Screening and Characterization of the High-Cellulase-Producing Strain Aspergillus glaucus XC9

Abstract  Cellulose is a kind of renewable resource that is abundant in nature. It can be degraded by microorganisms such as mildew. A mildew strain with high cellulase activity was isolated from mildewy maize cob and classified as Aspergillus glaucus XC9 by morphological and 18S rRNA gene sequence analyses. We studied the effects of nitrogen source, initial pH, temperature, incubation time, medium composition, and surfactants on cellulase production. Maximal activities of carboxymethylcellulase (6,812 U/g dry koji) and filter paperase (172 U/g dry koji) were obtained in conditions as follows: initial pH, 5.5–6.0; temperature, 30°C; cultivation period, 3–4 days; inoculum ratio, 6% (vol/vol); sugarcane bagasse/wheat bran ratio, 4:6. When bagasse was used as substrate and mixed with wet koji at a 1:1 (wt/wt) ratio, the yield of reducing sugars was 36.4%. The corresponding conversion rate of cellulose to reducing sugars went as high as 81.9%. The results suggest that A. glaucus XC9 is a preferred candidate for cellulase production.

Keywords  Aspergillus glaucus, cellulase activity, saccharification

1 Introduction

Cellulose is a kind of reproducible resource that is abundant in nature. It produces lignocellulose, primarily of lignin in plants. The total amount of biomass generated worldwide by plant photosynthesis is about 1.14×10^{12} t annually. Additionally, more than 7×10^{8} t of crop residues, such as wheat straws, rice straws, corn stalks, and groundnut shells, is produced annually in China. However, most of these materials are generally burnt in the field, resulting in low energy utilization efficiency (10%) and environmental pollution [1]. On the other hand, food shortage, energy crisis, and environmental pollution are now major problems all over the world, which makes the utilization of cellulose quite urgent. Cellulosic materials can be decomposed into fermentable sugars, which can be converted into other valuable products such as ethanol, single-cell proteins, and hydrogen [2]. Acid treatment and enzymatic hydrolysis are the most common ways to break down cellulose into glucose. Compared with acid treatment, enzymatic hydrolysis consumes less energy and is more environment-friendly [3]. But the low activity of cellulase limits its applications for the degradation of lignocellulosic materials [4]. To facilitate industrial usage of lignocellulosic materials, it is necessary to screen microbial strains with high cellulase activities. In this paper, we described the screening and characteristics of the high-cellulase-producing strain Aspergillus glaucus XC9.

2 Materials and methods

2.1 Culture media

This study used Dubos cellulose medium, Hutchison medium [5], and cellulose–Congo red medium [6,7]. The premedium was 10% wheat bran extract. The basal solid medium for cellulase production was composed of 4 g of alkali-treated rice straw powder, 1 g of destarched bran, and 15 ml of salt solution (4 g/l KH_{2}PO_{4}, 1.6 g/l (NH_{4})_{2}SO_{4}, and 1 g/l MgSO_{4}).

2.2 Isolation and screening of microorganisms

Rotten branches, mildewy materials, and soil samples were collected from the area surrounding the Xiamen University
campus. Rotten branches and mildewy materials were mashed, added to normal saline, and shaken until the soil had dispersed in normal saline. The suspension was then inoculated in Dubos cellulose medium and Hutchison medium. The enrichment culture was repeated thrice, without cessation. The culture did not end until the filter paper had decayed in the medium. Inoculated and streaked with the enrichment culture, the cellulose–Congo red agar plate was incubated at 30°C for growth of microorganisms. The colonies on the plate with surrounding clear zones were picked for isolation. Single well-isolated colonies with cellulase activity were obtained.

2.3 Identification of microorganisms

**Analysis of morphology** A very small sample of a colony was removed from the agar plate, mixed with a drop of water, and smeared on a glass slide [8]. Then observations were made with a microscope.

**Analysis of 18S rRNA gene sequence** Four milliliters of mycelium culture withdrawn after a 12-h incubation was centrifuged, and the liquid was discarded [9]. The deposit was subjected to 400 μl of 1% cetyl trimethyl ammonium bromide and centrifuged again. Then 400 μl of the solution (pH 7.5) containing 4 mol/l guanidine hydrochloride and 0.1 mol/l Tris–HCl was added to the second deposit for cell splitting. After incubation at room temperature for 30 min, the sample was extracted with 1:1 phenol–chloroform and chloroform successively. After centrifugation, 2× vol of anhydrous alcohol and 1/10 vol of sodium acetate (3 mol/l, pH 5.2) were added to the supernatant to precipitate DNA. The sediment obtained was dissolved in TE buffer, and the 18S rRNA gene was amplified by PCR using common primers (5′ AGGGCAAGTCTGGTG 3′ and 5′ CCGATCCCTAG TCGGCATAG 3′). PCR products were purified and then sequenced by Takara Company.

2.4 Preparation of crude cellulase solution

A total of 3.6 g of wet koji (equal to 1 g of dry koji) was mixed with 20 ml of distilled water and incubated at 30°C for 1 h. The mixture was filtered through two layers of gauze, and the filtrate was centrifuged at 4,000 rpm at 4°C for 10 min. The supernatant was harvested as crude cellulase solution.

2.5 Enzyme assays

**Cottonase** A total of 0.5 ml of diluted crude enzyme solution was transferred into a screw-capped tube containing 50 mg of absorbent cotton and 2 ml of acetate buffer (pH 4.8) [10–12]. The tube was covered with a stopper after adding a drop of toluene and then incubated at 50°C in a water bath for 24 h. One unit of enzyme activity was expressed as the amount of enzyme required to produce 1 mg of reducing sugars for every 24 h.

**Carboxymethylcellulase** The measurement of carboxymethylcellulase (CMCase) activity was similar to that of cottonase activity. Two milliliters of CMC solution was used as substrate, and the incubation time was 30 min. One unit of enzyme activity was defined as the amount of enzyme required to produce 1 mg of reducing sugars for every 30 min.

**Filter paperase** To determine filter paperase (FPA) activity, a piece of filter paper (Xinhua no. 1, 1 cm×6 cm) was reacted with the crude enzyme solution substituting for absorbent cotton. The reaction was performed in the same water bath for 1 h. One unit of enzyme activity was defined as the amount of enzyme required to produce 1 mg of reducing sugars per hour.

Meanwhile, the crude enzyme solution pretreated in a water bath of 100°C for 5 min was used as control sample. The amount of reducing sugars in the control sample should be subtracted while calculating for enzyme activity.

2.6 Analysis of reducing sugars and total cellulose

The amounts of reducing sugars and total cellulose were measured according to the methods described by Zhang et al. [9] and Yan et al. [13], respectively.

### 3 Results and discussion

3.1 Screening of microorganisms with high cellulase activity

Forty-one strains that can decompose cellulose were isolated. Four (marked as XC2, XC9, XC15, and XC23) of them with higher cellulase activities were selected for further study. *Trichoderma* sp. and *Trichodermakoningii* (Microbial Culture Collection Center of the Chinese Academy of Science) were used as contrasts. Six strains were inoculated to cellulose–Congo red agar plates. Then the clear zone diameter/colony diameter (H/C) ratio was calculated. Activities of cellulase were examined when the strains were grown on the basal solid medium. Experiments were performed in triplicate, and average values were represented (Table 1).

As shown in Table 1, the CMCase, cottonase, and FPA activities of strain XC9 isolated from mildewy maize cob were all higher than those of the other five strains, suggesting that strain XC9 was a valuable strain with higher cellulase activity.

3.2 Strain identification

The colony of strain XC9 initially diffused into the shape of a white floccule on the PDA agar plate, then turned into