

Bioconversion of cellulose into fermentable sugars by *Saccharomyces cerevisiae* cells for the production of ethanol using Cellulolytic fungi isolated from soil

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Citation

R Devi, S Shankar. *Bioconversion of cellulose into fermentable sugars by Saccharomyces cerevisiae cells for the production of ethanol using Cellulolytic fungi isolated from soil*. The Internet Journal of Microbiology. 2008 Volume 7 Number 2.

Abstract

The fungal species with cellulase activity was isolated from the soil using standard procedures. Two isolates were found to produce relatively high amount of cellulase. The production of cellulase by the two isolates was achieved in liquid shake culture containing Carboxymethyl cellulose as substrate. The filter paper activity and CMCase activity of the two isolates were also compared. The 9th day culture filtrate of the two isolates also exhibited highest activity. Ethanol production was checked in the media containing CMC as substrate after saccharification and fermentation by *Saccharomyces cerevisiae* cells. The ethanol yield was found to be maximum on the 9th day culture of both the isolates. Thus the current work deals with comparison of cellulase activity of the two isolates and alcohol production of two isolates by simultaneous saccharification and fermentation.

INTRODUCTION

Cellulose is the most abundant renewable natural product in the biosphere. Much of the cellulose in nature exists as waste paper. The potential of cellulose as an alternative energy source has stimulated research into bioconversion process which hydrolyze cellulose to soluble sugars for feedstock in alcoholic fermentations and other industrial processes (Bakare et al., 2005).

Study of fungi from the taxonomic point of view is of practical value. Apart from their disease causing nature, their beneficial effects have an increasing role in the industrial field. Fungi are general manager in nutrient recycling department of nature. Fungi have generally been considered the main organisms responsible for decomposition of cellulose. The researchers are trying to discover more cellulolytic fungi and are developing mutant strains to enhance the production of cellulases (Khalid et al., 2006).

Bioethanol produced from renewable biomass has received considerable attention in recent years. (Patel et al., 2007). Bioconversion of cellulosic biomass into fermentable sugar for the production of ethanol using cellulose degrading fungi, makes bioethanol production economic, environmental friendly and also renewable. Cellulase is a complex enzyme having chiefly endo and exo1-4 glucanase and β -glucosidase activities. A synergistic action of these

enzymes is required for the complete hydrolysis of cellulose (Pothiraj et al., 2006).

The *Saccharomyces cerevisiae* cells have been used in baking and fermenting alcoholic beverages for thousands of years. The ability of yeast to convert sugar into ethanol has been harnessed by the biotechnology industry, which has various uses including ethanol fuel. The fermentation method generally uses three steps: (1) the formation of a solution of fermentable sugars, (2) the fermentation of these sugars to ethanol, and (3) the separation and purification of the ethanol, achieved by distillation methods (Badger, 2002).

In recent years, growing attention has been devoted to the conversion of biomass into fuel ethanol, considered the liquid fuel alternative to fossil fuels (Lin and Tanaka, 2005). Bioethanol fermentation is by far the largest scale microbial process.

MATERIALS AND METHODS

ISOLATION OF CELLULOLYTIC FUNGI

Mandel's medium (Patel et al., 2007) was prepared by adding the following reagents (g 1000 ml⁻¹). Urea (0.3 g), (NH)₂SO₄ (1.4 g), KH₂PO₄ (2.0 g), CaCl₂.2H₂O (0.3 g), MgSO₄.7H₂O (0.3 g), Bactopectone (1.0 g), Tween 80 (0.1 g), Carboxymethyl cellulose (10g). Trace elements: FeSO₄.6H₂O (5 mg), MnSO₄.H₂O (16 mg), ZnCl₂.2H₂O

(17mg), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (2 mg).

The Mandel's medium was prepared and its pH was adjusted to 5.0. The conical flasks were plugged with cotton and sterilized at 15 lbs per sq.inch for 20 minutes. Each flask was inoculated with various soil isolate. These flasks were incubated at room temperature for 5 days on a shaker at 125 rpm. After five days, the mycelium was separated by filtration through Whatman filter paper No. 1. The filtrate was used for further analyses of the study.

DETERMINATION OF REDUCING SUGARS AND CELLULASE ACTIVITY

The total amount of reducing sugars in the sample was determined by Dinitrosalicylic acid (DNS) method (Miller, 1959). Cellulase activity (CMCase) of the fungal sample was determined as followed by filter paper activity (FPA), a method proposed by Ghose, (1987).

ESTIMATION OF ETHANOL AFTER FERMENTATION BY CELLS

The culture filtrate was further inoculated with *Saccharomyces cerevisiae* strain and allowed to ferment for 7 days. After fermentation it was filtered and ethanol content of the cultures was estimated. Ethanol content of the fermented culture filtrate was assessed quantitatively using spectrophotometric method (Cemeg and Cymru, 2006).

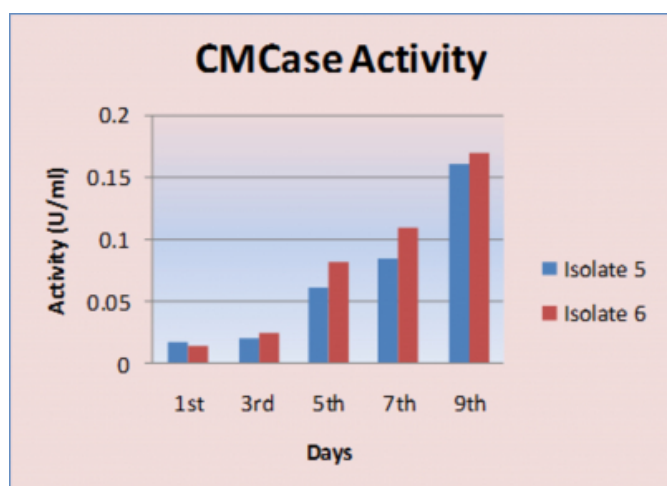
RESULTS AND DISCUSSION

CMCASE ACTIVITY

The CMCase activities of the two cultures were observed on the 1st, 3rd, 5th, 7th and 9th day of the growth.

Figure 1

Figure 1



It is clearly indicated from the figure 1 that the enzyme activity of both the isolates were gradually increasing when CMC was used as a substrate and also the activity of isolate 6 was found to be slightly higher when compared with isolate 5.

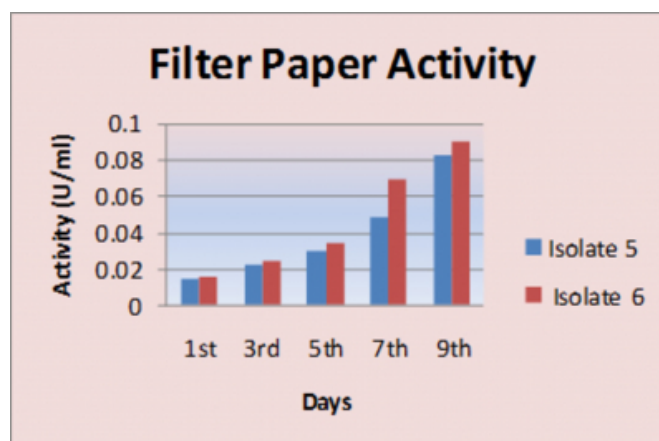
Among various soluble organic carbon sources and lignocelluloses tested in a study by Narasimha et al. (2005), carboxymethylcellulose and sawdust at 1% supported maximum production of cellulase by *A. niger*.

FILTER PAPER ACTIVITY

Figure 2 represents the filter paper activity of the selected isolates.

Figure 2

Figure 2



From the graph, it is observed that as the number of days increase, the cellulolytic activity of both the fungal isolates increased. Though similar trend was observed for both the methods, cellulase activity was found to be slightly lesser in FPA activity when compared to CMCase activity.

Our results are in accordance with Rajoka (2004), who has reported that the medium containing soluble carbon sources like CMC, the organism synthesizes low enzyme for filter paper activity, while it synthesizes a high level of enzymes when grown on other cellulosic substrates.

In a study by Ojumu et al., (2003), the filter paper activity of *A. flavus* on saw dust gave the highest cellulase activity of 0.0743 U/ml.

Estimation of alcohol in the selected sample

Since the enzyme activity was found to be more on 7th day and 9th day cultures, alcohol estimation was done only for

these two cultures. These two cultures were allowed to undergo fermentation using *Saccharomyces cerevisiae* at a temperature of 37°C for 7-9 days. The sample was centrifuged and the supernatant was used for alcohol estimation.

Figure 3

Figure 3



The graph clearly shows that alcohol production was found to be more on 9th day.

In a study by Karimi et al., (2006), the ethanol yield by *Saccharomyces cerevisiae* on complex substrate like straw yielded only 10.20 g/L of ethanol.

Production of alcohol using the fungal isolates and *Saccharomyces* cells could be achieved as more sugar molecules could be produced from the substrate like CMC.

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