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Enzymatic analysis of Natural and Artificial Banana Leaf waste in Vermi-composting and Composting Technique.

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Abstract: In the present study, the possible utilization of natural and artificial banana leaf waste which were discharged from Marriage halls were collected and investigated for the enzymatic studies. The Natural and artificial banana leaf waste were mixed with cowdung in the ratio of 1:1 and kept aside in a shed for vermicomposting and composting. The isolated microbes were subjected for qualitative and the quantitative enzyme analysis for the naturaland artificial banana leaf waste. In that the presence of enzymes such as amylase, cellulase , protease and lipase contents in vermicompostand compost were identified. Thus the vermicompost and the compost enriched with enzymes greatly enhance the fertility of the soil. These technologies help in reduction of the organic load on land fill. According to obtained results the left over and old banana leaves processed to as vermicompost and compost may help boost crop yields in terms of economical production and organic farming.

Keywords: Vermicompost, organic farming, Natural and artificial Banana leaf waste.

Introduction:

Solid waste generation and accumulation in a large amount creates the ecological and technical problems leads to the declining trend in global productivity and environmental protection. (Aveyard 1988, wani and lee 1992, wani et al. 1995).In Indian cities, rural and urban areas waste generation estimates to 700 million tons of organic waste annually filled in the landfill areas or burned. (Bhiday, 1994). To resolve this Composting and vermicomposting are two of the best known-processes for the biological stabilization of a great variety of organic wastes (Domínguez& Edwards, 2010a). Vermicomposting is the process of using worms and microorganismsto convert organic matter intonutrient-rich humus. However, more than a century had to pass until vermicomposting, i.e. the processing of organic wastes by earthworms was truly considered as a field of scientific knowledge or even a real technology, despite Darwin (1881) having already highlighted the important role of earthworms in the decomposition of dead plants and the release of nutrients from them. In recent years, vermicomposting has progressed

considerably, primarily due to its low cost and the large amounts of organic wastes that can be processed. Banana is the second largest produced fruit after citrus, contributing about 16% of the world's total fruit production. India is the largest producer of banana, contributing to 27% of world's banana production. Banana is highly nutritious and easily digestible than many other fruits rich in potassium and calcium and low in sodium content. Whole banana plant is useful in food, feed, pharmaceutical, packaging, and many other industrial applications .Peel is rich source of vitamins, starch , crude protein , crude fat , total dietary fibre, and polyunsaturated fatty acids, particularly linoleic acid and a-linolenic acid, pectin, essential amino acids (leucine,valine, phenylalanine and threonine), and micronutrients (K, P, Ca, Mg) The objective of this study was to investigatethe potential of banana-agro waste (dried leaves) mixed with cow dung into vermicompost using earthworm *Eiseniafoetida,*to generate the comparative data on enzyme production and to go for qualitative and quantitative analysis of the enzyme.

MATERIALS AND METHODS: In the present study the natural and artificial banana leaf waste collected was composted by eiseniafoetida and to obtain the final product as vermicompost. The finally procured vermicompost were used for analyzing enzyme identification and quantification.

Collection of earthworm: The exotic earthworm, *Eiseniafoetida* commonly known as red wrigglers collected from the farm in and around Trichy area. The collected worms were cultured in the substrate of farm soil mixed with cowdung under laboratory condition with the temperature, a worm tolerable one.

Collection of substrate: The substrate used for this project was natural and artificial banana leaf (musaparadisica) waste. The artificial paper banana leaf waste was collected from the general merchant store in the Trichy town. The natural banana leaf waste was collected from the local market area in Trichytown. The collected natural banana waste and paper banana leaf waste was chopped into small pieces for easier decomposition. After that it was mixed with cowdung in the ratio of 1:1

Collection of animal waste:

Fresh cow dung excreta wastes were procured from farms located in Trichy city, India, on the first morning of mixing the waste with the cowdung.

Experimental set-up:

The experimental containers were prepared as per method described by Suthar2007**.** The experiments were conducted in plastic tub container had 20 cm length, 14cm width, 15 cm depth with the top of containers were perforated for aeration .The paper and natural banana leaf waste were chopped into small pieces (maximum size 0.05m) for to enhance the rate of vermicompost. Each tub had chopped waste and cowdung in the ratio of 1:1.Each experimental percentages

were run in triplicates to minimize any experimental error. After 15 days of decomposition of waste, *Eiseniafoetida* earthworm weighing around 0.04 to 0.05 kg numbering 20 were randomly introduced into each treatment. But one tub free of worms for each waste was maintained as control. The moisture level of these wastes was maintained by periodic sprinkling of adequate quantity of tap water around 70 to 80% throughout the study period.At maturation the vermicompost assumes dark to brown coloration becomes non sticky and odorless. Now the vermicompost product is ready for various analyses. The work started with many replications and afterwards restricted to minimum numbers of 3 replications in order to get accurate results for statistical authentication. To prevent moisture loss the containers were placed in a humid and dark room with a temperature of 28.5+_0.4^{o}.c. The isolated microorganisms were treated for the presence of enzyme qualitatively and also quantified for further analysis. Theenzymatic isolation procedures were discussed as below

Qualitative Assay:

For the enzymes identification from the vermicompost and compost, the following Amylase, Protease, cellulase, and lipase producing organisms were analyzed.

Enzymatic procedure for qualitative analysis:

1 gm of soil sample was taken and mixed with 9 ml of serial distilled water and the dilution was noted as 10-1. About 1 ml suspension from dilution 10-1 was taken and was further diluted upto 10-8. 0.1 ml suspension from dilution 10-6, 10-7, and 10-8 respectively was taken and was spread on the respective plates containing starch agar for amylase, Czapek mineral salt agar for cellulase, and skimmed milk for protease. The results were confirmed by zone formation and the isolated colonies were taken and stored in slant for further studies.

Quantitative Enzyme Assay:

After confirming the organism by the zone formation by qualitative analysis, the isolated colonies were subjected for amylase, protease, cellulase and lipase.

Enzymatic procedure for quantitative analysis:

Amylase:

A total of 6samples were collected for theisolation of amylase. Purified 6 bacterial isolates were screened for amylase degradation.

Amylase enzyme assay was performed by DNS (dinitrosalicyclic acid) method using glucose as standard. 1 unit of enzyme is determined by the amount of reducing sugar released per ml of sample (Miller, 1959). 1% starch solution was prepared by dissolving 1 g of starch in 100 ml

sodium acetate buffer and 40% of potassium sodium tartarate. From this 1 ml of starch solution was taken and 1ml of diluted enzyme solution was added. It was then incubated at 27^{o}c for 15 min. and this reaction was stopped by adding 2ml of DNS. The solution was heated in boiling water bath for 5min followed by addition of 1ml of potassium sodium tartarate and was cooled in running tap water. The volume was made upto 10 ml by adding water and the absorbance was noted at 560nm.

Protease:

A total of 6samples were collected for theisolation of Protease. Purified 6 bacterial isolates were screened for protease degradation.The isolated organism was tested for protease activity by observing the zone of clearance in skim milk agar plate.

Protease enzyme assay was performed by denaturalizing 5 ml of casein by heating and by dissolving in 0.05M phosphate buffer (pH 7.5). To this 1ml of culture filterate was added to the mixture and was kept for 10 min at 37^{o}C.After incubation period, 5ml of TCA was added. It was left for centrifugation at 4000rpm for 10 min. the Tyrosine released was determined using folin calorimetric method (Nanniperi et al., 1980).

Cellulase degrading Bacteria & Halo Zone Formation:

A total of 6samples were collected for theisolation of cellulose degrading bacteria. Purified 6 bacterial isolates were screened for cellulose degradation on 1% CMC-Na medium and confirmed by the Congo red dye test where only three isolates showed best hallow zones (Fig. 3). Among the three bacterial isolates, B. megaterium S3 showed the largest zone and was selected for further study. Lu et al. (2005) reported fifteen bacteria that grew vigorously and showed the ability to develop clearing zones around their colonies on cellulose Congo-red agar during aerobic incubation. The clearing zone size and colony diameter of the isolatewere indicating that the isolate has high ability of cellulase production. Isolation and characterization of cellulolytic bacteria have been reported by many workers (Maki et al., 2011; Irfan et al., 2012).the cellulose assay was determined by the method (Bailey, M.J and Nevalainen, K.M.H., (1981).

Lipase:

Lipase is the enzyme used to convert lipid into fatty acid. The presence of lipase in the organic waste is low so their role is limited in the carbon cycle while compared with other kind of waste which shows high fat content (Gea et al., 2007).

Lipase Enzyme assay was performed by preparing 20µl of sample in 1ml of culture medium which contains 0.5% of Peptone, 0.3 % of yeast,1% agar supplemented with 0.1% tributryin were added in a petridish .then the inoculated plates were kept for 7 days incubation. The level of

lipase production was analyzed by measuring the width of clear zones (Halo) around the colonies with the hydrolysis of tributryin was determined by using tributryin assay method (N.Griebeler et al., 2011).

Results and discussion:

Enzymes are the main agents for the degradation of different types of wastes (Tiqui 2001).Here the enzyme activities are recorded for the natural and artificial banana leaf waste for both the composting and vermicomposting Samples.

Isolation and Identification of bacterial strains

Naturally occurring microorganisms are the primary decomposers that accomplish composting. These microorganisms include bacteria, fungi, actinomycetes and protozoa. They break up waste debris and facilitate the decomposition. Microbial activity is considered to be greatly stimulated by favorable conditions (moisture content, pH, high concentration of mucus) in the anterior part of the gut; in the midgut this enhanced microbial activity results in the digestion of soil organic matter. The bacterial species identified in the vermin compost of lingo cellulosic waste (Table 1 and 2).

Table 1: Total Bacterial count of Vermicompost.

S.No	Vermicompost	Total Heterotrophic bacterial population(CFU/ml)	
		Before	After
1.	Natural banana leaf waste	THTC	106 X 10^{-4}
2.	Artificial banana leaf waste	THTC	158 X 10^{-4}

Table 2: Total Bacterial count of Compost.

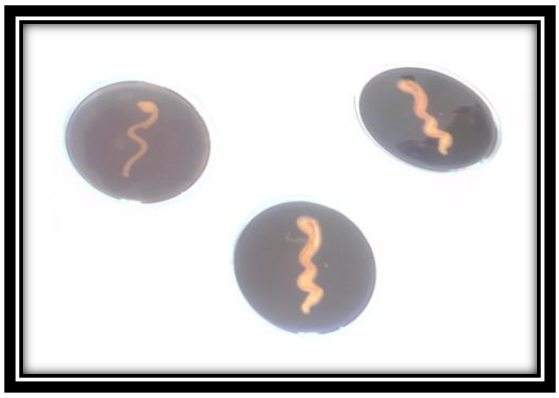
S.No	Vermicompost	Total Heterotrophic bacterial population(CFU/ml)	
		Before	After
1.	Natural banana leaf waste	THTC	92 X 10^{-4}
2.	Artificial banana leaf waste	THTC	122X 10^{-4}

Enzymatic Analysis:

Isolation of Enzymes activities: The isolated microorganism were analysed for their enzyme activity. The organism showed amylase, protease, and cellulaseand lipase activity. Hence this result would help us to understand presence of this organism plays an important role in the vermicompost and compost still more effective**.** The following table (3& 4) represents the qualitative enzyme activities of isolated bacterial strains with respect to the enzymes such as amylase, cellulase and protease and lipase.

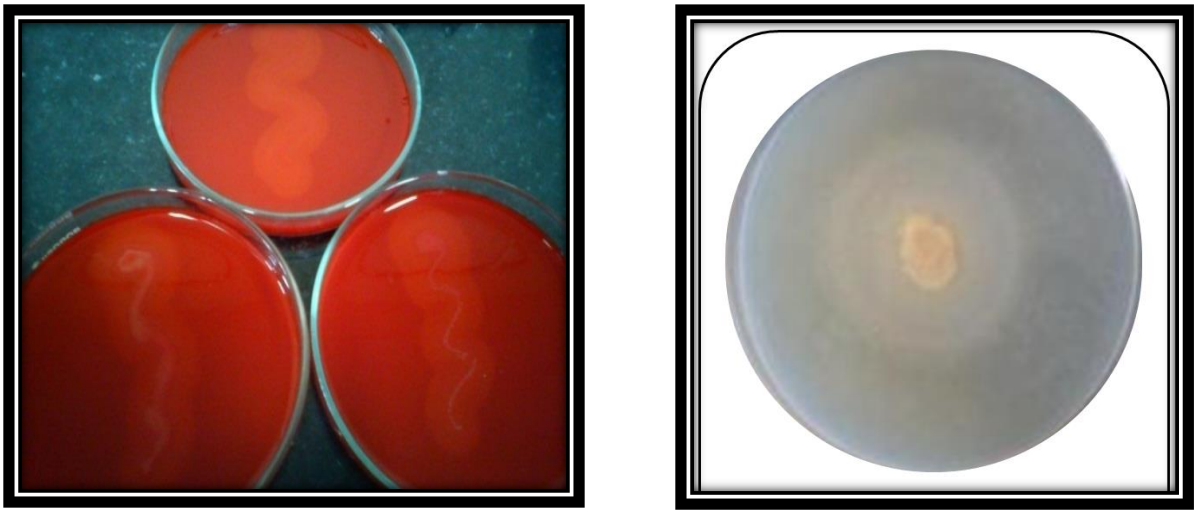
Table 3: Qualitative Enzyme assay of the isolated bacterial strains from different wastes (Natural and Artificial banana leaf waste) for Vermicomposting:

Enzyme	***azotobacter***	***azospirillum***	***Bacillus***
Amylase	Positive	Positive	Positive
Amylase	Positive	Positive	Positive
protease	positive	positive	Positive
lipase	positive	positive	Positive



PRESENCE OF AMYLASE

PRESENCE OF PROTEASE



PRESENCE OF CELLULASE PRESENCE OF LIPASE

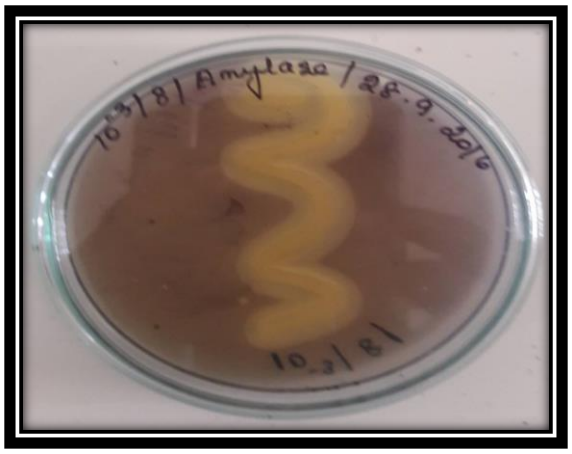
Fig 1: ZONE OF FORMATION FOR NBL AND ABL BANANA LEAF WASTE IN VERMICOMPOSTING

The Natural banana leaf waste was reported to have the strains such *as azotobacter and azospirillum*whereas the artificial banana leaf waste had the bacillus strain for the vermicompostwhich were subjected for enzymatic analysis. The results found to be positive for the amylase, cellulase, protease and lipase. Their presence of zone of formation were shown in the plates.

Table. 4. Qualitative Enzyme assay of the isolated bacterial strains from different wastes (Natural and Artificial banana leaf waste) for composting:

Enzyme	***azotobacter***	***azospirillum***	***bacillus***
Amylase	Positive	Positive	positive
Cellulase	Positive	Positive	Positive
protease	positive	positive	positive
lipase	positive	positive	positive

The Natural banana leaf waste was reported to have the strains such *as azotobacter and azospirillum*whereas the artificial banana leaf waste had the bacillus strain for the compost which were subjected for enzymatic analysis. The results found to be positive for the amylase, cellulase, protease and lipase. Their presence of zone of formation were shown in the plates

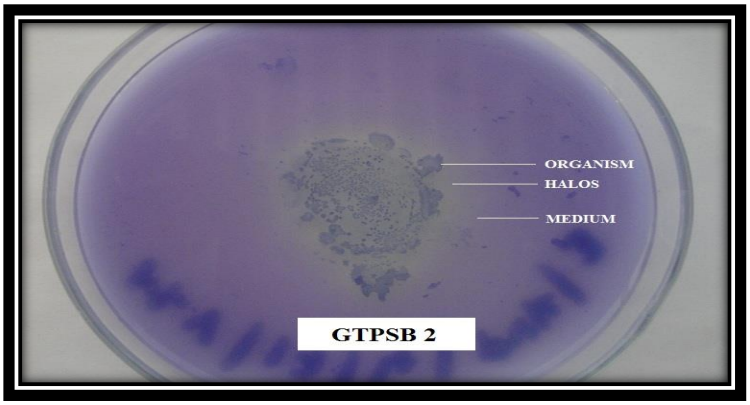


Isolation of amylase producing organism

Isolation of protease producing organism

Isolation of cellulase producing organism

Fig 2; ZONE OF FORMATION FOR NBL AND ABL BANANA LEAF WASTE IN VERMICOMPOSTING



Isolation of cellulose degrading Bacterial organism (*Bacillusmegaterium)*

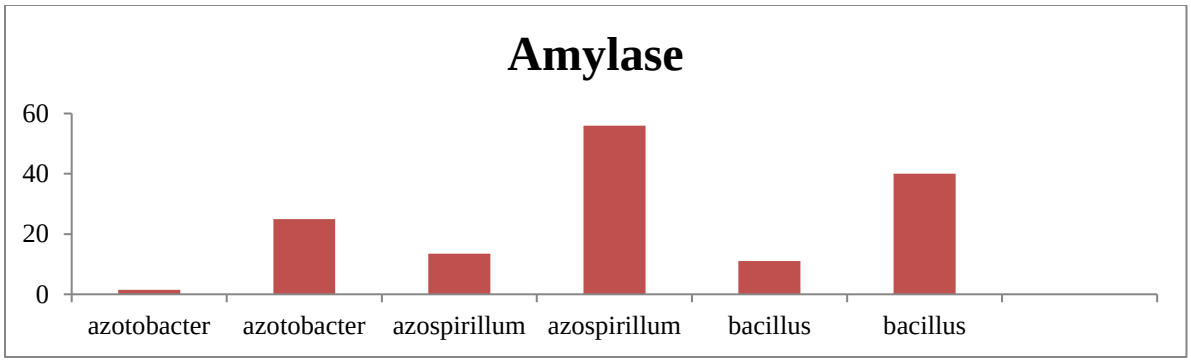
Fig 3: HALO FORMATION OF BACTERIA:

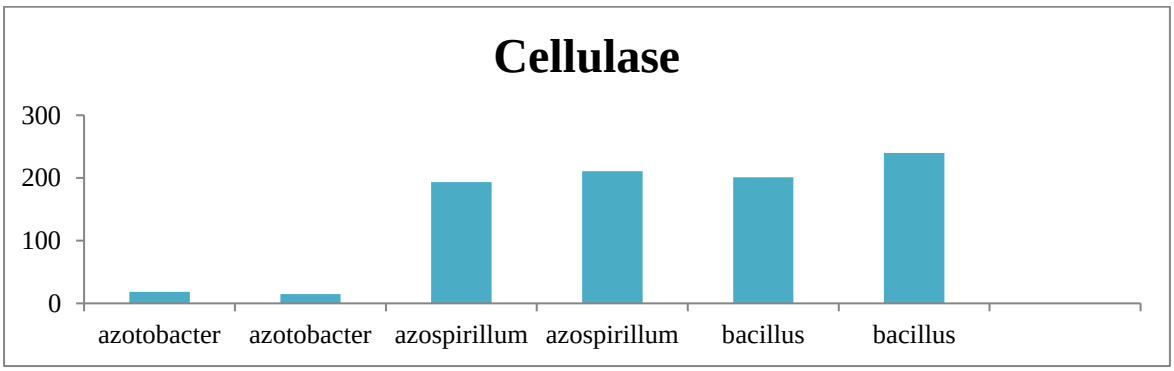
For the cellulase production alone bacteria reported to produce the Halo zone formation which was confirmed significantly from the above plate.

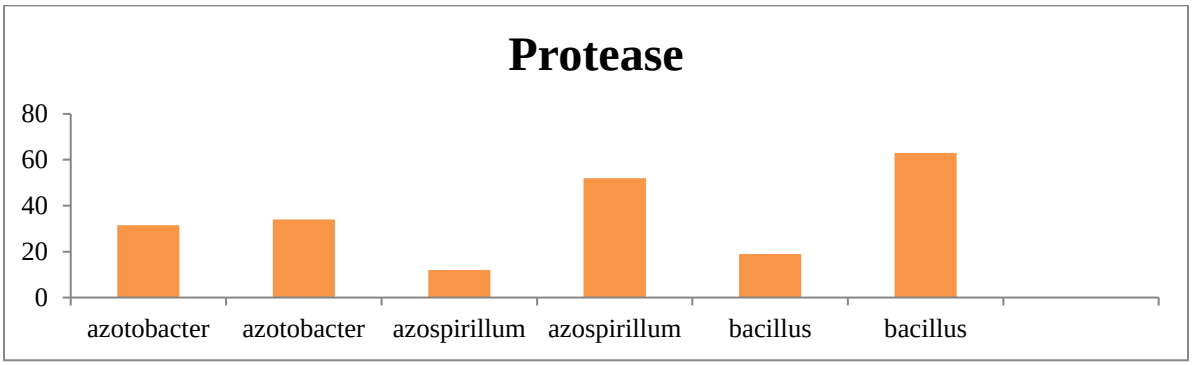
Table: 5: Quantitative enzyme assay of the isolated Bacterial Culture:

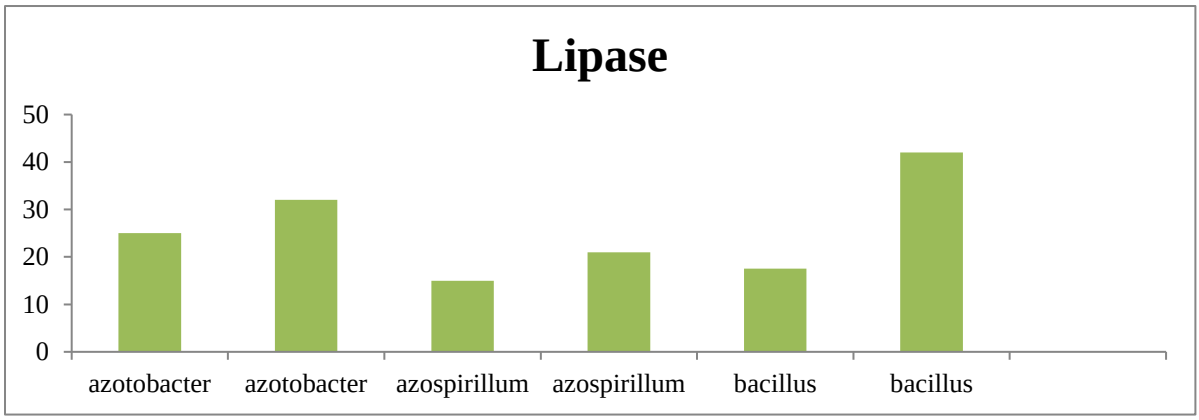
The cellulose enzyme produced by the isolated bacterial strains wasestimated.Totally 7 strains were isolated and the presence of enzymes had been confirmed such as amylase, protease and cellulase respectively. By quantification we obtain the following results

S.No.	Iso late	Identification	Strain	Lipase	Cellulase	Amylase	Protease
1	R1	Azotobacter	AZR1	25 U/ml	18.5 U/ml	1.5 U/ml	31.5 U/ml
2	R2	Azotobacter	AZR3	32 U/ml	15 U/ml	25 U/ml	34U/ml
3	R3	Azospirillum	AZM	15 U/ml	193.5 U/ml	13.5 U/ml	12 U/ml
4	R4	Azospirillum	AZM	21 U/ml	211 U/ml	56 U/ml	52 U/ml
5	R5	Bacillus	BM1	17.5 U/ml	201 U/ml	11 U/ml	19 U/ml
6	R6	Bacillus	BM3	42 U/ml	240 U/ml	40 U/ml	63 U/ml


Amylase
60
40
20
0
azotobacter
azotobacter
azospirillum
azospirillum
bacillus
bacillus


Cellulase
300
200
100
0
azotobacter
azotobacter
azospirillum
azospirillum
bacillus
bacillus


Protease
80
60
40
20
0
azotobacter
azotobacter
azospirillum
azospirillum
bacillus
bacillus


Lipase
50
40
30
20
10
0
azotobacter
azotobacter
azospirillum
azospirillum
bacillus
bacillus

Discussion: The vermicompost and compost contains high content of humic-like substances which are rich in microbes and enzymes enhance faster degradation of biodegradable waste.Thisstudy help us to determine the role of specific enzymes like amylase, cellulase, protease and lipase both qualitatively and quantatively from the bacterial strains isolated from the vermicompost and the compost.

Quantitative analysis: For the Natural and artificial Banana leaf waste in vermicomposting and composting totally produced 6 strains. From which the isolated bacterial strains named are Azotobacter, azospirillum and Bacillus were analysed for their enzyme production. Almost all strains produced the positive results but the halo zone formation only for the Bacillus species that are shown in the plate above.furtherthe isolates were checked for their respective quantitative production of the respective enzymes.

Qualitative Analysis: The isolates which displayed amylase, cellulase,protease and lipase activity in the respected plates were further subjected for quantification of respective enzyme assay by using different substrates.

The isolated strains from NBL and ABL OF natural and artificial banana leaf waste had produced azotobacter strain for 1.5,25 mg of glucose /ml , azospirillum strain for 13.5,56 mg of glucose /ml, bacillus strain for 11, 40 mg of glucose /ml using starch as the substrate there by proving its potential to produce the amylase enzyme in an efficient manner. the protease producing bacteria by quantification exhibit the results as follows azotobacter strain for 31.5, 34mg of glucose /ml , azospirillum strain for 12, 52 mg of glucose /ml, bacillus strain for 19, 63, mg of glucose /ml in an efficient manner. The cellulase production for the isolates were reported to be for the azotobacter strain for 18.5, 15mg of glucose /ml,azospirillum strain for 193.5, 211 mg of glucose /ml, bacillus strain for 201, 240bmg of glucose /ml in an efficient manner.For the lipase production, the isolate reported to produce azotobacter strain for 25,32mg of glucose /ml , azospirillum strain for 15, 21 mg of glucose /ml, bacillus strain for 17.5,42 mg of glucose /ml in an efficient manner.

Conclusion: The results confirm that these isolates had facilitated the organic matter decomposition.it may be because earthworm could digest the organic matter only with the enzymes such as amylase, cellulase, protease, and lipase produced by the gut microflora (Aira et al., 2005).the enzyme activity in vermicompost and compost of Natural and artificial Banana leaf waste clearly indicates these can be used as an efficient fertilizer for the soil and the plants consumed by humans to live their long and healthy life and help the chain to continue with the good and efficient vermicompost.

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