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Diversity of lignocellulolytic bacteria in native soil of sugarcane trash and to assess their biodegradation potential

Viji Jayabalan*, Neelanarayanan Periyasamy

Centre for Eco-friendly Agro-Technologies (CEAT), Research Department of Zoology, Nehru Memorial College (Autonomous and Affiliated to Bharathidasan University), Puthanampatti, Tiruchirappalli, Tamil Nadu, India

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Abstract

The main goal of this research was to investigate the diversity of lignocellulolytic bacteria in the native soil of sugarcane trash and to assess their biodegradation potential. From naturally degrading sugarcane waste, bacteria were identified. 35 distinct bacterial species were discovered; these bacteria were then employed to determine each one's potential for breaking down the lignin and cellulose found in sugarcane waste. After selecting a potential strain of bacteria, Congo red and iodine tests were created for the purpose of screening bacterial species, and they were then utilized to further the biodegradation of sugarcane waste. Thirteen of the thirty-five investigated bacterial species generated cellulase and ligninase enzymes, and *Pseudomonas fluorescens* was identified in the secondary screening as a suitable strain among these. Hence, this *P. fluorescens* was employed in the degradation of sugarcane waste. When this lingo-cellulolytic bacterium was introduced to sugarcane waste, the rate of degradation of the waste was enhanced. Significant reduction in lignin and cellulose contents were observed in sugarcane trash inoculated with *P. fluorescens* compared to other experiments. *P. fluorescens* lowered the C:N ratio in soil-mixed sugarcane waste from 70:1 to 10:1. The macronutrients of the compost taken from experimental trays seeded with *P. fluorescens* showed a substantial increase as well. It is evident from these results that the *P. fluorescens,* lingo-cellulolytic bacteria may be used for the degradation of sugarcane trash. Hence, we conclude that *P. fluorescens* can be recommended for the degradation of sugarcane trash which would result in the production of good quality compost containing higher amounts of total nitrogen, total potassium and total phosphorus contents.

* **Corresponding Author:** Viji Jayabalan ✉ vijikannan.j@gmail.com

Introduction

Sugarcane is one of the most significant cash crops in India and is extremely essential to both the agricultural and industrial economies of the nation. One of the world's top producers of sugarcane is India. India produces 320 million tonnes of sugarcane annually. In Tamil Nadu, 102 tonnes of sugarcane are produced per acre (Mohan and Ponnusamy, 2011).

More than 3000 ha of sugarcane are grown in the Tiruchirappalli district of Tamil Nadu, with an average production of 100 tonnes ha-1. Over 6.5 million tonnes of sugarcane waste are created year when the crop is harvested. The state government of Tamil Nadu, through Krishi Vigyan Kendra, Sirugamani, and Tiruchirappalli District, conducted numerous education and training initiatives on how to effectively manage trash from harvested sugarcane, although trash burning is still a common practise (Dhanushkodi *et al.*, 2019). By releasing harmful gases into the atmosphere, such as methane and carbon dioxide, this activity pollutes the atmosphere and endangers both human health and the ecosystem. Moreover, this approach significantly depletes the soil of minerals, micronutrients and microbes.

The two best alternatives for managing sugarcane waste effectively are composting and mulching, but both require a lot of work. The combined approaches of composting with microbial inoculants can be used to reduce this issue, and it currently attracts a lot of interest from researchers (Swetha *et al.*, 2010).

The aforementioned facts make it clear that using microorganisms is necessary for the efficient and rapid decomposition of sugarcane waste. The breakdown process is typically accelerated by the microorganisms that are naturally present in the garbage. In this work, we looked into a biological technique for employing bacteria to hasten the breakdown of the main chemical components found in the waste from sugarcane. It is one of the greatest options for quickly turning the available, unused organic biodegradable wastes, such as sugarcane garbage, into compost.

This study aims to examine the diversity of lingo-cellulolytic bacteria in native soil, or naturally decomposing sugarcane waste sites, and afterwards to test their biodegradation capacity in order to make recommendations to farmers in the future.

Materials and methods

1g of soil from naturally decomposing sites sugarcane trash was aseptically collected at a depth of 10 cm and placed in a sterile polythene bag. The soil samples were then kept at 4°C for future use. In order to cultivate and isolate bacteria, dilutions of 10-4, 10-5, and 10-6 were made from the mixture of the collected samples and plated out on Nutrient agar. Plates of nutrient agar were incubated for 24 hours at 27°C. To obtain their pure culture, one colony of bacteria was incubated and then moved aseptically onto nutritional agar. In preparation for future usage, the remaining soil samples were kept at 4°C. Thormann *et al.* (2002) and Hart *et al.* (2002) methods were used to determine the bacteria's principal lignolytic and cellulolytic capabilities.

For the manufacture of enzymes, the promising bacteria showing significant clearing zones in Congo red and iodine tests were employed, using the procedures recommended by Miller (1959) for endoglucanase, Pointer (1999) for exoglucanase, Herr (1979) for glucosidase, Das *et al.* (2008) for laccase, Ledwozyw *et al.* (1986) for manganese peroxidase (MnP), and Tien and Kirk (1988) for lignin peroxidase (LiP), the secondary ligno-cellulolytic enzymes.

According to Cappucino and Sherman (1999), the chosen bacteria were discovered using three colony morphological characteristics, three microscopic characteristics, and twelve different biochemical investigations. A conical flask containing 10 mL of molasses was filled with 90 mL of distilled water, which was then thoroughly mixed in. 500g of jaggery and 400mL of water were combined with 1 mL of a pure culture of *P. fluorescens* that was produced from this

study. This mixture was thoroughly combined, kept for 7 days, and then employed as an inoculum for the decay of sugarcane waste.

The sugarcane waste was gathered and cut into little pieces from regions around our campus. After drying them in the sun, 25 kg of chopped sugarcane debris were used. Each of the six plastic trays, measuring 45 by 15 by 30 cm, contained 4 kg of rubbish made of chopped sugarcane. Three trays from this group were used to inoculate *P. fluorescens*. To compare with the trays inoculated with *P. fluorescens*, the three remaining trays were kept as controls (i.e., sugarcane debris combined with soil). These trays were left in the shade unattended. Regular twice-daily watering was done to keep the medium's ideal temperature and moisture content throughout the duration of the degradation period. At the end of degradation i.e., 30 days, the samples were collected and analyzed.

The chief chemical constituents of sugarcane trash such as cellulose, hemicelluloses and lignin were estimated both in the raw waste and compost by following the method suggested by Updegraff (1969); Chang and Hudson (1967) and Bhat and Narayan (2003), respectively.

The changes in major nutrients were estimated at the end of 30 days. The pH, Organic Carbon, Total Nitrogen, Total Phosphorus and Total Potassium were estimated by following the methods of Tandon (2005). C:N ratio was calculated by dividing the percentage of organic carbon with percentage of total nitrogen (Anon, 2006).

Paired samples "t" test was used to determine the difference between experimental (sugarcane trash + *P. fluorescens*) and control (Sugarcane trash + Soil) analysis. All the statements reported in this research are at the $p<0.001$ levels. SPSS statistical package (windows version 16.0) was used for data analysis.

Results and discussion

Totally 35 bacteria were isolated from the native soil of sugarcane trash. Each bacterial strain was given an isolate number. Out of 35 bacterial strains tested, the cellulolytic and lignolytic activity were detected only in thirteen colonies (STB1, STB2, STB4, STB13, STB20, STB 21, STB22, STB28, STB29, STB30, STB31, STB33 and STB 35) (Table 1).

Table 1. The primary screening of ligno-cellulolytic activity of bacteria isolated from the naturally decomposing sites of sugarcane trash. Each value represents the mean (Mean ±S.D.) of three observations

SL	Isolate number	Cellulolytic activity (cm) Mean ± S.D.	Lignolytic activity (cm) Mean ± S.D.
01	STB 1	3.8 ± 0.1	2.7 ± 0.00
02	STB 2	2.8 ± 0.17	2.46 ± 0.20
03	STB 3	-	-
04	STB 4	4 .1 ± 0.00	3.7 ± 0.17
05	STB 5	-	0.4 ± 0.1
06	STB 6	-	0.4 ± 0.00
07	STB 7	-	-
08	STB 8	-	-
09	STB 9	-	-
10	STB 10	-	-
11	STB 11	0.1 ± 0.00	-
12	STB 12	0.73 ± 0.11	-
13	STB 13	0.4 ± 0.1	0.1 ± 0.00
14	STB 14	-	0.4 ± 0.1
15	STB 15	-	0.4 ± 0.00
16	STB 16	-	-
17	STB 17	-	-
18	STB 18	-	-
19	STB 19	-	-
20	STB 20	1.13 ± 0.05	1.5 ± 0.1
21	STB 21	0.5 ± 0.2	0.3 ± 0.1
22	STB 22	2.66 ± 0.20	1.76 ± 0.15
23	STB 23	-	-
24	STB 24	-	0.26 ± 0.11
25	STB 25	-	0.4 ± 0.17
26	STB 26	-	-
27	STB 27	-	-
28	STB 28	0.6 ± 0.1	0.60 ± 0.42
29	STB 29	0.2 ± 0.00	0.56 ± 0.05
30	STB 30	2.66 ± 0.20	1.33 ± 0.40
31	STB 31	0.4 ± 0.26	0.43 ± 0.28
32	STB 32	0.1 ± 0.00	-
33	STB 33	1.6 ± 0.1	1.73 ± 0.11
34	STB 34	0.43 ± 0.28	-
35	STB 35	0.6 ± 0.26	1.26 ± 0.20

These 13 bacterial species had active lignocellulose degrading substances and further these strains were tested for production of lingo-cellulolytic enzymes by following secondary screening. The maximum amount of cellulolytic enzymes (Endoglucanase: 3.62 ± 0.00; Exoglucanase: 3.03 ± 0.04 and β-glucosidase: 3.24 ± 0.01) and lignolytic enzymes (Laccase: 4.06 ±

0.00; Manganese Peroxidase: 4.55 ± 0.00 and Lignin Peroxidase: 4.52 ± 0.01) production was observed only in one bacterial strain *i.e.,* STB 4 (Table 2).

By using colony morphology and biochemical characterization, the selected bacterial strain STB 4 was identified as *Pseudomonas fluorescens* (Table 3).

Table 2. The secondary screening of ligno-cellulolytic activity (Endoglucanase, Exoglucanase, β Glucosidase, Laccase, Manganese Peroxidase and Lignin Peroxidase) of bacteria isolated from the native soil of sugarcane trash. Each value represents the mean (Mean ±S.D.) of three observations

Isolate number	Endo µg reducing sugar g^{-1} hr^{-1} Mean ± S.D.	Exo µg reducing sugar g^{-1} hr^{-1} Mean ± S.D.	β gluco µg reducing sugar g^{-1} hr^{-1} Mean ± S.D.	Laccase IU/mL Mean ± S.D.	MnP IU/mL Mean ± S.D.	LiP IU/mL Mean ± S.D.
STB 1	0.14 ± 0.02	0.93 ± 0.02	1.12 ± 0.01	0.05 ± 0.00	0.06 ± 0.02	0.10 ± 0.00
STB 2	1.12 ± 0.00	1.00 ± 0.00	0.97 ± 0.00	1.18 ± 0.01	1.19 ± 0.01	1.02 ± 0.00
STB 4	3.62 ± 0.00	3.03 ± 0.04	3.24 ± 0.01	4.06 ± 0.00	4.55 ± 0.00	4.52 ± 0.01
STB 13	1.11 ± 0.01	0.92 ± 0.01	1.14 ± 0.00	1.14 ± 0.00	1.17 ± 0.01	1.1 ± 0.01
STB 20	0.32 ± 0.03	0.61 ± 0.00	0.22 ± 0.01	0.62 ± 0.01	0.65 ± 0.00	0.71 ± 0.00
STB 21	0.64 ± 0.02	0.94 ± 0.01	0.25 ± 0.01	0.02 ± 0.00	0.05 ± 0.00	0.07 ± 0.01
STB 22	0.46 ± 0.00	0.27 ± 0.01	0.67 ± 0.01	0.02 ± 0.00	0.03 ± 0.00	0.01 ± 0.00
STB 28	0.14 ± 0.02	0.93 ± 0.02	1.12 ± 0.01	0.05 ± 0.00	0.06 ± 0.02	0.10 ± 0.00
STB 29	1.12 ± 0.00	1.00 ± 0.00	0.97 ± 0.00	1.18 ± 0.01	1.19 ± 0.01	1.02 ± 0.00
STB 30	1.10 ± 0.00	1.25 ± 0.00	1.01± 0.01	1.01 ± 0.00	1.01 ± 0.00	1.04 ± 0.00
STB 31	0.12 ± 0.00	0.53 ± 0.01	0.25 ± 0.00	0.25 ± 0.00	0.32 ± 0.01	0.20 ± 0.00
STB 33	0.90 ±0.02	0.9 ± 0.02	1.14 ± 0.02	0.06 ± 0.00	0.11 ± 0.01	0.09 ± 0.02
STB 35	0.17 ± 0.02	0.12 ± 0.00	0.12 ± 0.02	0.05 ± 0.04	0.98 ± 0.07	1.02 ± 0.00

Table 3. The colony morphology, microscopic characters and biochemical characterization of *P. fluorescens*

Characters		*P. fluorescens*
Colony morphology	Colour	Yellowish white
	Shape	Irregular
	Surface	Flat
Microscopic characters	Spore staining	Positive
	Gram staining	Rod-negative
	Motility test	Positive
Biochemical characters	Indole test	Positive
	Methyl red test	Positive
	Voges Proskauer test	Negative
	Citrate utilization test	Positive
	Catalase test	Positive
	Oxidase test	Positive
	Triple sugar Iron test	A/K/+/-
	Starch hydrolysis test	Negative
	Urease test	Positive
	Nitrate reduction test	Positive
	Gelatin hydrolysis test	Positive
	Hydrogen sulphide test	Negative

The *P. fluorescens* was utilized for the degradation of sugarcane trash. After 30 days of treatment, the sugarcane trash was transformed into black granules. It indicated that there was complete/maximum degradation of sugarcane trash. But in control trays, the degradation was observed to be very slow.

The propensity of major chemical constituents' changes in the raw sugarcane trash and decomposed sugarcane trash after decomposition with soil and *P. fluorescens* are given in Table 4. On 30th day, cellulose, hemicellulose and lignin were drastically reduced to the tune of 48.93%, 53.89% and 51.08%, respectively in the experimental trays inoculated with *P. fluorescens*.

A significant decrease (t test; $p<0.001$) in the pH was observed in the end product harvested from the experimental trays inoculated with *P. fluorescens* than the control (Fig. 1a). The pH level in the vermicompost was lowered by the combined influence of two oppositely charged groups, namely ammonium (NH4+) and humic acids (Pramanik *et al*., 2007).

P. fluorescens caused significant decrease (t test; $p<0.001$) in the level of organic carbon content harvested from the experimental trays than the control trays (Fig. 1b). The amount of organic carbon (OC) in soil is a crucial component of its health and fertility. The greater rate of decomposition aided by bacteria could be the cause of the lower organic carbon percentage in final products derived from organic sources infected with germs (Bharadwaj, 1999).

Table 4. The propensity of major chemical constituents of the raw sugarcane trash and decomposed sugarcane trash after decomposition with soil and *P. fluorescens*

Name of the chemical constituents	Raw sugarcane trash	Decomposed sugarcane trash with soil	Percent reduction	Decomposed sugarcane trash with *P. fluorescens*	Percent reduction
	Percentage Mean ± S.D.	Percentage Mean ± S.D.		Percentage Mean ± S.D.	
Cellulose	31.33 ± 0.82	27.01 ± 0.42	13.78	16 ± 1	48.93
Hemicellulose	23.12 ± 1.12	18.21 ± 0.21	21.23	10.66 ± 0.58	53.89
Lignin	21.12 ± 0.98	18.12 ± 0.34	14.20	10.33 ± 2.30	51.08

Each value represents the mean (Mean ± S.D.) of three observations

P. fluorescens inoculation resulted in significant increase (t test; $p<0.001$) in Total Nitrogen (TN) in the experimental trays than the control (Fig. 1c). The inoculation of *P. fluorescens* for the decomposition of sugarcane waste as well as the immobilisation and conservation of Nitrogen during the composting process may be to blame for the increase in total nitrogen percent. The increased oxidation of non-nitrogenous organic materials and partially the N_2-fixation by non-symbiotic nitrogen fixers, as indicated by the increase in organic nitrogen, may be the causes of the rise in total nitrogen percent, claim Barakah *et al.* (2013).

The Total Phosphorus (TP) increased significantly (t test; $p<0.001$) in the end product obtained from the experimental trays inoculated with *P. fluorescens* than the control (Fig. 1d). The bacterial inoculation P. fluorescens, which mobilises the soil's inaccessible P content through the synthesis of organic acids and P from garbage, may be responsible for the enhanced TP. The primary process of phosphorous solubilization during the microbial breakdown of organic materials is acid generation (Shakara, 2003).

Inoculation of *P. fluorescens* resulted in significant increase (t test; $p<0.001$) in Total Potassium (TK) in the experimental trays than the control trays (Fig. 1e). The effective management of trash resulted in increased potassium content as the trash is a rich source of K. The results are in accordance with the findings of Graham *et al.* (2000).

There was a significant reduction (t test; $p<0.001$) in C:N ratio of the end products harvested from the experimental trays inoculated with *P. fluorescens* after decomposition (Fig. 1f).

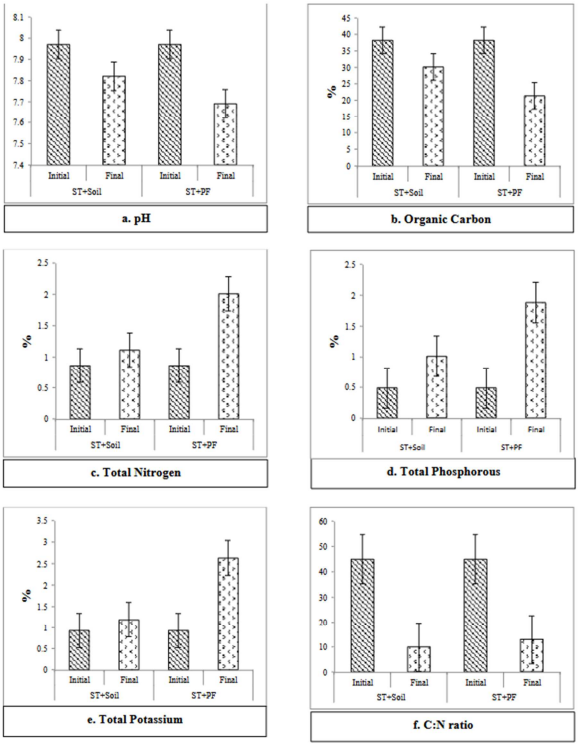

Fig. 1. Chemical nutrient changes during degradation of sugarcane trash with soil (ST + Soil) and P. fluorescens (ST + PF). Error bars represent standard error (n=3)

Changes in the C: N ratio can be used to measure compost maturity and the rate at which organic ingredients degrade. The C:N ratio, which decreases as a result of organic carbon being broken down by microorganisms during the composting process, serves as a gauge for the maturity level of the final product. The compost is deemed stable when the C:N ratio stops declining (Bernal *et al.*, 2009). Our findings are in opposition to those of Abdelhamid *et al.* (2004), who claimed that a C:N value of 20 or less may be regarded as satisfactory. According to research by Khalil *et al.* (2001), the optimal C:N ratio

for mature compost is around 10, however this ratio is rarely reached because of the existence of refractory organic compounds, or materials that resist decomposition due to their physical or chemical characteristics. Our findings were consistent with Moldes *et al.* (2007)'s assertion that compost can be deemed mature when the C:N ratio is 17 or below, barring the presence of ligno-cellulolytic components.

Conclusion

Inoculation of lingo-cellulolytic microorganisms is deemed to be a useful strategy to accelerate the degradation potential of lignocellulose in the chosen agricultural waste. It is concluded form the present study that among 35 bacterial colonies, *P. fluorescens* exhibited best lingo-cellulolytic performance both quantitatively and qualitatively. The *P. fluorescens* used in the present research work could able to degrade the sugarcane trash within a short span of time, i.e., 30 days. The effect of *P. fluorescens* on structural components revealed its efficiency in the degradation of cellulose, hemicellulose and lignin contents enzymatically which ultimately resulted in good quality compost. Macro and micro nutrients were observed at the desired levels in the compost harvested from the experimental trays inoculated with *P. fluoescens* than the sugarcane trash mixed with soil. Therefore, it may be concluded that the sugarcane trash could be decomposed with the chosen bacterial inoculants, i.e., *P. fluoescens*. Further, research work may be attempted with *P. fluoescens* and earthworms to convert the sugarcane trash into vermicompost.

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