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Screening, Identifying of *Penicillium* K-P Strain and Its Cellulase Producing Conditions

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Abstract

Cellulase production by *Penicillium* species are of greater interest in microbial enzyme technology. *Penicillium* sp. was cultivated in liquid culture medium at different carbon sources, nitrogen sources in different pH and temperature conditions. The strain of K-P with high cellulase activity was screened. The cellulase activity was 198 U/mL in the presence of fructose on day fifth. Maximum activity was recorded 154 U/mL in with the presence of ammonium nitrate on the fourth day. And maximum cellulase activity was obtained when the pH was 3.0 (129 U/mL) on day fourth. But the highest cellulase activity recorded (274 U/mL) in the presence of fructose, ammonium nitrate, pH 3.0 on the fifth day. The results showed the profiles of cellulase were produced maximum level according to which enzyme is most active in that particular environment.

1. Introduction

Penicillium is known to be the efficient producer of cellulose. Cellulase is an industrially important enzyme with applications in food, feed, textile, pulp and paper industries [1, 2]. Soluble raw materials, such as glucose, fructose, sucrose and corn Stover are commonly used as the main carbon source and it has been known as an ideally suitable substrate for cellulase production [3, 4].

The production of cellulase is a major factor in the hydrolysis of cellulosic materials. The widely accepted mechanism for enzymatic cellulase hydrolysis involves synergistic actions by endoglucanase (EC 3.2.1.4), exoglucanase or cellobiohydrolase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) [4, 5]. Therefore, isolation and selection of highly efficient cellulase producing strain with complete enzyme system appears to be crucial. In this study, large number of samples was collected and a cellulase producing strain K-P with highest enzyme activity was selected from them. Enzyme production conditions of K-P were optimized.

The enzyme production was affected significantly under different concentration of nitrogen source [3, 5, and 6]. The pH has influence on the production of cellulase. Earlier reports suggested the production of cellulase under acidic condition [7]. In this article, we studied the enzyme production by *Penicillium* sp. at different carbon sources, nitrogen sources on different pH conditions.

2. Materials and methods

Culture medium

SDA medium and cellulose-congo red medium [6] were used as differential medium. The selection medium was sodium carboxymethylcellulose (CMC) medium as described previously [7]. The liquid fermentation medium for the enzyme production was prepared as described by Li [8].

Isolation and screening

Samples were collected from the Air and soil. Air samples were collected from the fifth Floor of Life Science Building, University of Madras, Maraimalai campus, Guindy, Chennai. The soil samples were collected from Gandhi Mandapam, Chennai. The fungal strains were grown in Potato Dextrose Agar medium. Nine air samples and five soil samples were selected and it was maintained in the Sabouraud Dextrose medium. This culture solution was streaked on a cellulose-congo red medium at 28°C and incubated for 3 days. For screening the strain, the colony with hydrolyzed circle was selected and was again streaked on the isolation medium. The above mentioned steps were repeated until the pure culture of the strain was obtained.

Identification of K-P

Morphological analysis was carried out based on the Hand Book of Fungus Identification by Wei [9].

Preparation of crude enzyme

When fermentation was completed, fermentation broth was leached by single-layer gauze and then preserved at 4°C after centrifugation (10,000 rpm, 20 min at 4°C) to

remove the mycelium. Supernatant was the crude enzyme.

Enzyme assays

Cellulase activity was analyzed with the reducing sugar liberated in the reaction mixture measured by the dinitrosalicylic acid (DNS) method [8]. The widely accepted DNS method was applied in enzymatic activity assay. The determination of exoglucanase (CBH) activity was carried out by referring to the methodology described by Yoon [9]. After incubation at 50°C for 120 min, the reducing sugar liberated in the reaction mixture was measured by the DNS method. The cellulase was expressed as U/mL of crude enzyme. One unit (U) of enzyme activity is defined as the amount of enzyme required to liberate 1 μg of reducing sugars.

3. Results and discussion

Screening of cellulase producing strains

In the screening of strains, 14 cellulase producing strains were selected from SDA medium in primary screening, and then two strains with high cellulase activity were selected from cellulose-congo red medium in secondary screening. Because K-P strain was higher than another strain, it was selected for further study, which is shown in Table. 1.

Table. 1 Comparison of cellulase activity of two strains

Strain	Cellulase activity (U/mL)
K-P	18
K-G	6

Identification of cellulose-decomposing strain

Morphological analysis was carried out to identify the strain and found to be *Penicillium* sp.

Enzyme production conditions of K-P

Effect of different carbon sources

The strain K-P was cultured in liquid fermentation medium with (1% w/v) CMC, Glucose, Fructose, Galactose, Maltose, Sucrose and Corn steep liquor, plant carbon source (*Vigna radiata, Cajanas cajan, Cicer arietinum, Vigna mungo, Zea mays, Oryza sativa* and *Triticum vulgare* seeds), respectively.

From Figure 1 and 2, it can be observed that all kinds of cellulase synthesis were, respectively, corresponding to the optimum carbon sources. When CMC was the sole carbon source, cellulase activity reached the peak value; when Fructose, *Oryza sativia*was the sole carbon source. In terms of microbial degradation of cellulose, cellulase production will be effected by several inducers, whereas the production of cellulase will be inhibited in the presence of the utilizable sugar, such as glucose [5]. The strain was resistant to repression of glucose.

Fig. 1. Effect of different carbon sources on cellulase activity

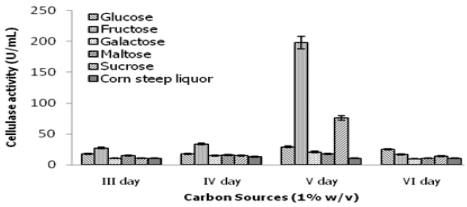
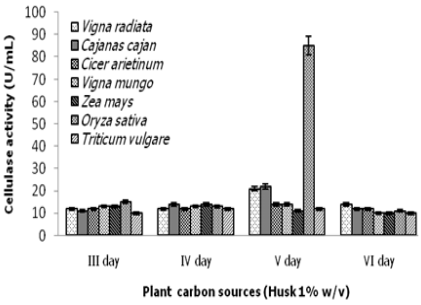


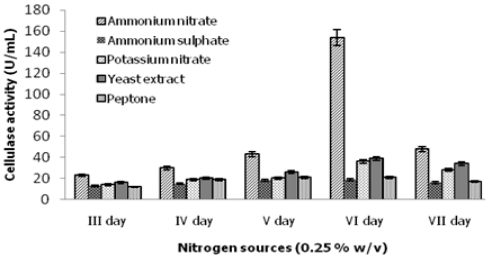
Fig. 2. Effect of different plant carbon sources on cellulase activity



Effect of different nitrogen sources

The effect of nitrogen sources on enzyme activity was investigated by separately adding 0.25% to each nitrogen source. Such as ammonium nitrate, ammonium sulphate, potassium nitrate, yeast extract and peptone in the culture medium. In Figure 3, it can be observed that enzyme activity reached the peak value when ammonium nitrate was the sole nitrogen source as ammonium nitrate was good for cellulase synthesis.

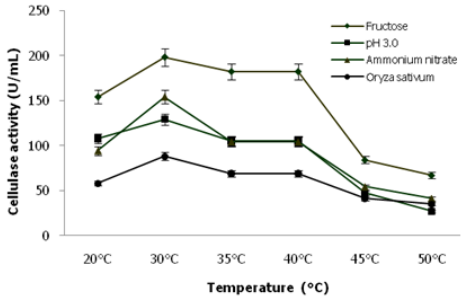
Fig. 3. Effect of different nitrogen sources on Cellulase activity



E

Under the different culture temperatures (25°C, 30°C, 35°C, 40°C and 45°C) the cellulase activity was detected after seven days in fermentation culture strain K-P. As shown in Figure 4, cellulase activity reached the highest value under 30°C.

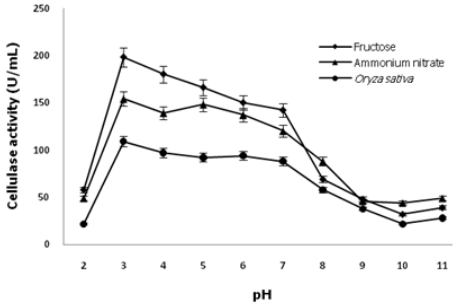
Fig.4. Effect of different temperature on cellulase activity



Effect of initial pH value

Effect of pH was detected by varying the pH (2.0- 11.0) of liquid fermentation medium of the fermentation culture found to the enzyme activity after cultivation for 4 days is shown in Figure 5. Cellulase reaches the highest content under pH 3.0. The activity was observed to be maintained under pH 3.0- 7.0.

Fig. 5. Effect of initial pH value on cellulase activity



Optimum culture condition

The strain, K-P was cultured in the optimal conditions, the cellulase activity was determined, and the results are shown in Table. 2. The optimized cellulase activity reached the highest value of 274 U/mL on the fourth day in the presence of Fructose, *Oryza sativa,* ammonium nitrate, pH 3.0 and temperature 30°C. Compared with the control, cellulase was found to be increased by 50 times. Time for achieving the peak enzyme activity was found to be shortened considerably.

Table. 2 Comparison of cellulase activity between the un-optimized and the optimized

Cellulase	Incubation time (Day)					
	1Day	2 Day	3 Day	4 Day	5 Day	6 Day
C*	62	88	104	148	**274**	23
C	4	9	27	34	**165**	17

Note: The enzyme with the "*" superscript was optimized

3. Discussion

As biotechnological process as are likely to base on crude enzymes, it is important to increase their activities in the culture supernatants by selecting the exact carbon source and optimizing their concentrations [12]. Enzyme production was affected significantly under different concentrations of nitrogen sources [13, 14]. Besides these temperature, moisture, pH had also influenced the cellulase activity [15, 16].

In this article, the experimental result showed that strain K-P has a high cellulase activity (Optimized to 274 U/mL). So with this result the fructose and ammonium nitrate, *Oryza sativa* at pH 3.0 such types surceases influence the high yield for the production economically important cellulase.

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