Production and partial purification of cellulose by *Basillus subtilis* Fumigatus fermented in coir waste and sawdust

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SUMMARY

Basillus subtilis* was used as fermentative organism for the production of cellulose enzyme using cheap substrate such as coir waste and sawdust. Maximum cellulose enzyme productivity was noted on coir waste compared with saw waste. Cellulose productivity was optimized in various physico-chemical parameters, such as pH, temperature, carbon and nitrogen sources. Cellulose enzyme protein fraction was analyzed by SDS-PAGE method. Coir waste can be used as the substrate for the large scale production of cellulose enzyme using *Basillus subtilis.

Key words : *Basillus subtilis*, Cellulose enzyme, SDS-PAGE, Coir waste and sawdust.

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Cellulose is commonly degraded by an enzyme called cellulose. This enzyme is produced by several microorganisms, commonly by bacteria and fungi (Bahkali, 1996; Magnelli and Forchiassin, 1999; Immanuel *et al*., 2006). Although a large number of microorganisms are capable of degrading cellulose, only a few of these produce significant quantities of cell free enzymes capable of completely hydrolyzing crystalline cellulose *in vitro*. Fungi are the main cellulose producing microorganisms, though a few bacteria and actinimycetes have also been reported to yield cellulose activity.

The specific cellulolytic activity shown by the bacterial species is found to be depending on the source of occurrence. Some features of cellulosic materials are known to inhabit their degradation / bioconversion. There are degree of crystalinity, lingnification and the capillary structure of cellulose to cellulolytic enzymes and other hydrolytic agents (Fan *et al.*, 1987). However, many physical, chemical and microbial pre-treatment methods for enhancing bioconversion of cellulosic materials have been reported (Kumakura, 1997; Kanosh *et al.*, 1999).

The bioconvertion of various complex cellulosic waste materials such as baggase (Kanosh *et al.*, 1999) have been reported. *Basillus subtilis* has been used for a bioware stimulant during project SHAD. Hence, the present study was carried out to determine the cellulolyticenzyme activity of bacterium, *Basillus subtilis* against coir waste and saw dust as carbohydrate source.

MATERIALS AND METHODS

The strain of *Bacillus subtilis* MTCC 1305 used in the present study was obtained from IMTECH at Chandigarh. The organism was cultured and maintained on Czapek medium for further study. The raw substrates were sun dried individually to reduce the moisture content to make them more susceptible for crushing. The crushed substrates were then sieved individually to get powder form. Then the substrates were soaked individually in 1% sodium hydroxide solution (NaOH) in the ratio 1: 10 (substrate: solution) for two hours at room temperature. After which, they were washed for free of chemicals and autoclaved at 121°C for one hour. The treated substrates were then filtered and washed with distilled water until the wash water became neutral (Gharpuray *et al.*, 1983).

The cultures of *Bacillus subtilis* were maintained as stock culture on Czapek- Dox agar slants. They were grown at 37°C for 24 hours and stored at 4°C for regular sub-culturing. 100 ml of inoculum was prepared for each culture using Czapek- Dox broth in 250 ml flasks. The inoculum was kept in shaker (200 rpm) at 37°C for 24 hrs before it was used for the fermentation process. To 100 ml of the optimized culture medium, 10ml of broth culture was inoculated under controlled conditions. Then it was kept in a shaker (200