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Isolation and Preliminary Screening of Paddy Straw Degrading Thermphilic Fungi

KEYWORDS: Paddy straw, Thermophilic fungi, Isolation, Screening

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ABSTRACT *The present study was aimed at isolation, purification and screening of paddy straw degrading (lignocellulolytic) thermophilic fungi for enhancing digestibility. A total of 80 cultures were isolated from paddy straw, farm yard manure and soil. These isolated cultures then purified and screened qualitatively and quantitatively on agar plates. Remazol brilliant blue (RBB) dye and guaiacol used as substrate to evaluate lignolytic activity in eighty isolated thermophilic fungi. A total of 68 fungi decolorized the RBB during the growth and only 19 fungi showed redness zone on guaiacol. Three isolates namely T10, T14 and T17 are the potential paddy straw degraders which can used for enhancing biogas production.*

Paddy straw, being a lignocellulose, predominantly contains cellulose (35-40%), hemi-cellulose (20-24%), lignin (8-12%), ash (14-16%) and extractives (10-12%) which are associated with each other (Maiorella, 1985). Hemi-cellulose serves as a connection between lignin and cellulose fibres and provides more rigidity to the whole cellulose-hemicellulose-lignin network (Laureano-Perez et al. 2005). Lignin provides structural support to the plant, impermeability and resistance to degradation. Rice plant, a typical silicon accumulating organism, accumulates about 10% silicon in the paddy straw obtained from rice plant (Van Soest, 2006). This silicon forms complex with lignin as lignin-silica complex which further restricts the accessibility to the cellulose.

MATERIALS AND METHODS

Isolation and purification of lignocellulolytic fungi

The fungal cultures were isolated from different samples like soil, compost, digested slurry and plant debris. One gram of sample was vortexed with 99 ml of sterilized distilled water to make uniform suspension. Heavy particles were allowed to settle and clear supernatant was used for serial dilution. One ml of serially diluted sample was pour plated on paddy straw agar medium (PSA), each containing chloramphenicol (50 mg/l) and incubated at 50±2°C. The isolated colonies were transferred thrice on fresh agar plates to purify the cultures.

Screening of lignocellulolytic fungi

The isolated and purified cultures were qualitatively screened for its lignocellulose degradation potential by agar plate assay method (Okino et al. 2000). Remazol brilliant blue (RBB) and guaiacol were used as indicator dyes for lignin degradation and potency index was calculated by the following formula:

Potency index = size of clearance zone (cm^2)/size of colony (cm^2)

The concentration of RBB used was 0.05% and that of guaiacol was 0.075ml/l. The clearance zone on RBB plates indicates the presence of lignin degrading enzymes which may be lignin peroxidase (LiP), manganese peroxidase (MnP) or laccase. The presence of red zone on guaiacol containing medium indicates the presence of lignin peroxidase and presence of clearance zone indicates either manganese peroxidase or laccase. For cellulose degradation, colonies grown on paddy straw agar (PSA) medium were flooded with 0.15% I_2 solution and clearance zone around the colony indicates the presence of lignocellulolytic enzymes.

Growth profile of lignocellulolytic isolates

Growth profiles of lignocellulolytic isolates were studied by measuring the colony size (cm^2) on paddy straw agar medium up to 5 days of incubation period.

RESULTS AND DISCUSSION

Results from Table 1 showed potency index of isolated cultures (T1 to T80) i.e. Cellulase activity, RBB activity and Guaiacol activity. The potency index for cellulase activity ranges from 1.5 to 4.9, forming three different groups i.e. high, moderate and low cellulase producing cultures. The potency index for high cellulase producers ranges from 3.5 to 4.9 which includes isolates no. T2, T3, T6, T8, T9, T11, T13, T16, T20, T21, T22, T29, T30, T33, T41, T43, T44, T45, T46, T47, T53, T56, T58, T59, T62, T65, T71, T73, T76, T78, T79 and T80. Isolate number T22 showed maximum cellulase activity with potency index of 4.9. The potency index for moderate cellulase producers ranges from 2.1 to 3.4 including isolate numbers T1, T4, T10, T12, T18, T19, T23, T24, T25, T26, T27, T31, T32, T34, T35, T36, T37, T38, T39, T40, T42, T48, T50, T51, T52, T54, T55, T57, T60, T61, T63, T64, T66, T68, T69 and T70. The potency index for low cellulase producers range from 1.5 to 2.0. Isolates numbers T5, T7, T14, T15, T17, T49, T67, T72, T74, T75 and T77 are included in this category. The potency index for RBB ranges from 0.5 to 2.9. Isolate numbers T10 showed maximum RBB activity having potency index 2.9 followed by T17 (PI = 2.6). Most isolates gave negative results on guaiacol containing media. Only isolate numbers T1, T4, T5, T7, T10, T12, T14, T17, T25, T32, T35, T42, T44, T47, T56, T65, T72, T74 and T77 showed positive results i.e. presence of redness zone on guaiacol plates, thus indicating presence of lignin peroxidase.

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Table 1: Potency index of isolated cultures

Isolate No.	Cultural characteristics	Potency Index		
		Cellulase activity	RBB activity	Guaiacol activity
T1	Off white colored colony, thick mass, pale colored from reverse	2.6	1.8	+ve (0.5 cm)
T2	Green colored colony like mat/ layer, center of colonies pulled up (pointed), powdery, no pigmentation	3.9	2.1	-ve

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Isolate No.	Cultural characteristics	Potency Index		
		Cellulase activity	RBB activity	Guaiacol activity
T3	Thin layer of dull green colored colony, no pigmentation	4.1	1.7	-ve
T4	Concentric rings of dark and light green colored colonies, wrinkled toward center, powdery, no pigmentation	2.3	1.5	+ve (1.0cm)
T5	Dull grayish green colored wrinkled and clumped, colony, powdery, fast growing, no pigmentation	1.7	2.4	+ve (1.1cm)
T6	White outside, green inside (2nd day incubation), overall green (4th day incubation), forming a mat on media, no pigmentation	4.1	1.6	-ve
T7	Black colored spores, powdery, no pigmentation, fast growing	2.0	2.3	+ve (0.7 cm)
T8	Greenish grey colored colony, powdery, no pigmentation	4.1	1.2	-ve
T9	Black colored colony, thick mass, no pigmentation	4.5	-ve	-ve
T10	Green colored powdery colony, no pigmentation	2.1	2.9	+ve (0.6 cm)
T11	Black colored very small spores, no pigmentation	3.6	1.4	-ve
T12	Appears as dots of dark or black color on media of circular shape, no pigmentation	3.3	1.6	+ve (0.5cm)
T13	Grayish colored, no pigmentation, powdery, no pigmentation	4.1	1.7	-ve
T14	Light brownish colored colony, fluffy cottony, powdery, no pigmentation	1.8	1.3	+ve (1.3cm)
T15	White cottony mass with fluffy brown colored layer, no pigmentation	1.9	2.2	-ve
T16	Greenish colored, powdery, no pigmentation	4.5	2.0	-ve
T17	Dull brown colored spores forming mat on media, powdery, no pigmentation	1.5	2.6	+ve (1.3cm)
T18	Greenish grey colored colony, powdery, no pigmentation	3.2	1.6	-ve
T19	Black colored, no pigmentation, thick mycelium	3.4	1.5	-ve
T20	Dark grayish green colored spores, powdery, no pigmentation	4.1	1.5	-ve
T21	Brown colored colony, powdery, no pigmentation	3.6	1.6	-ve
T22	Light green colored colony which becomes dark purple colored on further incubation, pale colored pigment	4.9	-ve	-ve
T23	Brown black colored spores, no pigmentation	2.8	1.1	-ve
T24	Yellow green droplets on culture slant, reddish brown pigment	3.4	2.2	-ve
T25	Dull brown colored spores forming mat on media, pale from reverse	3.1	2.3	+ve (0.5 cm)
T26	Grey colored fluffy colony, no pigmentation	3.4	0.5	-ve
T27	Yellow buff colored colony, no pigmentation	3.1	0.7	-ve
T28	Dark green colored spores, powdery, no pigmentation, forming mat on media	3.9	1.0	-ve
T29	Dull grayish colored colony, powdery, fast growing, pale colored from reverse	3.7	1.6	-ve
T30	Brown colored colony, powdery, no pigmentation	3.8	2.1	-ve

Table 2: Qualitative screening of selected isolates

Sr. No.	Isolate No.	Paddy straw Agar	Lignin Agar
1	T1	3	3
2	T4	3	3
3	T5	4	3
4	T7	3	3
5	T10	4	3
6	T12	2	3
7	T14	4	4
8	T17	4	4
9	T22	4	2
10	T25	3	3
11	T32	3	3
12	T35	3	2
13	T42	2	3
14	T44	3	3
15	T47	3	1
16	T56	3	2
17	T65	3	3
18	T72	2	3
19	T74	2	2
20	T77	2	3

1: poor growth; 2: fair growth; 3: good growth; 4: excellent growth

Paddy straw agar: paddy straw (1%), agar (1.5%), malt extract (1%); Lignin agar: lignin sulphonic acid (0.3%), agar (1.5%), malt extract (1%)

Figure 1(a): Growth profile of selected isolates

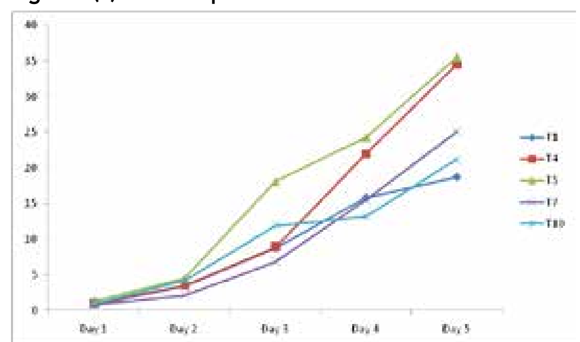
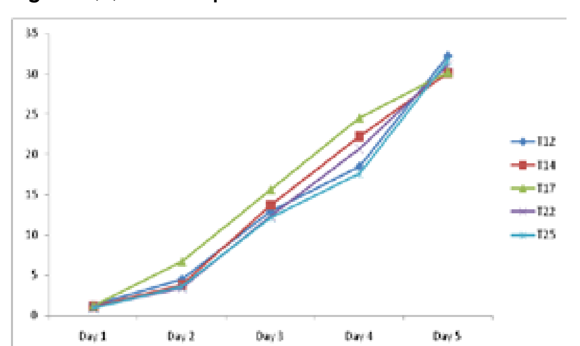


Figure 1(b): Growth profile of selected isolates



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