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Exploitation of Cellulase Producing Bacterial Strains from Mangrove Soils for Rapid Composting of Leaf Litter

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Abstract: Climate change has become unavoidable due to the increase in carbon dioxide emissions all over the world. The best way to counter this is would be increasing the tree cover. Reforestation or afforestation leads to accumulation of leaf litter which can be used as a source of composted fertilizer obtained by the decomposition taking place naturally at the site itself but it is observed that certain bacterial strains are capable of rapidly decomposing the leaf litter and converting it to nutrient rich compost and this is the need of the hour. In the present investigation cellulose producing bacteria were isolated from the soil samples collected from Pichavaram in Tamil Nadu, India, which is mangrove forest area, rich in micro-organisms that break down woody branches and fallen leaves in a saline environment. The bacteria isolated were screened for cellulase activity both qualitatively and quantitatively and out of the 20 isolates, 15 tested positive for cellulase activity. Of these two strains BN2 and BN11 showed highest activity and highest level of quantified enzyme. These two strains were used to inoculate leaf litter to bring about decomposition and compared to a control that was allowed to decompose naturally. The composting was completed in 45 days in the treated set up as compared to the natural composting that took about 120 days. The generated composts were analysed for nutrients and phytohormones at regular intervals from 30 days onwards which was gradually increasing showing a maximum in compost generated by the bacterial strain BN2 and minimum in the naturally generated compost. This study is significant in helping to identify a rapid composting method as well as providing a source for producing an industrially important enzyme namely cellulase.

Keywords: Compost, Nutrients, Phytohormones, Mangrove forest, Industrial enzyme

Forests play an important role in the maintenance of the biological equilibrium and act as the lungs of the planet. Raising forests has become very essential as environmental degradation is taking place at an accelerated rate. Whatever forests are available serve as a source of leaf litter that can be put to good use if properly processed. Leaf litter and other woody wastes generated in forests is naturally decomposed by the soil microbes and results in the cycling of nutrients. But in areas where tree density is high, it becomes necessary for the periodical removal of litter and a faster means of composting would yield commercially valuable manure. Areas like Mangroves have a concentration of salt in the substratum with an enormous production of woody wastes on a daily basis. Mangroves as an ecosystem are highly productive and here bacteria are involved in bio-mineralisation and biotransformation of biological wastes (Gonsalez-Acosta et al 2006). The major products of recycling include the detritus which is rich in a large microbial population helping in the cycling of carbon, sulphur, phosphorus and nitrogen (Rojas et al 2001). About 30 to 50% of the organic matter of mangrove leaves have tannins and sugars that are leachable and the remaining is the structural component having cellulose which is a linear polysaccharide composed of glucose units linked by 1-4glycosidal linkages.

The carbon and energy for the animal consumers is transferred from mangrove detritus through the grazing of lignocellulosic material decomposed by the bacterial and fungal populations in the detritus. These microbes have an enzyme called cellulase that is able to degrade cellulose and are important enzymes used in the food, feed, textile and pulp industries (Behera et al 2014). Hence the present study was undertaken to identify cellulase producing bacterial strains from Pichavaram, a mangrove forest located 250 kilometer from Chennai, Tamil Nadu, India, and to screen them for production of the enzyme cellulase and utilize the prospective strains for rapid composting of leaf litter.

MATERIAL AND METHODS

Sampling location and sample collection: Pichavaram is a village near Chidambaram in Cuddalore district, Tamil Nadu, India. It is located between the Vellar estuary in the north and Coleroon estuary in the south. This Vellar-Coleroon estuarine complex forms the Killai backwaters and the mangroves are rooted in three to five feet in water having geographical location is latitude 11°29'N and longitude 79°46'E. The soil samples were collected from different locations of the mangrove forest which were chosen based on the presence of degrading plant litter at a minimum

distance of 1000 m between the sampling sites. About 100 g of the collected samples were stored at 4°C in sterile containers, ensuring the samples are free from unwanted materials. Nutrient Agar was the medium used for culturing the bacteria in the samples and the cultured bacteria were subjected to serial dilution and plating to obtain pure cultures that could be screened for cellulolytic activity

Cellulolytic bacterial screening: To screen for cellulolytic organisms, the isolates were grown in minimal agar plate consisting of yeast extract (0.2%), KH_2PO_4 (0.1%), $MgSO_4$ (0.5%)and carboxy methyl cellulose (CMC) -(0.5%). Negative control plates inoculated with laboratory *E. coli* strain (HB101) were included in all tests. The test plates were incubated at 30°C for 2 days. The hydrolysis zones were visualized by flooding the plates with an aqueous solution of Congo red (1 mg/ml) for 15 minutes and washed with 1M NaCl (Teather and Wood 1982). Cellulolytic organisms produced a clear zone around the colonies because of the digestion of carboxy methyl cellulose (CMC) the zone of digestion was measured and documented.

Cellulase enzyme activity assay: Carboxy methyl cellulase activity was assayed using methods described by Mendells and Weber (1969). The activity was estimated using 1% solution of carboxy methyl cellulose in 0.05 M citrate buffer (pH 4.8) as substrate. The reaction mixture contained 0.5 ml of substrate solution and 0.5 ml of suitably diluted enzyme solution and reaction was carried out at 50°C for 30 minutes and the reaction was stopped by adding 3 ml of DNS solution to the reaction mixture. The reaction mixture was then boiled at 100°C for 5 minutes. The optical density was taken at 540 nm. One unit of carboxy methyl cellulose activity expressed at 1µmol of glucose liberated per ml of enzyme per minute. The values were compared with a glucose standard curve.

Method of composting: Pot study was carried in the laboratory to produce the compost by setting up the vermicompost, bacterial compost and natural compost system each in separate pots. Vermibed was prepared with a layer of good moist loamy soil of 5 cm thickness placed at the bottom and above it coarse sand and broken bricks of 4 cm thickness were placed with small holes drilled at the bottom for air circulation and drainage. The pots were of equal size with 60 cm height and circumference of 36 cm at bottom and 88 cm at the top. In each pot 3 kilogram of substrate (leaf litter) was used for all the treatments. Twenty-five adult individuals of *Eudrilus euginae* (earthworms) were introduced on the top of the substrate for vermicompost and 10 ml of bacterial inoculum was added in the bacterial composting setup. The third pot which was the control contained only the substrate. The process of composting in the 3 setups were carried out until the leaves were completely decomposed.

Physico-chemical parameters: The pH of the compost was measured using a digital Ph meter and electrical conductivity using an electrical conductivity meter. Mineral nutrients like phosphorus was determined using the method of Cook (1997), potassium and calcium by the method of Kalra (1971).

Quantification of plant growth regulators (PGR's): Extraction and estimation of growth regulators like indole acetic acid (IAA), gibberellic acid (GA3) and kinetin (KN) in the 3 extracts of generated composts were performed according to the method of Unyayar et al (1996).

RESULTS AND DISCUSSION

The sampled soil when cultured on nutrient agar medium showed the presence of different bacterial strains. 20 colonies with varying morphology were selected from the mother plate and subculture on nutrient agar plates using quadrant streaking method. Pure colonies were obtained in all the 20 plates after 24 h of incubation.

Bacterial screening Of the isolated bacterial strains, 15 cultures showed cellulolytic activity on CMC plates. The highest zone of 2.1 cm was observed in strains BN2 and BN20 while a zone diameter 2 cm was formed in strain BN11 followed by strains BN13 and BN16 that each showed a zone of 1.9 cm in diameter. The least zone of digestion was observed in strain BN19 while strains BN 5, BN 6, BN 10, BN 17 and BN18showed no zone of digestion and were not used for further analysis (Table 1). Padhan et al (2023) have isolated cellulase producing bacteria like *Pseudomonas flourescens*, *Bacillus subtilis* and *Serratia macrescens* from the soil and studied the factors affecting cellulase production.

Quantification of cellulase enzyme activity: In the present study, 15 strains that showed cellulolytic activity in the plate method were subjected to enzyme quantification studies by spectrophotometric assay. The strain BN2 had the highest cellulase activity of 0.521 U/ ml followed by the strain BN11. The activity in strain BN20 was 0.489 U/ ml and the least was observed in strain BN19 (0.245 U/ml) (Table 2). Gupta et al (2012) recorded a cellulase activity ranging from 0.162 IU/ ml to 0.400 IU/ ml . Sreena et al (2016) reported an activity level of 5.06 U/mg for *Bacillus cereus* isolated from termites. Ali et al (2019) have screened bacteria from a sub-terranean species of termite and the cellulase activity of the 5 isolated strains ranged from 0.1U/ ml to 1.47 U/ ml. Bhagat and Kokitkar (2021) have isolated cellulose degrading bacteria from the soil and have optimized the cellulase activity.

Composting: Among the different strains, the one that had high cellulolytic activity with high level of cellulase enzyme was strain BN2 and this was used to bring about composting of leaf litter. This was compared with vermicomposting and natural composting.

Treatment I: Natural composting: The setup containing only leaf litter was allowed to undergo decomposition naturally, and at the start of the experiment the temperature of the substrate was high and then decreased gradually from 34°C to 26°C, stabilizing at 25°C and composting was completed in 120 days

Treatment II: Leaf litter treated with earthworms (*Eudrils euginae)* for vermicomposting: At the start of the experiment, the temperature rose to 34°C and decreased to 23°C on maturation and composting was completed in 90 days.

Treatment III: Leaf litter treated with bacterial strain BN2: The temperature was high initially and rose up to 35°C and then it fell gradually reaching 22°C with a stable pH. The composting was completed in 45 days.

Physico-chemical parameters of the generated composts: The study of the physico-chemical parameters revealed that there was a progressive decrease in pH and increase in electrical conductivity as composting progressed and this was more in the case of bacterial composting. The nutrients in the bacterial compost including calcium, phosphorus and potassium were in the bacterial compost as compared to vermicompost and natural compost (Table 2). The level of plant growth regulators (PGR's) was high in the bacterial compost in comparison to the other composts generated (Fig. 2a to 2c).

Completion of the composting process is revealed by the lowering of temperature and then the stability of temperature and pH, increase in the C/N ratio and an increased germination index as reported by Oktiawan et al (2018). The use of microbial inoculants to reduce composting time has been earlier reported by Pan et al (2012). The fall in temperature of

Table 1. Zone of digestion in CMC agar plates showing cellulase activity

Isolate	Zone (cm)
BN-1	1.8
BN-2	2.1
BN-3	1.8
BN-4	1.8
BN-5	0.0
BN-6	0.0
BN-7	1.3
BN-8	0.7
BN-9	1.5
BN-10	0.0
BN-11	2.0
BN-12	1.4
BN-13	1.9
BN-14	0.8
BN-15	2.0
BN-16	1.9
BN-17	0.0
BN-18	0.0
BN-19	0.5
BN-20	2.1

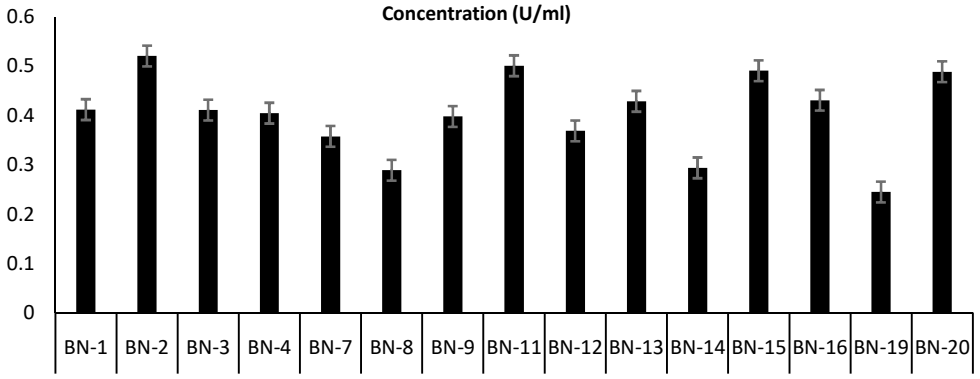
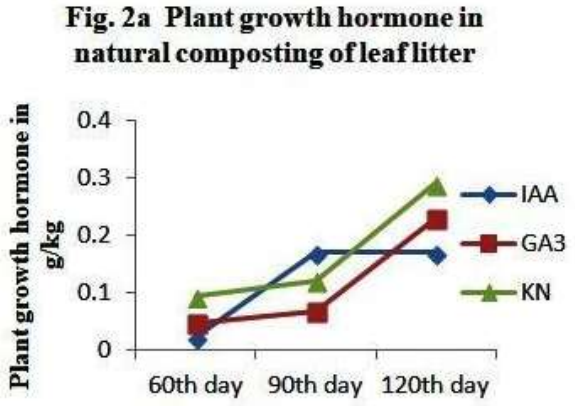
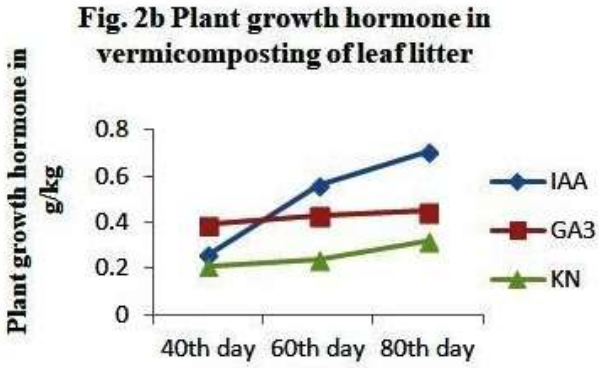


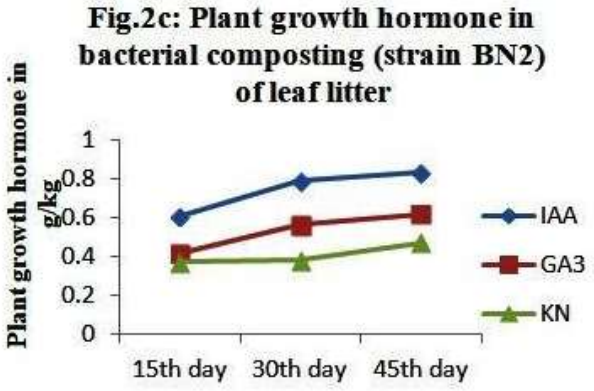
Fig. 1. Quantification of enzyme cellulase from the selected isolates

Table 2. Physico-chemical properties of the generated composts

Treatment	pH	EC (µS)	Phosphorus (P) mg/gm	Potassium (K) (mg/gm)	Calcium (Ca) (mg/gm)	Nitrogen (mg/gm)
Control (110th days)	6.3	1.29	0.29	0.273	0.192	0.49
Treatment I (80th)	5.9	1.31	0.063	0.341	0.319	1.97
Treatment II (42nd day) strain BN2	5.7	1.43	0.13	0.49	0.671	2.41







the compost indicates low microbial activity and the low amount of organic material as was converted to inorganic nutrients. This is evident from the high level of mineral nutrients present in the composts. This has also been observed in the earlier studies of Zakriya et al (2018) during the composting of rice straw. The pH according to them, varies from day1 to day 30 and is on the increasing trend due to decrease in organic matter. In the present study the decrease in pH in the bacterial compost was due to the high lignin content of the leaf litter used for composting (Toumela et al 2000). Athirai et al (2021) have shown increased level of micronutrients like phosphorus, potassium and calcium and have attributed this to the speedy mineralization brought about by composting which has also been further confirmed by Alade et al (2019)

CONCLUSION

The isolates BN2 and BN11 have high cellulase activity and can be effectively used as a potential source for industrial production of enzyme cellulase. The use of these strains can help in bioethanol production from cellulose containing bioresources. Moreover the strain BN2 has been effective in rapid composting of high lignin-containing leaf litter to produce nutrient-rich compost which would be a boon to sustainable agriculture in the present day.

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