

Isolation and Identification of Cellulose Degrading Microbes

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ABSTRACT: Cellulose degrading bacteria was isolated from soil where as cellulose degrading fungi was isolated from soil and decaying wood. The bacteria were identified as *Bacillus subtilis* and the fungi were identified as *Aspergillusniger* and *Trichodermaviride*. Their cellulase degrading ability was screened by Congo red plate assay. Their enzyme activity was tested with CMC assay, and the protein estimation was done by Lowry's method. *Aspergillusniger* showed the highest cellulase activity of 1861.61IU/ml, followed by *Trichodermaviride* 780.676IU/ml and *Bacillus subtilis* showing the least cellulase activity of 450.389IU/ml. Protein estimation was also carried out, in which *Aspergillusniger* showed 0.18 mg/ml, *Trichodermaviride* showed 0.15mg/ml and *Bacillus subtilis* showed 0.31mg/ml.

KEYWORDS: Cellulose, *Bacillus subtilis*, *Aspergillusniger*, CMC assay, protein assay.

I.INTRODUCTION

Increase in energy demand due to growth and development of nations has led to the exhausting of the non renewable sources leading to energy crises. Every day the energy requirement is increasing and the existing sources are unable to cope up with the energy demand. Increasing in the price of fossil fuels is also a major concern, and it affects badly the social economy of the country. And these fossil fuels also cause environmental pollution. Alternative sources of energy must be utilized as to balance or satisfy the growing energy demand. These alternative sources are easily renewable and don't cause any environmental pollution. Bio ethanol comes as a good alternative solution. It can be produced from waste organic matters. The bio ethanol produced can be blended with the existing fossil fuel or can be used. Ethanol is commonly used as gasohol in the United States. Ethanol produced from corn had been utilized in United States of America as gasoline since 1980s [1].For India, its ethanol market is more mature than its biodiesel market. In India during the year 2003, the Ministry of Petroleum and Natural Gas has launched Ethanol Blended Petrol (EBP) Program in which 5% ethanol is blended in gasoline and give in nine states and four union territories [2]. Ethanol production from lignocellulosic biomass is possible however it requires more refinement in extracting of reducing sugars for the ethanol production.

In this study isolation and identification of cellulase degrading microbes is studied and compared between cellulose degrading bacteria and fungi.

II. MATERIAL AND METHODS

- A. *Sample Collection:* The soil sample and decaying wood were collected for the isolation of cellulose degrading microbes. 1g of soil sample was taken and was dissolved in 10ml of distilled water and serial dilutions were made from 10^{-1} and 10^{-7} . Then 1ml from each dilutions of 10^{-5} , 10^{-6} and 10^{-7} , were plated on nutrient agar

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plate. 1g of decaying wood sample was serially diluted and dilutions were made from 10^{-1} to 10^{-3} , then 1ml of 10^{-1} and 10^{-2} dilutions were pour plated on potato dextrose agar plate.

- B. *Identification of Microbes:* Bacterial identification was done by following Bergeys manual of determination [3] and fungi identification was done by lacto phenol cotton blue.
- C. *Screening for cellulase activity:* Screening of cellulase activity was done by Congo red plate. Congo-Red agar media with the following composition: KH_2PO_4 0.5 g, MgSO_4 0.25 g, cellulose 2 g, agar 15 g, Congo-Red 0.2 g, and gelatine 2 g; distilled water 1000ml [4]. 100ml of media was prepared. It was autoclaved at 121°C at 15psi. The media was cooled at room temperature before pouring in sterile Petriplate. After pouring the media with the help of sterile gel puncher small wells were made on the plates. The spore suspensions of the isolated microorganism were prepared and were inoculated on to the well and were incubated for 48 hours and the colour change of Congo red plate was observed.
- D. *Cellulase enzyme production and assay:* The bacteria species was grown for production of cellulase in a minimum salt medium containing MgSO_4 0.1 g, $(\text{NH}_4)_2\text{SO}_4$ 5g, KH_2PO_4 2g, K_2HPO_4 7g, Sodium citrate 0.5g, distilled water 1000ml supplemented with CMC (1%) as carbon source. The fungi species were placed in a minimal salt medium, containing FeSO_4 0.002g, NaNO_3 , 3g, Na_2HPO_4 1g, MgSO_4 0.1g, KCL 0.5g, distilled water 1000ml supplemented with CMC 1% as carbon source [5]. Both bacterial and fungal species were grown on the medium for seven days and each day the cultural filtrate was taken for testing of enzyme activity. Cellulase activity was assayed by the determination of reducing sugar released from CMC assay (CMC). 0.5 ml of culture supernatant fluid was incubated with 0.5 ml 1% CMC in 0.05M citrate buffer, pH 4.8 at 50°C for 30mins. The reducing sugar product was assayed by the Dinitrosalicylic acid (DNSA) method using glucose as the sugar standard [6]. The enzyme activity was calculated by the formula [7]:

$$\frac{IU}{ml} = \frac{mg/ml}{incubation\ time \times volume\ of\ substrate \times Mol.\ wt.\ of\ glucose} \times 1000$$

- E. *Protein estimation:* Protein estimation was done based on Lowry's Method. 1ml of the cultural filtrate was taken, centrifuged and then was analysed for protein content taking bovine serum albumin as standard.

III. RESULT

- A. *Identification of cellulose degrading microbes:* The cellulose degrading bacteria was identified as *Bacillus subtilis* based on the Bergy's manual of determination. And on the basis of lactophenol cotton blue staining the fungi were identified as *Aspergillus niger* and *Trichoderma viride*.

Table 1: Physiological and biochemical characteristic of Bacteria

CHARACTERISTICS	RESULTS
Gram staining	+
Morphology	Rod shaped
Aerobic test	+
Starch hydrolysis test	+
Voges-Proskauer test	+
Citrate test	+
6.5% NaCl test	+
Catalase test	+

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B. *Screening for cellulase activity:*The spore suspension of bacteria and fungi were prepared in their respective broth. Congo red media was prepared, autoclaved and poured onto petriplate. With a sterile gel puncher well were created in the centre of petriplate and the spore suspension of bacteria and fungi were inoculated. After 48 hours the plates were checked for cellulase activity. The change in the colour of media from red to purple confirms the ability of the microorganism to produce cellulase enzyme.

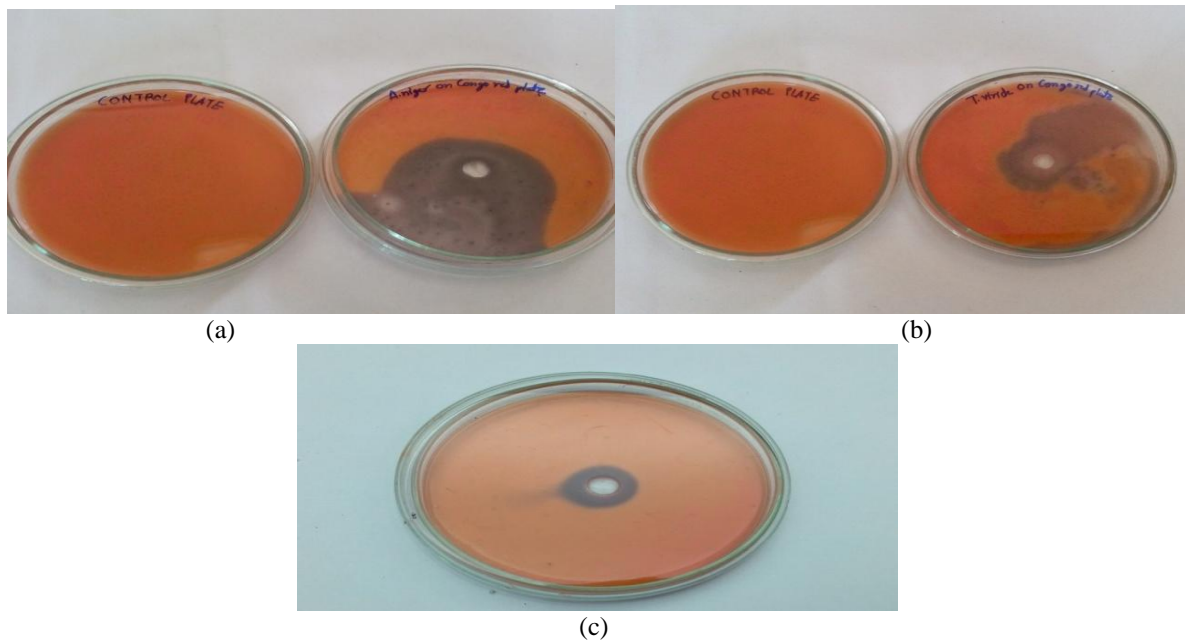


Figure 1: Congo red plate assay of *Aspergillusniger* (a),*Trichodermaviride* (b),*Bacillus subtilis* (c)

C. *Cellulase enzyme assay:*The cellulase enzyme was produced in MSM with Carboxymethyl cellulose as substrate. The enzyme activity was done by CMC assay by using glucose. It was found the *Aspergillusniger* showed the highest enzyme activity of 1861.61IU/ml, followed by *Trichodermaviride* at 780.67IU/ml. *Bacillus subtilis* showed the least activity of 450.38IU/ml.

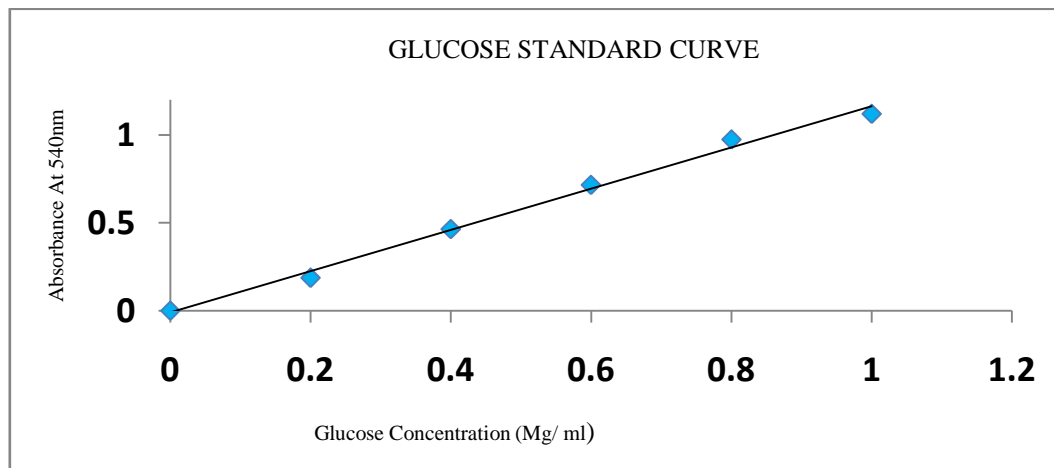


Figure 4: Standardization of Glucose

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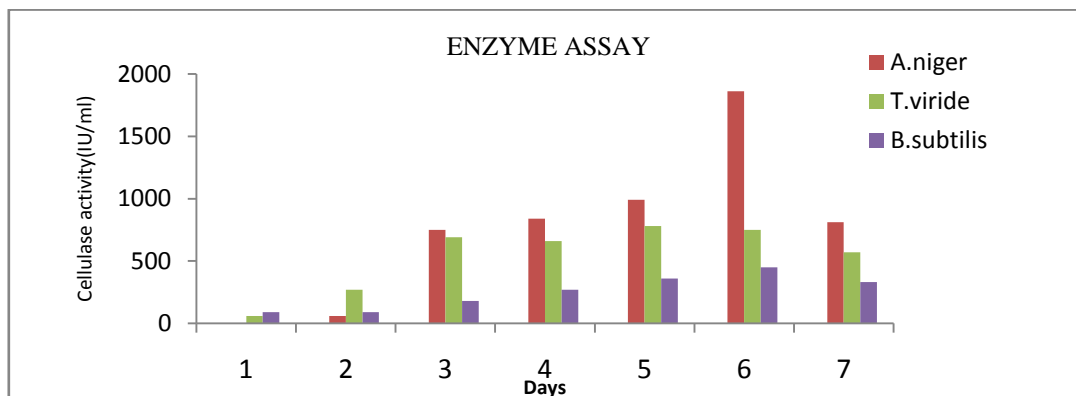


Figure 5: Enzyme activity of *Aspergillusniger*, *Trichodermaviride*, *Bacillus Subtilis*

Table 2 : Enzyme activity by CMC assay

Cultural filtrate (No of Days)	<i>Aspergillusniger</i> (IU/ml)	<i>Trichoderma viride</i> (IU/ml)	<i>Bacillus subtilis</i> (IU/ml)
1	3.00	60.05	90.07
2	60.05	270.23	90.07
3	750.64	690.59	180.15
4	840.72	660.57	270.23
5	990.85	780.67	360.31
6	1861.61	750.64	450.38
7	810.70	570.49	330.28

D. Protein estimation:Protein estimation was done by Lowry’s method using bovine serum albumin as standard. Cultural filtrate was withdrawn in 24 hour time interval.1ml of cultural filtrate was taken to estimate the protein content. *Bacillus subtilis* showed the highest protein content at 0.31mg/ml, *Aspergillusniger* showed 0.18mg/ml, and *Trichodermaviride* showed the least protein content of 0.15mg/ml

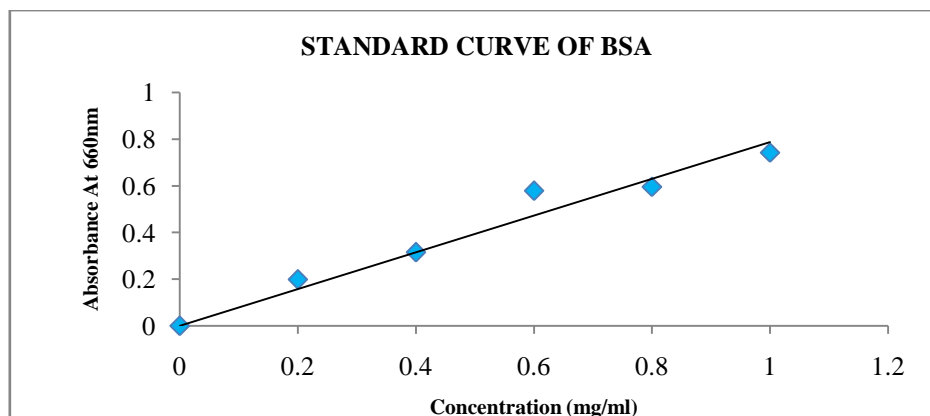


Figure 6: Standardization of Protein

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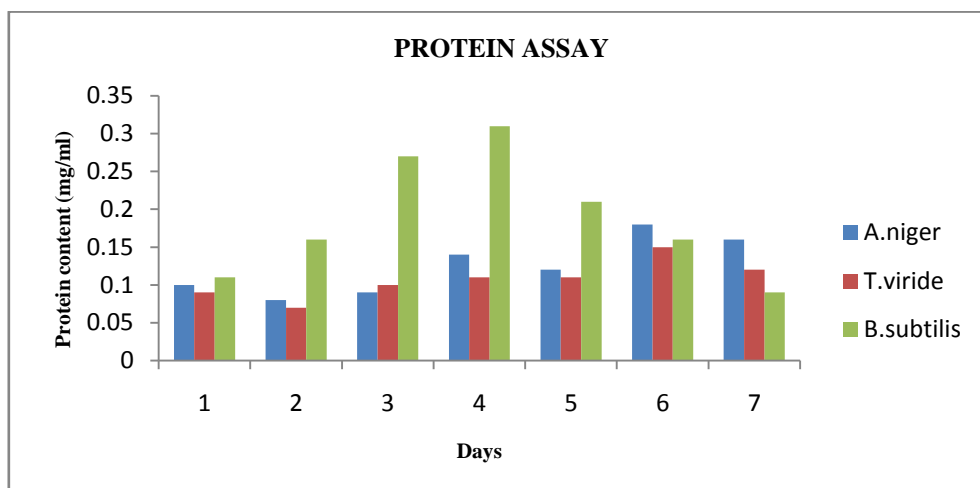


Figure 7: Standardization of Protein

Table 4: Protein estimation of cellulase enzyme

Cultural Filtrate(days)	<i>Aspergillusniger</i> (mg/ml)	<i>Trichodermaviride</i> (mg/ml)	<i>Bacillus subtilis</i> (mg/ml)
1	0.1	0.09	0.11
2	0.08	0.07	0.16
3	0.09	0.1	0.27
4	0.14	0.11	0.31
5	0.12	0.11	0.21
6	0.18	0.15	0.16
7	0.16	0.12	0.09

IV. DISCUSSION

In this present work cellulase activity of the isolated bacteria *Bacillus subtilis* and fungi *Aspergillusniger*, *Trichodermaviride* were studied. It was found that fungi *Aspergillusniger* showed the highest cellulase activity compared with *Bacillus subtilis* and *Trichodermaviride*. In a study conducted the fungi which were isolated from forest litter soil out of which three belong to genus *Aspergillus* and one belonged to *Penicillium*. Studies showed that *Aspergillus* species showed the highest cellulase activity by CMC assay and FPA. [8]. In A similar study conducted by [9] it was found that *Aspergillusniger* is easily capable of producing large amount of cellulase enzyme and can be used as to break the lignocellulosic biomass for the production of bioethanol, and also the most common occurring fungi found in the environment. In one of study found that the percentage index of *Aspergillusniger* was 91.7%. It is clear that the fungi are most efficient in producing cellulase enzyme when compared with bacteria. Optimization for the production of cellulase enzyme for these fungi is being studied. In a study conducted for the optimization of cellulase enzyme by using sugarcane waste as a substrate for *Aspergillusniger* and *Trichodermaviride* it was found that the waste acted as a good carbon source for the production of cellulase enzyme and *Aspergillusniger* produced highest cellulase activity compared to *Trichodermaviride* [10].

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V. CONCLUSION

The current study was based on the comparative study of cellulase degrading bacteria and fungi. The bacterial identification was done by Bergey's manual of determination and fungi identification was done by lactophenol cotton blue staining. The isolated microbes were found to be *Bacillus subtilis*, *Aspergillusniger*, *Trichodermaviride*. Their enzyme activity was confirmed by congo red plate screening assay. It was found that *Aspergillusniger* showed the highest cellulase activity when compared with *Trichodermaviride* and *Bacillus subtilis*. Protein estimation using Lowry's method was found to be highest in *Bacillus subtilis* at 0.31mg/ml.

VI. FUTURE SCOPE

The isolated microorganism can be further studied for the optimization of cellulase enzyme production by fermentation which can be useful for the breakdown of the lignocellulosic biomass into reducing sugars for the production of bioethanol.

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