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RESEARCH ARTICLE

Studies on Microbial Consortium for Composting of Coirpith Waste by Enzymatic Activities

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A B S T R A C T

Composting of coir pith is a time taken process and to reduce the composting period an appropriate technology should be evolved. The relationship between microorganisms and its enzymatic activities lead for the better under standing of technology to develop microbial consortium for effective composting. In the present study the microorganisms were screened from coir pith which produces enzymes like cellulase, xylanase at higher levels of temperature and pH. The temperature ranging from 40 - 60°C and the pH of 6.0 to 7.0 were found to be favourable for enzyme production. Further the three isolates namely *Bacillus sp, pseudomonas fluorescens* and *Trichoderma reesei* were found to be better when compared to other isolates and it also used to make a microbial consortium for effective composting of coir pith.

INTRODUCTION

Coir pith is a major by product of coir fiber extraction industries [1]. It decomposes very slowly due to its high lignin content. Coir pith waste were accumulates every year leads to pollution of the environment. In recent years, these waste materials were converted to biofertilizers or compost using several microbes and their enzymatic activities [2]. Therefore alternative ways to dispose of coir pith, Such as composting, is of critical importance. For reduction in overall time taken for composting, developing efficient microorganisms using the same substrate prove its best.

Enzyme activities have been indirectly used as an index of microbial population on organic matter decomposition. Enzyme activities are due to the enzymes present with in a living or dead cells, cell debris, free enzymes [3]. Thus necessitates determining the relationship between microorganisms and enzymatic activities, which would lead to better understanding for the development of microbial consortia for efficient composting.

In the present investigation, an attempt was made to isolate and screen the organisms capable of producing enzymes cellulose, xylonite at higher levels of temperature and pH yielded potential isolates that could be used to develop a microbial consortium. For effective composting of coir pith waste.

MATERIALS AND METHODS

The coir pith waste was collected from Khazhi Biotech (p) Ltd., Yanaikara Sathiram, Nagapattinam District. Cellulase and xylanase degrading fungi and bacteria were isolated from coir pith waste. For isolation, using sterile pestle and motar coir pith was ground and made in to slurry using sterile distilled water an aliquot of this sample was serially diluted with an appropriate dilution blank and plated on Nutrient Agar medium for bacteria and Potato Dextrose Agar for fungi. After incubation, the isolated bacteria and fungal cultures were purified and characterized by standard methods.

Assay of cellulolytic enzymes

1,4 – Endo glucanase

The endoglucanase activity was assayed based on the ability of the enzyme to reduce the viscosity of carboxyl methyl cellulose (CMC) [4].

1,4 – Exoglucanase

The exoglucanase activity was assayed by the method followed by Denison and koehn [5].

The enzyme activities were determined by incubating the culture under different pH (5.0, 6.0 and 7.0) and temperature (30°C, 40°C and 80°C).

Assay of xylanases

The purified strains were grown in respective medium with one percent xylan and incubated at 27°C. The cultures were centrifuged at 4°C at 1000 rpm for 15min and the supernatants were used as crude enzyme extract.

RESULT AND DISCUSSION

In the present study 12 isolates were obtained from coir pith waste. The isolates were screened for their xylanase activity at different temperature and the results were presented in table1. All the isolates recorded xylanase activity at 30°C and the isolated cp1 recorded maximum xylanase activity of 21.88 percent reduction in viscosity followed by CP7 (21.78% reduction in viscosity) and Cp3 (13.70% reduction in viscosity) and 30°C.

The temperature of 80°C was unfavorable for all the isolates to produce xylanase. Both 40°C and 60°C were found favourable for the production of enzymes. The temperature 40°C was better than 60°C. The isolates cp1, cp7, cp3 were alone produced xylanase enzymes at 80°C. All the isolates exhibited maximum activity at 40°C. Due to the production of large amount of metabolic heat, the fermented substrate temperature shoots up [6]. At lower incubation temperature, practically xylanase activity remained unchanged and at higher temperature, all the enzyme activities decreased. At higher temperature, there may be a heat build up which causes evaporative water loss and reduction in vegetative growth; where as a controlled evaporation with continuous water replacement promote heat dissipation and thus assure productive vegetative growth [7].Ray et al., [8] found that 35±2°C was found to be optimum for enzyme production

Table 1 Effect of temperature on xylanase activity of microbial isolates

Culture no	Name of the isolates	Xylase (μm^{-1})			
		30°C	**40°C**	**60°C**	**80°C**
CP1	*Bacillus sp*	21.88	29.65	22.50	11.50
CP2	*Pseudomonas sp*	4.89	6.12	4.89	NP
CP3	*p.fluorescens*	13.70	20.89	13.95	9.12
CP4	*Flavobacterium*	1.23	NP	NP	NP
CP5	*Erwina sp*	1.32	NP	NP	NP
CP6	*Xanthomonas sp*	1.24	6.00	3.12	NP
CP7	*Trichoderma reesei*	21.78	26.15	16.75	10.70
CP8	*Phanerochaete chrysosporium*	3.12	4.14	3.12	NP
CP9	*Aspergillus niger*	1.38	5.98	4.92	NP
CP10	*Penicillium sp*	1.89	5.96	4.99	NP
CP11	*Fusarium sp*	4.24	6.88	5.83	NP
CP12	*A.fumigatus*	1.28	5.62	3.72	NP
	SE	0.81	1.00	0.81	0.69
	CD (P=0.05)	1.63	2.32	1.63	1.42

Table 2 Effect of p^H on xylanase activity of microbial isolates

Culture no	Name of the isolates	Xylanase (μml^{-1})		
		P^H		
		5.0	**6.0**	**7.0**
CP1	*Bacillus sp*	14.17	16.45	6.25
CP2	*Pseudomonas sp*	1.82	2.62	NP
CP3	*p.fluorescens*	16.23	19.00	10.25
CP4	*Flavobacterium*	NP	NP	NP
CP5	*Erwina sp*	1.32	NP	NP
CP6	*Xanthomonas sp*	1.32	2.48	NP
CP7	*Trichoderma reesei*	13.33	16.25	8.25
CP8	*Phanerochaete chrysosporium*	3.06	4.17	NP
CP9	*Aspergillus niger*	1.23	2.46	NP
CP10	*Penicillium sp*	1.32	2.33	NP
CP11	*Fusarium sp*	1.23	2.42	NP
CP12	*A.fumigatus*	2.29	4.89	NP
	SE	0.83	0.60	0.05
	CD (P=0.05)	1.71	1.42	0.12

which can be compared favourably with the present study.

The screening of isolates at various pH levels viz, 5.0, 6.0 and 7.0 for xylanase activity are presented in table 2. The pH7 was found to be unsuitable for most of the isolates. The isolates cp3 recorded maximum xylanase activity at pH 6.0 (19.00% reduction viscosity). Cp1, and cp7 were comparable in xylanase enzymes. The three isolates cp7, cp3 & cp1 were capable of with standing not only higher temperature and pH but elaborated xylanases anzymes at sizable levels. Opimum pH value for xylanase production has been reported to be 4.0-6.0 [9].

The production of endo and exoglucanase by various temperature and pH are presented in table 3 and 4. the isolate cp7 trichoderma reesei recorded 72.62 percent reduction in viscosity for endoglucanase and 2.42 μmg^{-1} Protein of exoglucanases activity at 30°C. *p. fluorescens* (CP3) recorded 70.12 per cent reduction in viscosity and 2.31μ mg^{-1} protein at 30°C. *Bacillus sps* (cp1) recorded 62.78 per cent reduction in viscosity and 2.12 μ mg^{-1} protein at 50°C. The isolate cp1, cp3 and cp7 were found to be capable of producing cellulase at higher temperature of 80°C. The temperature of eithore 40 and 60°C were found favourable for all the isolates.

Table 3 Effect of temperature on cellulase activity of the microbial isolates

Culture no	Name of the isolates	Endo-1,4, glucanase (% Reduction in viscosity)				Exo-1,4-glucanase (μ mg^{-1} protein)			
		30°C	40°C	60°C	80°C	30°C	40°C	60°C	80°C
CP1	*Bacillus sp*	62.78	64.12	51.06	47.27	2.12	3.17	1.91	1.60
CP2	*Pseudomonas sp*	35.14	23.92	21.52	17.13	1.64	2.69	1.44	1.37
CP3	*p.fluorescens*	70.12	71.30	53.10	50.27	2.31	3.38	1.85	1.62
CP4	*Flavobacterium*	37.00	38.64	13.90	NP	1.51	2.72	1.52	NP
CP5	*Erwina sp*	27.81	28.98	14.92	NP	1.36	2.40	1.24	NP
CP6	*Xanthomonas sp*	37.81	37.18	31.40	NP	1.63	2.71	1.48	NP
CP7	*Trichoderma reesei*	72.62	74.24	63.18	52.80	2.42	3.56	2.03	1.61
CP8	*Phanerochaete chrysosporium*	24.21	27.92	18.82	18.80	1.52	2.60	1.21	1.10
CP9	*Aspergillus niger*	43.90	47.91	41.00	26.17	1.43	2.48	1.25	1.12
CP10	*Penicillium sp*	44.51	49.51	37.78	23.12	1.39	2.42	1.31	1.09
CP11	*Fusarium sp*	36.00	41.21	32.92	13.00	1.30	2.36	1.23	1.11
CP12	*A.fumigatus*	38.23	40.64	16.92	NP	1.25	2.34	1.20	NP
	SE	1.991	2.109	1.875	1.430	0.129	0.108	0.060	0.021
	CD (P=0.05)	4.079	4.320	3.840	2.930	0.265	0.223	0.123	0.044

Table 4 Effect of P^H on Cellulase activity of the microbial isolates

Culture no	Name of the isolates	Endo-1,4, glucanase (% Reduction in viscosity)			Exo-1,4-glucanase (μ mg^{-1} protein)		
		P^H			P^H		
		5.0	6.0	7.0	5.0	6.0	7.0
CP1	*Bacillus sp*	62.62	75.15	44.15	1.72	3.05	1.64
CP2	*Pseudomonas sp*	43.12	56.20	32.27	1.20	2.36	1.32
CP3	*p.fluorescens*	60.12	73.10	44.45	1.43	2.95	1.62
CP4	*Flavobacterium*	34.90	22.60	19.23	1.16	2.31	1.24
CP5	*Erwina sp*	35.74	44.12	46.20	1.32	2.51	1.39
CP6	*Xanthomonas sp*	23.10	25.16	12.20	1.13	2.23	1.29
CP7	*Trichoderma reesei*	60.22	72.92	43.15	1.52	2.83	1.41
CP8	*Phanerochaete chrysosporium*	23.60	35.30	35.70	1.25	2.09	1.26
CP9	*Aspergillus niger*	45.69	41.23	25.57	1.40	2.50	1.29
CP10	*Penicillium sp*	41.13	41.03	23.33	1.13	2.17	1.12
CP11	*Fusarium sp*	30.27	38.27	30.17	1.11	2.19	1.10
CP12	*A.fumigatus*	30.78	32.03	30.43	1.19	2.21	1.12
	SE	1.787	1.733	1.609	0.024	0.042	0.037
	CD (P=0.05)	3.661	3.550	3.297	0.049	0.086	0.076

The isolates cp1, cp3 and cp7 were found to produce enzyme at all levels
of pH viz., 5.0, 6.0 and 7.0 studied, while the remaining isolates were able to record anzyme activity at pH 4.0 and 6.0 at comparable level, where as the enzyme production was low of pH 7.0. The isolates cp1 and cp3 recorded maximum endo._ 1,4 – glucanase activity at pH 6.0 (75.15 and 73.10% reduction in viscosity).

The results revealed that temperature ranging from 40-60°C and pH ranging from 6.0 to 7.0 were found to be favourable for enzyme production. It was supported by lynd and zhang [10] who reported the thermophilic and themotolerent cellulolytic microbes exhibit substantially higher growth rates than any of the mesophiles.

CONCLUSION

Twelve bacterial and fungal isolates were screened from coir pith waste and characterized. Further to develop effective consortia, their efficiency to produce enzymes at different temperature and pH was studied. The three isolates *T. reesei, p.fluorescens* and *Bacillus sp* could be used to develop a microbial consortia for composting.

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