for example, heat-stable DNA polymerases for PCR) and in washing agents. Thermophiles are classified into obligate and facultative thermophiles: Obligate thermophiles (also called *extreme thermophiles*) require such high temperatures for growth, whereas facultative

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thermophiles (also called *moderate thermophiles*) can thrive at high temperatures but also at lower temperatures (below 50 °C). Thermophiles are group of fascinating microorganisms for enzyme studies and protein design. Due to the unusual properties of the enzymes isolated from thermophilic microorganisms such as: Thermostability, Stability against denaturing substance like detergents, organic solvents, Stability against high alkalinity & acidity, Suitable for fermentative processes etc, the present investigation was carried out to isolate thermophilic microorganisms from compost samples and study their enzymatic and biocontrol potential.

MATERIALS AND METHODS

Sample Collection

The compost samples were collected from various places. The Agriculture compost, mushroom compost was collected from the Agriculture field, the Department of Plant Pathogen, OUAT. The cow dung sample was collected from the OUAT Field. The Garbage waste was collected from Acharya Vihar road side and the Vegetable Waste was collected from the Unit-1 vegetable market. The fungal samples were obtained from the rotten citrus fruits and banana plants infected with banana wilt diseases. These samples were then brought to the laboratory for further analysis.

Isolation

Isolation of bacteria was done by 10fold serial dilution method. The diluted sample was taken from the tubes and the plated containing Tryptone soya Agar sterilized in 121º C and 15lb pressure for 20 mins. To the plates 1 to 2 drops of diluted sample was added and it was uniformly spread over the media with the help of L-shaped loop and rotor. The plates were then incubated for 24hrs at 50-90 ^{0}C inside an incubator. The number of colonies formed were observed & counted with the help of a colony counter. Then the colony forming units (CFU) was calculated.

Maintenance of Pure Culture

The colonies which were streaked out in the plates were maintained in the sterilized slants which containing TSA for further use. The total process was undertaken in LA under aseptic condition. After pure cultures were obtained they were maintained in the refrigerator and different parameters of the isolates were studied.

Growth, Morphology and Staining of the Organism (Bacteria)

Morphological characters of the bacteria were studied following the methods (Norris and Swain, 1971).Staining characteristics of the organism were studied by Gram's staining. After the microscopic examination the Gram negative bacteria and Gram positive rods were processed separately for identification.

Physiological and Biochemical characters

Physiological viz. temperature tolerance and halotolerance and biochemical characters of the organisms were checked following the standard identification method. Different biochemical tests like Oxidase test, catalase test , urease test, indole production test, methyl red , voges-proskauer (acetone production) test, nitrate reduction test, citrate utilization test, carbohydrate metabolism were studied.

Enzymatic activity

All the isolates were screened on pseudo-selective media for production of various industrially important extracellular enzymes like amylase, cellulase, Lipase, Trybutyrin hydrolysis test, Gelatinase, Pectinase, Caesinase and DNase etc following standard microbiological methods of Collins and Lyne (1970).

Quantitative Assay for amylase

Preparation of inoculums

The bacterial isolates were transformed from stock culture to 100ml nutrient broth. The inoculated flasks were incubated overnight at 37 °C. The broths were then centrifuged at 10,000rpm at 4ºC for 10minutes. After centrifuging, the pellets were resuspended in 10ml of sterile water and the absorbance was observed at 660nm to have a suspension of 4.5 x 10,000 cells/ml. The experiments were conducted in triplicate.

Assay of amylase - (By 3, 5-Dinitro salicylic acid (DNSA) method of Miller, 1959)

DNSA assay method was composed of 0.5% soluble starch, 0.3% Di-potassium hydrogen phosphate and 0.1% $MgSO_4.7H_2O$. A volume of 100ml of the medium was put in a conical flask and inoculated with 1ml of bacterial suspension. After 96 hours incubation, the broth was centrifuged at 8,000 rpm for 10 min. In 2 ml of supernatant, 100 µl of 1% starch was incubated with 1 ml of phosphate

Table 1. pH and Temperature of the compost samples

Samples	pH	Temp. (°C)
Agriculture compost	6.12	40.3
Mushroom compost	6.25	40.0
Cow dung	6.98	40.3
Garbage waste	8.27	40.3
Vegetable waste	7.75	40.1

Table 2. Aerobic Plate Count of different types of compost

Sample	Aerobic Plate Count (APC) in CFU/ml
Agriculture compost (AG)	23.5×10^2
Mushroom compost (MC)	24.7×10^2
Cow dung compost (CW)	27.1×10^2
Garbage waste (GW)	12×10^2
Vegetable waste (VW)	10×10^2
Mean	19.4×10^2

buffer (pH 6.5). The mixture was incubated for 20 minute before stopping the reaction by adding 0.5 µl DNSA reagent and cooling in a water bath for 10 minutes. A volume of 2.5 ml of distilled water was added, the absorbance was read at 540 nm using UV visible spectrophotometer against glucose as standard curve. One unit of enzyme activity is defined as the amount of enzyme which release 1µm of reducing sugar as glucose per unit, under the assay condition (1U/ml/min).

RESULTS AND DISCUSSION

The Present study was carried out in P. G. Department of Microbiology, CPGS, OUAT. The compost samples were collected from various places and brought to the department of microbiology for further study and maintained as pure cultures after identification.

The pH of the above five compost samples varied from 6.00 to 8.2 and the Temperature remained all most uniform i.e. mean at 40.2°C.

Determination of Aerobic Plate Count (APC)

This variation in PH may accounts for the differences in the no of CFU of bacteria in a particular sample. The Aerobic Plate Count (APC) of the bacteria present in the compost samples were determined Table 2.

Numerous authors have reported the presence of *Bacillus spp*. Yusaku Fujio and Shigeru Kume(1991) isolated twelve strains of thermophilic bacteria from sewage sludge compost under aerobic and anaerobic condition at 60^0C. On the basis of their physiological and biochemical

Table 3. Identification of Gram Positive organism

Gram positive organisms	
Agriculture compost (AC)	Organisms
AC-1	*Bacillus pantothenicus*
AC-2	*Bacillus Coagulans*
Cowdung (CW)	
CW-1	*Paenibacillus Polymyxa*
CW-2	*Paenibacillus alvei*
CW-3	*Bacillus coagulans*
Garbage Waste (GW)	
GW-1	*Bacillus epiphytes*
GW-2	*Bacillus stearothermophilus*
GW-3	*Bacillus stearothermophilus*
Vegetable waste (VW)	
VW-1	*Bacillus pantothenicus*
VW-2	*Bacillus megaterium*
VW-3	*Bacillus firmus*
VW-4	*Bacillus subtilis*
VW-5	*Bacillus licheniformis*

Table 4. The identification of Gram negative organisms

Gram Negative Organisms	
Mushroom compost	
MC – 1	*Proteus mirabilis*
MC – 2	*Providencia Spp*
MC – 3	*Vibrio spp*
MC – 4	*Providencia Sp.*
MC – 5	*Vibrio sp.*
Cow dung compost	
CW – 3	*Salmonella subgenus*
CW – 4	*Proteus vulgaris*
CW – 5	*Aeromonas hydrophila*

characters, nine strains were identified as *Bacillus stearothermophilus* and two strains were tentatively identified as *Thermus* species. These isolates were growing in the temperature range between 40-78^{0}C.

Test for the growth of the bacterial isolates at different Temperatures

This test was performed to see the temperature tolerance of these 21 isolates in varying temperature. Most of these bacterial isolates were found out to grow well at 50^{o}c to 70^{o}c and growth rate decreases after 80^{o}c. The detailed result is given in the table no.5. Some of the isolates i.e. AG1, AG2, GW2, GW3 were found to grow up to 90^{o}c showing its growth.

Out of five composts studied maximum no. of facultative thermophilic microorganisms was found in cowdung compost (27.1×10^2) followed by Mushroom compost as the temperature of cowdung compost was found to be the highest(40.3^{0}C) and Mushroom compost (40.2^{0}C).

Isolation and identification of the isolates

A total of 21 isolates of bacteria were obtained from the 5 different compost samples. All the bacterial isolates were identified on the basis of their Gram reaction, Biochemical test Bryant TN (2004). and a number of other tests.

Of the 21 isolates, 20 organisms survived at 60^o c, 18 organisms at 70^o c, 11 orgnisms at 80^{o}c and 5 organisms at 90^{o}c, which indicates that some are facultative thermophiles and *Bacillus stearothermophilus, Bacillus pantothenicus* could survive upto 90$^{o\,c}$ which was also reported by Ray & Sethy (2011) who had obtained *Bacillus stearothermophilus* isolated from hot springs growing at 90^o C.

Tests for the Halotolerant Nature

Detailed results of the halotolerant nature of the bacterial isolates are given in Table 6. Most of the gram negative isolates were found out to grow at 5% Nacl incorporated in Nutrient Broth and all the gram positive isolates were tolerating 15% NaCl concentration.

Table 5. Temperature Tolerance Test

Isolates No.	Temperature (°C)				
	50	60	70	80	90
AC-1	+	+	+	+	+
AC-2	+	+	+	+	+
MC-1	+	-	-	-	-
MC-2	+	+	+	+	-
MC-3	+	+	+	-	-
MC-4	+	+	+	+	-
MC-5	+	+	+	+	-
CW-1	+	+	+	+	-
CW-2	+	+	+	-	-
CW-3	+	+	+	-	-
CW-4	+	+	+	-	-
CW-5	+	+	+	+	-
CW-6	+	+	+	-	-
GW-1	+	+	+	+	-
GW-2	+	+	+	+	+
GW-3	+	+	+	+	+
VW-1	+	+	+	-	-
VW-2	+	+	+	-	-
VW-3	+	+	+	+	-
VW-4	+	+	-	-	-
VW-5	+	+	-	-	+

Table 6. Test showing Halotolerance properties

Isolates	control	Media with % NaCl Concentration (w/v)		
		5%	10%	15%
1	+++	+	-	-
2	+++	++	+	+
3	+++	-	-	-
4	+++	-	-	-
5	+++	-	-	-
6	+++	-	-	-
7	+++	-	-	-
8	+++	++	+	+
9	+++	++	+	+
10	+++	+	-	-
11	+++	+	-	-
12	+++	+	-	-
13	+++	-	-	-
14	+++	++	+	+
15	+++	++	++	+
16	+++	++	++	+
17	+++	+	-	-
18	+++	+	+	+
19	+++	+	+	-
20	+++	+	+	+
21	+++	+	+	+

Table 7. Enzyme Test Results

	Enzyme test results							
	Cellulase	Pectinase	Amylase	gelatinase	protease	lipase	caseinase	DNAase
Ag compost								
AC – 1	+	+	+	+	-	-	*	*
AC – 2	+	+	+	-	-	+	*	*
mushroom compost								
MC -1	+	-	+	+	-	+	-	-
MC -2	+	-	-	+	-	+	-	+
MC -3	+	-	-	+	+	+	-	+
MC -4	+	-	+	+	+	+	-	+
MC -5	+	-	+	+	-	+	-	+
Cow dung								
CW -1	+	-	+	+	-	+	*	*
CW -2	+	-	+	+	+	-	*	*
CW -3	+	-	+	+	+	-	-	-
CW -4	+	-	+	+	+	+	-	+
CW -5	+	-	+	+	-	+	-	+
CW -6	-	-	+	-	+	+	*	*
garbage waste								
GW -1	+	-	+	-	-	+	*	*
GW -2	+	-	+	-	-	+	*	*
GW -3	+	-	+	-	+	+	*	*
vegetable waste								
VW -1	+	-	+	+	-	+	*	*
VW -2	+	-	+	+	-	+	*	*
VW -3	+	-	+	+	+	+	*	*
VW -4	+	-	+	+	+	-	*	*
VW -5	+	-	+	+	-	-	*	*

Table 8. Quantitative estimation of amylase by bacterial isolates

Bacterial isolates	Amylase Unit (IU/ml/min)
Bacillus pantothenicus	0.085
Bacillus coagulans	0.080
Paenibacillus polymyxa	0.034
Bacillus stearothermophilus	0.095
Bacillus licheniformis	0.097

REFERENCES

1. Mathur, S. R. Composting process. In: Bioconversion of waste materials to industrial products. Martin, A. M. (ed.) Kluwer Academic publishers, *Dordrecht*, 1991; 147-183.
2. Norris, J. R. and Swain, H. Staining bacteria : *Material in Microbio*, 1971; **5A**: 105-134.
3. Collins, C. H. and Lyne, P.M. Microbiological methods, *University press, Baltimore*. 1970.
4. Bryant, TN. PIBWIN-software for probabilistic identification, *Journal Appl. Microbiol.* 2004; **97** (6):1326-1327.
5. Peter, F. Strom. Identification of Thermophilic bacteria in solid waste composting, *Environmental microbiol*, 1985; **50**(4): 906-913.
6. Yusaku , Fujio & Shigeru, Kume. Isolation and identification of thermophilic bacteria from sewage sludge compost. *Journal of Fermentation and Bioengineering*, 1991; **72**(5): 334-337.
7. Ray, P. and Sethy, A. Isolation and characterization and identification of thermophilic microorganisms of hot spring "Atri" of Orissa. *J. Microb. World*, 2011; **13**(1): p 50-54.

8. Lee, B. H. and Blackburn, T. H. Cellulase production by a thermophilic clostridium species, *Appl. Environ Microbiol*, 1975; **30**(3): 346-353.
9. Dash, T and Ray, P. Characterization and Quantification of Amylase from *Bacillus stearothermophilus*. *Journal of Pure and Applied Microbiology*, 2009; **4**: 303-308.
10. Bernfeld, P. α and β amylases. *Methods in Enzymol*; 1955, **1**: 149-158.
11. Aiyer, P. Amylase and their applications. *Afri. J. Biotechnol;* 2005.