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RESEARCH ARTICLE

ISOLATION AND SCREENING OF POTENTIAL CELLULOLYTIC FUNGI FOR THE MANAGEMENT OF MUNICIPAL SOLID WASTE

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ABSTRACT

Issues of Municipal Solid Waste (MSW) are a great concern of cities worldwide. Developments in municipal solid waste management technologies are lagging behind when it is compared to the growth scale of urbanisation and industrialisation. It also paves path for the greater dangers like global warming and epidemic diseases. The MSW is comprised of more than 60% of the organic matter. Since cellulose is the major component in the biodegradable fraction of the MSW, the aim of the present study is the detection of a potential cellulolytic fugal isolate for the bioconversion of MSW in to compost. Soil samples were collected from 5 different garbage dumping regions of Jabalpur and a total of 67 fungi were isolated, in which 30 showed cellulolytic activity. Seven fungal isolates were taken for the *in vitro* biodegradation of the MSW and after 45 days of incubation sample inoculated with *Cladosporium* sp. Showed maximum weight loss i.e. 30.40%. Therefore this fungal isolate is a potential cellulose degrader and useful for the municipal solid waste management.

INTRODUCTION

The issues of Municipal Solid Wastes (MSW) are no longer a menial task attended by the civic bodies, now it has emerged as a disastrous outcome of industrialization and rapid urbanization. The growing expenditure for MSW management is the common denominator in both developing and developed countries. The waste sector (including wastewater) contributes to 5% of the global greenhouse budget [1].

In the developing countries like India, biodegradable Municipal Solid Waste accounts for more than 60% of the total MSW generation [2]. It is the source of major vector borne diseases like Malaria, Cholera, Dengue, Yellow fever, Dysentery etc. Cellulose is the most abundant organic polymer found in the nature and in the MSW as well. Generally 40-50% of the MSW composed of cellulose material [3, 4].

Employing cellulolytic microorganisms in the waste management will lead to the efficient and cost effective management of the MSW as well as it could generate derive economic benefits from the end products like compost and biofuels.

The cellulolytic system of aerobic fungi and bacteria consists of three types of cellulases i.e. endoglucanases, exoglucanases and B- glucosidases [5]. Cellulase is a consortium of hydrolytic enzyme capable of hydrolysing cellulose in to simple glucose units. Several studies were carried out for the isolation and identification of the cellulolytic fungi possessing activity [6, 7].

MATERIALS AND METHODS

Collection of samples

The soil samples were collected from five different garbage dumping sites of Jabalpur i.e. Priyadarshini housing colony, Sivaji Ground Sadar, South Civil lines, Katondha Municipal Dumping Site and Gwarighat pooja stage. The samples were collected in sterile sealed polythene bags and brought to the laboratory for microbiological study.

Isolation of fungi

The soil samples were processed within 3-4 hours of collection. For isolation serial dilution method was used. This was done by adding 1 g of soil sample in 10 ml of distilled water and mixing properly at room temperature. This solution was immediately diluted up to 10^{-6}. Then 100 µl of these solutions were inoculated on different PDA (Potato Dextrose Agar) plates supplemented with chloramphenicol antibiotic [8]. The plates were made in duplicates for each dilution and the plates were incubated at 28 ± 2^0C for 5-6 days. After 6 days the colony forming units (cfu) were sub cultured into PDA slants and stored at low temperature (4^0C) for further study.

Screening for cellulolytic activity

The screening was done to select the most potential fungal isolate for the production of the extracellular cellulase. The isolated isolates were inoculated on basal salt medium supplemented with 1% Carboxy Methyl Cellulose and incubated for 6 days at 28 ± 2^0C. The inoculation was done by point inoculation method using inoculation needle. After 6 days of inoculation the plates were

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flooded with 0.1% congo red solution and kept for 10 minutes and then washed with 1 M NaCl and allowed to stand for 15 minutes [9]. Cellulolytic fungi creates a clearing zone around the colony, this zone was measured.

In vitro Biodegradation of Municipal Solid Waste

After screening six isolates showing more growth rate, zone formation and cfus were selected for the *in vitro* biodegradation of the MSW. For this 5 g of MSW was weighed and taken in petri dishes and autoclaved. These were inoculated with 5% spore suspension of the selected isolates [10]. The plates were made in triplicates for each isolate and incubated at 28 ± 2^0C for 45 days.

Dry Weight Calculation

After 45 days of incubation the weight loss were measured using the following formulae [11].

Weight loss (%) = W- W_1/W X100
Were, W=Initial weight
W_1=Final weight after 45 days of incubation.

Identification of the potential strain

Identification of the potential isolate was done microscopically by slide culture technique [12].

RESULTS AND DISCUSSION

Collection of samples and isolation of fungi

A total of 67 fungal isolates were isolated from the soil samples of five different dumping sites of Jabalpur. The number of fungal isolates isolated from each site is given in the table 1 (fig. 1)

Table 1 Number of samples isolated from each garbage dumping site

S. No	Garbage dumping site	No. of strains isolated
1	Priyadarshini housing colony, Dumna road	7
2	South civil line	21
3	Shivaji ground, Sadar	20
4	Katondha municipal dumping site	10
5	Gwarighat pooja stage	9

Gautam *et al*. [3] isolated 165 fungal species from the soil sample of MSW dumping sites. Among these the most frequent fungi were *Aspergillus niger*, *Curvularia lunata*, *A. nidulans*, *A. fumigatus*, *Penicillium sp.*, *Fusarium roseum*, and *Trichoderma viride*.

Screening for the cellulolytic activity

67 isolates were screened for cellulolytic activity after 6 days of incubation and only 30 showed shown clearing zone around the colony. Among these 30 only 7 showed significant cellulolytic activities, growth rate and colony forming units. The activity of these fungal strains is given in the table 2 (fig 1)

The soil contaminated with MSW is a rich source of cellulolytic fungi since cellulose is a major component in the MSW. These naturally occurring fungal isolates can be manipulated for the enhanced MSW treatment.

In vitro biodegradation of the Municipal Solid Waste

The Petri Dishes containing 5 g MSW inoculated with fungal isolates for the biodegradation were taken out after 45 days of incubation and the percentage of weight loss were measured.

Table 2 Activity of the major cellulolytic fungal isolates

S. No	Isolate Code	Diameter of the clearing zone
1	MSW A10	37 mm
2	MSW A8	12 mm
3	MSW A20	30 mm
4	MSW A42	29 mm
5	MSW A50	5 mm
6	MSW A63	10 mm

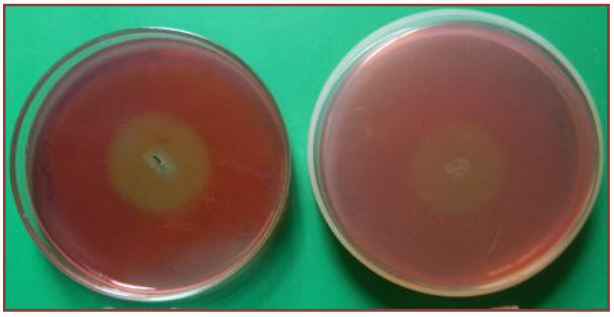

Fig 1 Cellulolytic screening of *Cladosporium* sp.

Table 3 Dry weight of the MSW samples after 45 days

S. No.	fungal isolate	Initial Weight (W)	Final Weight	loss in %
1	MSW A10	5g	3.48g	30.40%
2	MSW A8	5g	4.029g	19.42%
3	MSW A20	5g	4.065g	18.70%
4	MSW A42	5g	3.95g	21%
5	MSW A50	5g	4.096g	18.08%
6	MSW A63	5g	4.090g	18.20%
7	Control	5g	4.375g	12.50%

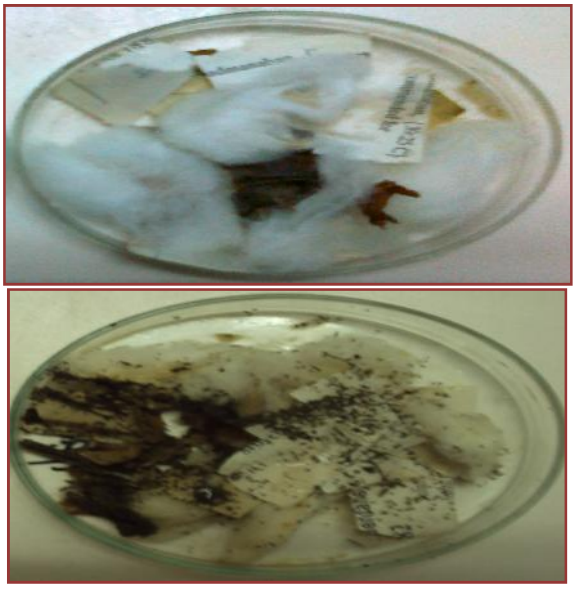

Fig 2 *In vitro* biodegradation (a): Control: After 45 days (b): Sample inoculated with *Cladosporium* sp. After 45 days

Maximum weight loss was observed in plate inoculated with fungal isolate MSW A10 whereas the weight loss was lowest in control (fig. 2). Hence the fungal isolate MSWA10 was found to be the most potential isolate for the biodegradation of Municipal Solid Waste.

Rahman *et al*. [11] reported that *T. harzianum*is the most effective fungal strain in kitchen waste decomposition. It provided the highest volume (31.80%) and weight (30.80%) losses in waste treated with spore suspension in their studies

Identification of the potential strain

The fungal strain MSW A10 was found to be the potential strain for the degradation of the municipal solid waste. The identification of the potential strain was done by slide culture technique. By this technique the potential strain was identified to be *Cladosporium* sp. Its colonies were effuse, pale grey or grayish brown, cottony. Conidiophores macronematous, straight or slightly flexuous, distinctly no dose; pale or mid pale brown, smooth, up to 500µ long or sometimes even longer in culture, 3-5µ thick terminal and intercalary swellings 6-8µ diam. Conidia from term in al swellings, which later become intercalary, in simple or branched, chains, cylindrical rounded at the ends, ellipsoidal, limoniform or sub spherical, sub hyaline or pale olivaceous brown, smooth, 5-30 x 3-6µ.

Fig. 3 *Cladosporium sp.*

The studies conducted by some workers proved that *Asprigillus s*p. and *Trichoderma* sp. exhibits good cellulolytic activity. This study indicates that *Cladosporium* sp. is also a major cellulolytic enzyme producer. Kumar and Swamy [6] reported *Aspergillus Niger* and *Trichoderma* sp. as the potential cellulolytic fungi for the degradation of areca nut husk waste.

CONCLUSION

Lignocellulolytic microorganisms, especially fungi, have attracted a great deal of interest as biomass degraders for large-scale applications due to their ability to produce large amounts of extracellular lignocellulolytic enzymes. Fungi play an important role in bioconversion/ composting of organic waste and can be an important contributor to optimal municipal waste bioconversion. From the results obtained in this study, it can be concluded that a great variety of microorganisms are found in the municipal solid waste which can effectively decompose the organic matter present in the municipal solid waste [13]. The fungal isolate *Cladosporium* sp. shows good potential for the bioconversion of the organic municipal solid waste [14].

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