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Analysis of Compost Parameters and a Molecular Approach on its Thermophilic Microflora

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Abstract- The present study deals on factors and parameters influencing composting. The temperature , pH, ash, moisture, carbon and nitrogen content estimated at every 15 days interval states that composting is high during thermophilic phase than mesophilic phase. The microbial loads of bacteria were estimated by SPC method in PBYG medium. charecterization was done by biochemical analysis, thermotolerance assay and by molecular methods. Amplification of 16s rRNA gene sequence and universal eubacterial primers 27F and 1492 R to give a product of approximately 1500 bp in length was amplified by PCR. Sequencing software windows VQ2, gene codes were used to assemble the fragment. The sequence data was analyzed by comparing in gene bank database using Basic local Alignment Search Tool (BLAST 2.2.1.6) in a nucleotide to nucleotide search of 16s rRNA gene sequence from type strain and species were identified as thermophilic *Bacillus pumilis and Pseudomonas stutzeri* showing 93% and 98% homology respectively.

Index Terms- Thermophiles, Compost, Composting Analysis

I. INTRODUCTION

The development of a proper crop residue management system is the only alternative method to provide optimum soil consistency with the minimization of environmental pollution. Rapid urbanization and industrialization in the recent years has also resulted in the production of enormous amount of wastes which pollute the environment. Composting of crop residues is a biochemical process in which diverse and mixed groups of microorganisms break down the organic materials into humus like substances. The resulting product has a lower bulk volume than the original raw organic materials It is stable , decomposed at a slow rate and can be returned to the soil without reduction in its high energy value. Balamurugan *et al.,* (1999). Composting is a microbial decomposition process in which easily degradable and putrescent organic waste is turned into a stable material compost and current method for understanding the communities in composting ecosystem composting is a controlled decay of organic matter in a warm moist environment by action of bacteria, fungi and other organisms(Sequi , 1986). Hence it is programmed molecular identification of thermophiles and parameter analysis of compost.

II. MATERIALS AND METHODS

The vegetable market waste was collected from nearby the Jaya College of Arts and Science, Thiruninravur, Chennai, India. A pit of size 1m x 1m x 1m was made and piles of vegetables waste were laid. The non-biodegradable materials were sorted out manually. The degradable matter was shreded into small pieces and spread in layer of one foot and exposed to the sun for two days. The wastes were mixed with cow dung slurry and allowed to stand for fifteen days for predigest ion. After fifteen days the content was mixed with cowdung (1:1) and spread plastic troughts in triplicate. Water was sprinkled everyday so as to maintain the optimum moisture level. The bedding material was turned periodically without disturbing the worms for uniform maturation of the compost. Compost was collected as sample for every 15 days from 0th day to 90th day. The physical characteristics such as temperature, pH, ash content, moisture content, carbon content and C: N ratio were determined. The microbiological characteristics such as standard plate count, biochemical and physiological tests were done to identity and characterize strain according to Bergey's manual of determinative Bacteriology John Holt *et al* (1994). The samples were taken from the central surface of composting material. The temperature of compost was measured by a thermometer at the sampling points

The compost sample (5g) was suspended in ultra pure sterile water. The suspension was adjusted to 50 ml and shaken for 30 min at 1800 rev/min. After sub-sampling the suspension were centrifuged at1500xg, 4˚c for 10 min. The supernatant was filtered through 0.22 µm filter units. The filtrate was measured for pH and then used for analysis of dissolved components by the method of Furke, (1997). Water contents were determined by weight loss of triplicate 5gm composting samples after drying at 105˚c for longer than 24 hours. The dried samples were ground and mixed well large pieces (about > 0.1mm) of wood chips, pieces that were not finely ground were removed from samples for ash content and C/N ration measurement, because lignin, a main component of wood material is not degraded using the composting process. Ash contents were determined by weight loss of 2 gm samples after burning at 550˚c for 4 hrs(Breed, 1957) . Total dissolved organic carbon is measured by Jackson(1973).

Isolated pure cultures were incubated at different temperature. Growth of cells at varied temperature was tested in 3 ml. Tryptic soy broth in 13x100mm tubes for 72 hours in water bath set to 30, 35, 40, 45, 50, and 60˚c and examined for turbidity

at 24 hours varied pH were tested in same manner with pH 4, 5, 5.5, 6, 6.5, 7.0, 8 and 9.0 incubated at 37 and 60˚c.pH range evaluated in cells grown in aerated NB set at 0.3 interval of pH are examined for turbidity at 24-48 hours. Growth dynamics of the cells were studied in 100 ml buffered TSB at 30˚c for24 hours.

The bacterial genomic DNA was isolated and amplification of 16s r RNA gene sequence was done using universal eubacterial primers 27˚F and 1490 R to give a product of approximately 7500bp in length. The amplified product was purified with montage PCR Ausubel *et al* (1999). *S*equence software (Windows V42 gene codes) was used to assemble the fragment. The sequencing date was analyzed by comparison of the gene bank, database using Basic Local Alignment Search Tool in a nucleotide to nucleotide search (BLASTN2.2.1.6). The 16S r RNA gene sequence from type strain of selected species was obtained from gene bank was aligned . Plasmids were isolated using alkali lysis method and their weight was analyzed by gel electrophoresis.

III. RESULTS AND DISCUSSION

The physico- chemical and microbial characteristics of compost in Table -1,fig 2 3 and 4. The ranges of temperature were measured and show highest during 60th day. The pH does not show significant change but was found to be high during 60th day. Ash content increased with age and suggests that mineralization of organic materials occurred Mesophiles and thermophiles were isolated and characterized. They identified as based on their biochemical reactions depicted (Table 5 and 6). The thermophiles doubling time and generation time at 30˚c in aerated buffer tryptase. Soy broth are 30 and 32 minutes at pH 5.9, 49 minutes at pH 7.1 and 52-48 min at 7.5.No: of generation is 6 generation They produced acid from fructose, glucose, maltose, mannitol, mannose, sucrose, sorbitol, lactase, arabinose, dulketol, mellibiose. Amplification of 16s rRNA gene sequence and universal eubacterial primers 27F and1492 R to give a product of approximately1500 bp in length was amplified by PCR. Sequencing software windows VQ2, gene codes were used to assemble the fragment. The sequence data was analyzed by comparing in gene bank database using Basic local Alignment Search Tool (BLAST 2.2.1.6) in a nucleotide to nucleotide search of 16s rRNA gene sequence from type strain and species were identified as thermophilic *Bacillus pumilis* and *Pseudomonas stutzeri* showing 93% and 98% homology respectively. (Table 8) The bands were observed in agarose gel electrophoresis. *Bacillus* showed 2 bands which were compared with the standard markers (DNA) and the molecular weight was determined as >33500D, 8990D. In case of pseudomonas species, 3 bands were observed and compared with the standard marker. The net weight is determined as >33,500D, 33100D, 6000D respectively.

The bands were compared with the standard marker (□ □ Hind III) and their molecular weight was determined. Bacillus sps- 10.5 kb, Pseudomonas sps- 10kb- 10.5kb, the sequencing of 16S rRNA of A and B was performed and BLAST I done to identify the nearest homology. The A strain shows 95% homology with Bacillus B strain shows (93%) homology with *Pseudomona stutzeri.*

The number of microbes have declined in the high temperature phase; since the mean maximum temperature was nearly 62c (Table-1) the decline in numbers may be due to increase in temperature or due to release of toxic metabolites or to catabolite repression as observed in fungi. In the literature on composting microbial production of toxins does occur but is associated with the initial stage of decomposition and disappears long before the end of thermophilic phase.Thus number of organisms vary mainly due to the shift in temperature,the two organisms *Bacillus pumilis* and *Pseudomona stutzeri.* The high temperature withstanding and wide temperature range makes them an excellent consortia to maintain the number of the compost microbial load as nearly constant for its stable activity in compost. The enhanced microbial activity accelerated the decomposition process leading to humification thus oxidizing unstable organic matter to stable forms

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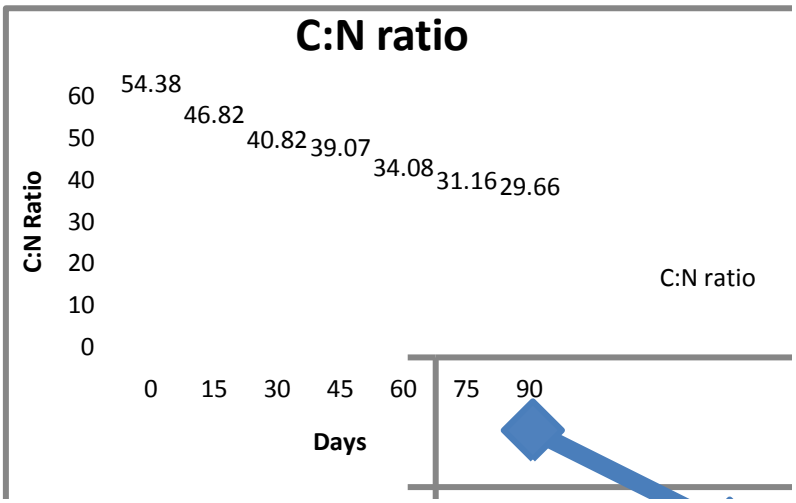
Table -1 .Physical parameters of compost

Days	temperature	pH	Ash content	Moisture content
0	29	6.9	50 ± 0.05	10.12 ± 0.32
15	31	7.2	56 ± 0.23	11.3 ± 0.65
30	47	7.5	56 ±0.23	13.3 ± 0.65
45	51	7.7	56 ±0.34	14.72 ±0.78
60	62	7.5	58 ±0.56	14.25± 0.65
90	56	7.4	59 ±0.45	14.59 ± 0.56

All the values are averages of 5 observations (Mean ± SE)

Fig: 1 Chemical parameters of compost.



All the values are averages of 5 observations (Mean ± SE)

Table -2 Enumeration of microorganisms from the compost.

Days	CFU/ml
Control	4.7×10^{7}
15	9.3×10^{6}
30	1.4×10^{6}
45	1.4×10^{6}
60	2.3×10^{4}
75	1.2×10^{5}

Fig 2-Optimal temperature and growth of mesophilic microorganism

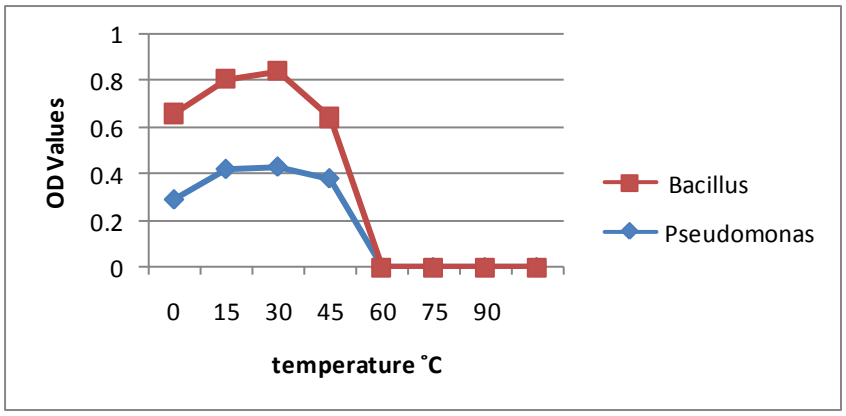
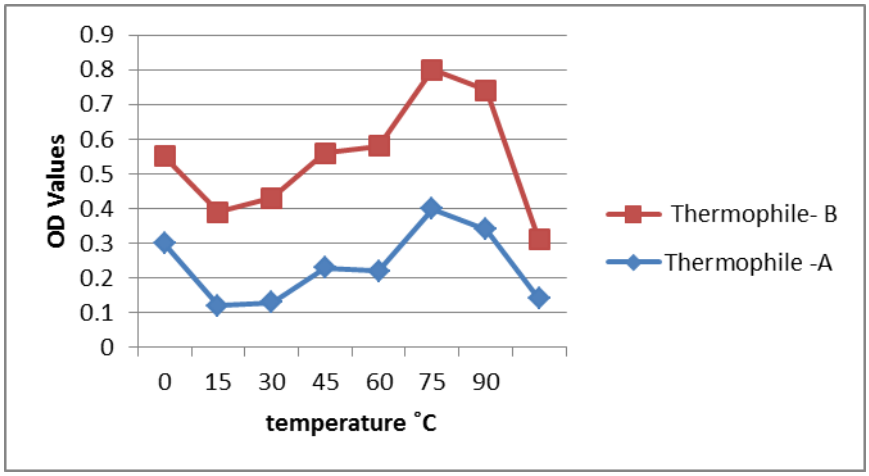


Fig 3- Optimal temperature and growth of Thermophilic microorganism



All the values are mean of ten observations