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RESEARCH ARTICLE

DEVELOPMENT AND USE OF AN IMPROVED CELLULOLYTIC STRAIN FOR THE EFFICIENT BIOCONVERSION OF MUNICIPAL SOLID WASTE (MSW) INTO VALUE-ADDED COMPOST

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ABSTRACT

The development and use of an improved cellulolytic bacterial strain, through chemical mutagenesis, for the efficient bioconversion of MSW into value-added compost was studied under *in vitro* condition. In the present study, the degrading soil (MSW) samples were collected from the dumping site of Chidambaram municipality. Five cultures of cellulolytic bacteria screened and designated as "AU" series and numbered randomly. The production of cellulase enzyme by representative isolates was assayed under *in vitro* condition and revealed that the isolate AU-3 recorded a maximum production of cellulases enzyme when compared to other isolates. The phenotypic characterisation of the efficient isolate viz., AU-3 revealed that the organism belonged to the genera *Bacillus* and species *subtilis*. A putative mutant AU-3-M screened from wild AU-3 after mutagenesis with N-methyl N-nitro N-nitrosoguanidine (NTG), as a chemical mutagenic agent. It was observed that the mutant AU-3-M produced a higher amount of cellulases when compared to the wild type AU-3 strain. Moreover, the MSW inoculated with the mutant strain, AU-3-M recorded the highest weight loss on 60th day of observation than the wild strain under *in vitro* condition. It is concluded that the strain improvement of cellulolytic bacteria by mutation has high biotechnological potential for the bioconversion of MSW into value-added product viz., compost.

INTRODUCTION

The phenomenal increase in world's population and accelerated pace of urbanization has lead to the increased production of waste which renders the problem of management of municipal waste throughout the world. In the present technoeconomic era, the energy and environment crises developed due to high amount of cellulosic materials, as municipal solid waste (MSW) [1] MSW is composed of 40-50% cellulose, 9-12% hemi cellulose and 10-15% lignin on dry weight basis [2]. By the year 2047, MSW production in India is expected to reach 300 MT; and the land requirement for disposal would be 169.6 KM. Unscientific disposal of MSW causes an adverse effect on all components of environment and human health. Several measures have been taken to tackle this problem. Among the various options available, the most modern and appropriate one is the recycling of this municipal waste in a natural way [3].

The municipal waste is rich in cellulose, which is a cheap, abundant bio polymer and renewable energy source. It is a polysaccharide having a fibrous crystalline appearance made up of the repeating units of D-glucose linked by -1,4-glucosidic linkage. It is a water soluble compound and has a high molecular weight.

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The cellulosic biomass can be hydrolyzed to fermentable sugars by cellulolytic enzymes and the cellulosic enzyme system consists of three major components: endo-β-glucanase (EC 3.2.1.4), exo-β-glucanase (EC 3.2.1.91) and β-glucosidase (EC 3.2.1.21) [4].

Microorganisms play a significant role in the bioconversion of MSW into value-added product, compost through the production of cellulases enzymes. Several microorganisms, including, fungi and bacteria capable of producing cellulases in which bacterial cellulases production is gaining momentum. Bacterial cellulases are often more complex and expressed in multi-enzyme complexes providing increased function and synergy. Most importantly, bacteria inhabit a wide variety of environmental and industrial niches which produce cellulolytic strains that are extremely resistant to environmental stresses *viz*., these are thermophilic or psychrophilic, alkaliphilic or acidophilic. So, these strains are able to survive in the harsh conditions, they often produce enzymes that are stable under extreme stress conditions for bioconversion process. The wide variety of bacteria in the environment permits screening for more efficient cellulases to help overcome current challenges in application of this enzyme. Several reports discussed the bacterial diversity on cellulase enzyme production and emphasized the need of strain improvement for enhancing the same production in order to speed up the bioconversion processes [5].

The present investigation has been undertaken with an aim to develop a potential cellulolytic bacterial strain through chemical mutagenesis and use the same for efficient degradation of MSW into value-added product, compost.

MATERIALS AND METHODS

Collection of municipal solid waste sample

The municipal solid waste dump yard of Chidambaram was selected for sample collection for the isolation cellulolytic bacteria. Five different sites were located in the yard and a sample of each 1 kg of the degrading soil (MSW) was collected. All the five samples were pooled and 1 kg was taken as representative sample and used for the isolation studies.

Isolation of cellulolytic bacteria from MSW sample

Serial dilution techniques were used for the isolation of bacteria. In this technique sample suspension was prepared by adding 1 g degrading soil (MSW) to 10 ml of sterile water (the stock) and shaken vigorously for at least 1 minute. The dilute was then sedimented for a short period. Sterile dilution blanks were marked sequentially starting from stock and10^{-1} to 10^{-6}. From the dilution 10^{-6}, 1ml sample was transferred to the cellulolytic medium (CMC 1%, $NaNO_3$ 0.2%, $MgSO_4$ 0.05%, K_2HPO_4 0.005%, $FeSO_4$ 0.001%, $CaCl_2$ 0.002% and $MnSO_4$ 0.002%, distilled water 1L and agar 10g) and used for isolation of cellulolytic bacteria. The plates were incubated for 72 hours at 37°C. Bacterial colonies were isolated and subcultured to obtain single strain. The purified cultures were maintained at 4°C for further analysis.

Screening for Cellulase Producing Activity

The carboxy methyl cellulose (CMCase) activity was assayed using a method [6] with some modifications [7]. A 0.5 ml of culture supernatant was added to 0.5 ml of 1% CMC prepared in 50 mM sodium citrate buffer (pH 4.8) in a test tube and incubated at 60°C for 30 min. The reaction was terminated by adding 3.0 ml of dinitrosalicylic acid (DNS) and subsequently placing the reaction tubes in a water bath at 100°C for 15 minutes. One ml of Rochelle salt solution (l40 gm Rochelle salt in 100 ml distilled water) was then added to stabilize the color. The absorbance/Optical Density (OD) was recorded at 575 nm wave length against a blank of 50 mM sodium citrate buffer. One unit of CMCase activity was defined as the amount of enzyme that liberated 1µmol of reducing sugar (glucose) in 1 min at 37°C and pH 7.0 [8].

Identification of Bacterial Isolates

Bacterial isolates were identified by using standard identification tests given in Bergey's Manual of Determinative Bacteriology [9].

Mutagenesis by N-methyl-N-nitro-N-nitrosoguanidine (NTG)

The wide type (AU-3) cells grown in CMC medium at 50°C for 24 h was harvested at logarithmic phase by centrifugation (10,000g, 20 min) at 4°C and washed twice with McIlvaine's buffer (containing 0.1 M citric acid and 0.2 M phosphate buffer) pH 5.0. The cell was resuspended in the same buffer at a concentration of 5.8×10^8 cell/ml. and NTG (1 mg/mL) was added into the cell suspension. After incubation for 1 h at 37°C in incubation shaker at 100 rpm, the cell was centrifuged and washed immediately with buffer. The treated sample was transferred into CMC plates [10] and incubated at 50°C for 48 h. The cellulolytic activity was assayed using congo red [11].

Biodegradation of Municipal Solid Waste

Municipal solid wastes were collected from different waste dumping points of chidambaram in polythene bags. Samples were cut into small pieces, and 5 g of each was aliquoted into petri plates which were then wrapped by using polythene bags.

The plates containing municipal solid waste were then autoclaved at 121°C for 15 min. After sterilization, the plates were inoculated separately with wild strain (AU-3) and the mutant strains (AU-3M, AU-3M1 and AU-3M2). Moisture content was maintained at 50-60% throughout the active biodegradation in the plates. Turning of the organic waste was provided once in every week to ensure aerobic condition in the plates. Changes in odour and weight loss of the decomposed organic solid waste were recorded up to 60 days. For measurement of weight loss (%), the following formula was used:

$$\text{Weight loss (\%)} = \frac{W - W1}{W} \times 100$$

Where W is initial weight, and W_1 is final weight.

RESULTS AND DISCUSSION

Table 1 Designation of the cellulolytic bacteria isolates from the degrading soil (MSW) of Chidambaram

Name of Municipality	Designation of isolate
Chidambaram	AU-1
	AU-2
	AU-3
	AU-4
	AU-5

The samples of the degrading soil (MSW) were collected from the dump yard of Chidambaram municipality from various locations. The number of cellulolytic microbes isolated from the sample and their designations are given in Table-1.

Results presented in Table-2 clearly indicate the cellulase enzyme production potential of different cellulolytic isolates, obtained from MSW of Chidambaram. Among the various cellulolytic isolates, the isolate, namely, AU – 3 recorded the highest cellulase enzyme production followed by the cellulolytic isolates AU-2, AU-5, AU-4 and AU-1. The variation in cellulase enzyme production potential of cellulolytic isolates has been discussed by many authors (12-13). In the present study also, the different cellulolytic isolates, obtained from MSW Chidambaram recorded a variation in cellulase enzyme production potential and in concomitant with the earlier above findings.

The morphological and biochemical characteristics of the five cellulolytic bacterial isolates viz., AU-1 to AU-5, were studied and the results presented in Table-3 and 4. The cellulolytic gram positive bacterial isolates included the genera *Bacillus*, *Micrococcus* and *Staphylococcus* and the gram negative bacterial isolates included the genera *Pseudomonas* and *Vibrio*, respectively. Researchers studying on cellulolytic activity have isolated various bacteria from different environmental sources.

Table-2 Morphological characteristics of the cellulolytic bacteria

Isolate No.	Gram Staining	Morph-ology	Motility	Colour	Colony shape	Margin
AU-1	+	Cocci	–	cream	Irregular	Undulate
AU-2	–	Rod	+	Cream	Circular	Entire
AU-3	+	Rod	+	Cream	Circular	Undulate
AU-4	–	Rod	–	Cream	Circular	Entire
AU-5	+	Cocci	–	Cream	Circular	Entire

Tabel-3 Morphological characteristics of the cellulolytic bacteria

Biochemical characteristics	Bacterial isolates				
	AU-1	AU-2	AU-3	AU-4	AU-5
Indole Production	–	+	+	+	+
Methyl red reaction	–	–	+	+	–
Voges-Proskauer reaction	+	+	+	+	–
Citrate utilization	–	+	+	–	+
Catalase reaction	+	+	+	+	+
Oxidase reaction	–	+	+	+	–
Urease production	+	–	–	–	+
Gelatin hydrolysis	–	–	+	–	+
Nitrate	–	+	+	–	+
Strain identified	Staphylo coccus	Pseudomo nas	Bacillus sp.	Vibrio sp.	Microco ccus sp.

Tabel 4 Determination of cellulase enzyme production potential of cellulolytic isolates, obtained from MSW of Chidambaram

Isolate No.	Enzyme activity (IU/mL)
U-1	0.8
AU-2	1.9
AU-3	**2.2**
AU-4	1.3
AU-5	1.7

Aerobic cellulolytic bacteria was isolated from forest and farming soils and it was determined that they had the ability to decompose cellulose. Eight cellulolytic bacterial strains were isolated from cow dung samples. *Bacillus sp. Clostridium, Pseudomonas* and *Erwinia* showed optimum cellulase production. Ten bacterial strains such as *Bacillus spp1, Bacillus spp2, Micrococcus spp. Staphylococcus spp. Clostridium spp, Acinetobacter spp, Pseudomonas spp1, Klebsiella spp, Proteus spp1* and *Enterobacter spp*, were isolated from decayed sawdust which showed better cellulolytic activity [14].

Effect of Mutagenic Agent on Cellulase Productions

The most efficient cellulolytic isolate of the previous experiment *viz*., AU-3 (*Bacillus sp.*) was subjected to mutagenic treatment using NTG for strain improvement. After the mutagenesis following AU-3M1, AU-3M2 and AU-M3 mutant colonies were obtained. The cellulase activity of the clones and the wild type were determined in CMC liquid medium in which the mutant strain AU3-M1 exhibited highest cellulase enzyme production (4.4 IU/mL) followed by AU3-M2, AU3-M3 and wild type (AU-3) (Table-5). A similar result was found in *cellulomonas sp.* TSU03 where NTG treated mutant strains increased the yield of cellulase [15]. NTG was suggested to affect the cellulase genes within this mutant. But, how NTG triggered the cellulase production in these mutants is not clear. NTG could have affected the regulatory genes of the stability of the mRNA leading to greater cellulase synthesis [16].

Table 5 Comparative performance wild (AU-3)

Bacterial strains		Enzyme activity IU/mL
Wild type	AU-3	2.1
Mutant	AU-3M	4.4
	AU-3M1	3.0
	AU-3M2	2.6

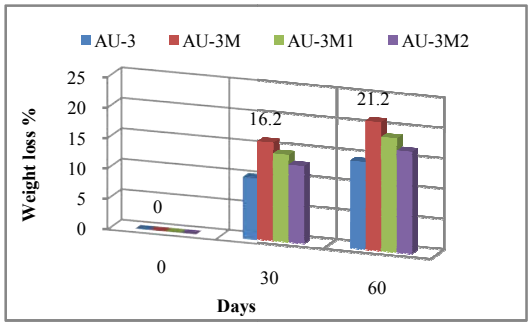
Percent Weight Loss of MSW

Table 6 Percent weight loss of Municipal Solid Waste (MSW) by wild (AU-3) and mutant strains in petri plate

Days	Weight loss (%)			
	AU-3	AU-3M	AU-3M1	AU-3M2
0	-	-	-	-
30	10.2	16.2	14.4	12.8
60	14.4	21.2	18.8	16.8

Bacteria play an important role in the decomposition of organic waste and can be important contributors to optimal waste bioconversion. For decomposition of MSW by preinoculation of *Bacillus sp*. and its NTG mutants, no bad smell was emitted after 60 days. It indicates the possible complete degradation of MSW in plates that contained 5g of MSW (Table-6). In control plates, bad smell continued after 60 days and indicated the slow degradation of MSW (Data not shown).

Percent weight loss of municipal solid waste by wild (AU-3) & mutant strains in petri plates



It was observed that there was a maximum degradation of MSW recorded with preinoculation of MSW with AU3-M, followed by AU-3M1, AU-3M2 and AU3. Preinoculation of efficient cellulolytic bacterial strain for the efficient bioconversion of MSW has already been suggested [17]. The passive role of NTG mutants in cellulase enzyme production and the efficient bioconversion of organic solid wastes have been already suggested [16]. In the present study also, the mutant strain AU3-M (NTG mutant) exhibited the highest cellulase production and efficient bioconversion of MSW and the results of the present study in concomitant with the above earlier findings.

CONCLUSION

From the above findings, it is concluded that a large number of bacteria were found in the degrading soil (MSW) and MSW is a suitable substrate for the efficient bioconversion into value-added product, compost because of the presence of high

percentage of organic matter. *Bacillus sp*. had promising effects in the process of bioconversion. However, strain improvement (*Bacillus*) by using NTG had a promising role in the augmentation of cellulase enzyme production when compared to the respective wild type. Moreover, the preinoculation of improved strain (AU3-M) resulted in higher degradation of MSW in 60 days followed by other mutants and wild strain. It is evident that strain improvement is the essential phenomenon for the efficient bioconversion of MSW into value-added compost and the subject needs further physiological and molecular research of the microbes concerned.

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