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Screening of Microbes Isolated from Natural Polluted Soils for Degradation of Pressmud-Spentwash

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Abstract

The byproducts released from distilleries and sugar industries mainly include spent wash and press mud respectively. Composting of these wastes paves a way for safe disposal. Press mud and spent wash degrading enzymes comprise a set of ligninolytic and cellulolytic enzyme complexes. These enzymes are present in bacteria, fungi and actinomycetes. As many as 184 bacteria, 48 fungi and 20 actinomycetes were isolated and screened to degrade these wastes. All the isolates were tested for Bavendamn tests using META, LNAM with phenol red and methylene blue indicators. Among 184 bacterial isolates, 26 isolates showed degradation on META media, while 11 bacterial isolates showed degradation on LNAM (MB) and 36 isolates could show degradation on LNAM (PR). Nine isolates showed degrading activity both on LNAM (PR) and META media and three on both LNAM (PR and MB) media. Out of 48 fungal isolates, 15 isolates showed degradation on META media, while 13 LNAM (MB), whereas on LNAM (PR), 24 fungal isolates showed degradation. Only seven showed degradation on both LNAM (PR) and META media and eight both on LNAM (PR and MB) media, and two showed degradation both on META and LNAM (MB) indicator media. Only two isolates showed degradation on all the three media. Out of 20 actinomycetes, seven isolates showed degradation on LNAM (MB) one on LNAM (PR) and one on both LNAM (PR and MB) indicator media. None of them showed degradation on META media.
Key words : Press mud, Spent wash, Enzymatic activity.

There are more than 300 molasses based distillerries in India. They also have sugar production unit with them. The byproducts released from distilleries include spent wash and press mud from sugar industries. The principal organic constituents in these industrial wastes include lignin, cellulose, hemicellulose and other components (1). Press mud is slowly degraded due to its high C/N ratio and higher lignin content. Molasses is the raw material in distillleries. Alcohol is produced from continuous type of fermentation, where fermentation is carried out for 12 h and the concentrated spentwash generated is in the tune of 2.8 liter/ liter of ethanol produced in contrast to the batch fermentation process where 15 liter of spentwash is generated per liter of ethanol produced. This spentwash is highly concentrated. It is acidic (pH 3.88 to 4.00) and loaded with organic and inorganic salts, resulting in higher EC values (30 to 45 ds/m). Being plant originated, spentwash also contains considerable amounts of NPK and secondary plant nutrients and organic matter. It can be effectively be used as a source of plant nutrient and as soil amendment. Pressmud-spentwash waste degradation can be accomplished by inoculation with lignolytic and cellulolytic microorganisms. Hence, it is essential to isolate and screen microorganisms which degrade the byproducts efficiently.

Methods

Isolation of Efficient Lignin Degrading Microorganisms from Nature

Soil samples were collected from diverse locations such as dumpsites of paper mills, commercial compost production units, elephant dung, soils with high EC. They were serially diluted and plated out on Nutrient agar, Martins Rose Bengal agar and Kuster's agar media to isolate bacteria, fungi and actinomycetes respectively. The representative microbes were purified and preserved in a refrigerator till use.

Screening of Microorganisms for Lignin Degradation Using Indicator Media

The representative microbes isolated were sub-

Table 1. Screening of bacterial isolates from the soil samples brought from Mysore Paper Mills, Bhadravathi.

Isolate code	LNAM (PR)	LNAM (MB)	META	Isolate code	LNAM (PR)	LNAM (MB)	META
MPM B 1/3	+	NG	+	MPM B 12/1	+	NG	–
MPM B 1/4	+	NG	+	MPM B 12/2	+	NG	–
MPM B 1/5	–	NG	+	MPM B 14/2	+	NG	–
MPM B 1/6	+	NG	+	MPM B 14/3	+	NG	–
MPM B 2/1	+	NG	+	MPM B 15/2	+	NG	–
MPM B 2/2	–	NG	+	MPM B 16/2	+	NG	–
MPM B 2/4	–	NG	+	MPM B 16/5	+	NG	–
MPM B 2/6	–	NG	+	MPM B 17/5	+	NG	–
MPM B 3/1	+	NG	–	MPM B 19/3	–	NG	+
MPM B 3/2	+	NG	–	MPM B 19/4	+	NG	+
MPM B 3/3	+	NG	–	MPM B 19/5	+	NG	+
MPM B 3/4	+	NG	–	MPM B 19/6	+	NG	+
MPM B 3/5	+	NG	–	Rn-30/1	+	–	–
MPM B 4/1	+	NG	–	Rn-30/3	+	+	–
MPM B 4/6	+	NG	–	Rn-30/6	–	+	–
MPM B 5/1	+	NG	+	Rn-SG/2	–	+	–
MPM B 6/1	–	NG	+	Rn-SG/3	–	+	–
MPM B 6/2	–	NG	+	Rn-SG/4	+	–	–
MPM B 6/3	–	NG	+	Rn-SG/7	–	+	–
MPM B 6/5	–	NG	+	RJW-11/2	+	–	–
MPM B 7/1	+	NG	–	RJW-11/3	+	+	–
MPM B 7/4	+	NG	+	RJW-11/4	+	+	–
MPM B 7/5	–	NG	+	RJW-11/7	–	+	–
MPM B 10/2	–	NG	+	RJW-11/8	–	+	–
MPM B 10/3	–	NG	+	RJW-1/4	+	–	–
MPM B 11/1	–	NG	+	RJW-2/2	+	–	–
MPM B 11/2	+	NG	+	RJW-2/5	+	–	–
MPM B 11/3	–	NG	+	RJW-3/6	+	–	–
MPM B 11/4	–	NG	+	EB-4	+	–	–
MPM B 11/5	–	NG	+	EB-5	–	+	–

jected to Bavendamm test (2). The microbes were spotted on indicator media like 3% Malt extract tannic acid medium (META) (per 1000 ml of distilled water) : malt extract, 30.0g; tannic acid, 1.0 g; agar, 20.0 g; (3) and Low nitrogen agar medium (LNAM) (per 1000 ml of distilled water) : glucose, 10.0 g; malt extract 10.0g; peptone, 2.0g; yeast extract, 2.0g; aspargine, 1.0g; KH_2PO_4, 2.0 g; $MgSO_4$, 10.0g; thiamine HCl, 10 ppm ; agar, 20.0g; amended with phenol red (0.02%) and methylene blue (0.02%) (2), separately with enzyme substrate viz, tannic acid (0.1%) (wt/vol), in meta media and asparagine (0.1% wt/vol) in LNAM media. The pH of the medium was adjusted to 6.5 with 1 N NaoH and 1 N HCl. After autoclaving media was cooled and poured to sterile petriplates aseptically. On solidification, the plates were spotted with bacterial cultures, whereas in fungi, the fungal organisms were inoculated at the centre with 1 cm^2 mycelium disc. The plates were incubated at 28 ± 1 C for a week. The respective enzyme activities were evaluated by observing the color change.

Results and Discussion

The representative microbes isolated were subjected to Bavendamm test. The microbes were spotted on indicator media like Malt extract tannic acid agar medium (3.0%) (META) and low nitrogen agar medium with phenol red (0.02%) (LNAM-PR) and methylene blue (0.02%) (LNAM-MB) separately. The colonies showing halo zones surrounding them on META medium and low nitrogen agar medium amended with methylene blue medium were considered positive for lignin degradation. The colonies showing red color surroundig them on low nitrogen agar medium amended with phenol red were consid-

Table 2. Screening of fungal isolates for lignin degradation from the soil samples brought from paper mill effluent dumpsites and from commercial compost production units and samples borrowed from Department of Agricultural Microbiology, UAS, Dharwad.

Isolate code	LNAM (PR)	LNAM (MB)	META	Isolate code	LNAM (PR)	LNAM (MB)	META
MPM-B2	–	–	+	Rn - 30/4	–	–	+
MPM-B5	+	+	–	Rn - 30/5	+	–	+
MPM-B8	+	–	–	Rn - 30/6	+	–	+
MPM-B9/1	+	+	–	Rn - 30/7	+	–	–
MPM-B9/2	–	+	+	Rn - 30/8	–	–	+
MPM-B11/1	+	+	+	Rn - 30/9	+	–	+
MPM-B12/2	–	+	–	Rn - 30/10	+	–	–
MPM-B13/1	+	–	–	Rn - 30/13	+	–	+
MPM-B13/2	+	–	+	Rn - 30/14	+	–	–
MPM-B17/2	+	–	–	Rjw - 8/1	–	+	–
MPM-B18/1	+	+	–	Rjw - 8/2	–	+	–
MPM-B18/2	+	+	–	Rjw - 10/1	+	–	–
MPM-B19/1	+	+	–	*Trichoderma*	+	–	+
MPM B-19/3	+	+	+	*Pleurotus*	–	+	+
MPM-B19/4	+	–	–	*Phenerocheate*	+	–	+
Rn - 30/2	+	+	–	*Aspergillus*	–	–	+
Rn - 30/3	+	–	–				

ered as positive. Positive reaction indicated production of lignin peroxidase, laccases and polyphenol oxidases. The use of indicator media has been suggested as an approach for rapid screening for lignin degrading ability (4).

As many as 184 bacterial isolate were isolated from the soil samples brought from the paper mill effluent dump sites, commercial compost production unit and from elephant dung sample. Among them, 36 showed positive on LNAM-PR media, 11 of them showed positive on LNAM-MB, 26 of them showed positive on META media, three of them showed positive both on LNAM-PR and LNAM-MB and nine of them showed positive both on LNAM-PR and META media (Table 1).

From the soil samples brought from the paper mill effluent dump sites and commercial compost production sites, 48 fungal isolate were isolated. Out of these, 24 showed positive on LNAM-PR, 13 of them showed positive on LNAM-MB, 15 of them showed positive on META media and eight showed positive both on LNAM-PR and LNAM-MB, seven of them showed positive both on LNAM-PR and META media, two of them showed positive on both LNAM-MB and META media and two of them showed positive on all the three media (Table 2). Fifty three native fungal isolate were isolated from soil, sludge and decomposing organic materials from forests, dump sites of paper mills, wood deposits etc. Out of these, 44 showed positive reaction on META, 16 on LNAM-MB and 22 on LNAM-PR (5). Seven indigenous white rot fungi were screened for the production of extracellular wood degrading enzymes on solid media by providing the apropriate enzyme substrates viz., tannic acid for ligninase, CMC for cellulase and locust bean gum for mannanases. Lignin degradation in wood wastes. Two of them showed lignin degrading activity as evidenced by the formation of dark brown zone surrounding the colony on META media (2). Fungal enzymes are being studied for their application in the degradation of aromatic pollutants causing environmental problems like pulp and paper mills (6).

From soil samples brought from the paper mill effluent dump sites, as many as 20 actinomycetes were isolated. Among them, only one showed positive on LNAM-PR, seven of them showed positive on LNAM-MB, none of them showed positive on META media (Table 3).

Many microorganisms and their enzymes have been discovered by means of extensive screening and are now commonly used in industrial applications. The discovery of new microbial enzymes through extensive and persistent screening has brought about many new and simple routes for synthetic processes

Table 3. Screening of actinomycete isolates for lignin degradation from the soil samples brought from paper mill effluent dumpsites.

Isolate code	LNAM (PR)	LNAM (MB)	META	Isolate code	LNAM (PR)	LNAM (MB)	META
B 11/1	–	+	–	B19/1	–	+	–
B 11/2	+	+	–	B 19/2	–	+	–
B 12/1	–	+	–	B 19/3	–	+	–
B 19/4	–	+	–				

and provided one possible way to solve environmental problems. Our work made an attempt in screening of such enzymes from microbes and their possible use in industries. The selected promising isolates have immense potential in the large scale commercial composting of Pressmud-spentwash.

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