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Screening Psychrophilic Fungi of Cellulose Degradation and Characteristic of Enzyme Production

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Abstract: A fungus (WR-C1) decomposed cellulose was isolated from a hypothermal litter layer using Congo red medium as the preliminary screening culture medium and then using a filter as the secondary screening medium at low temperature. The experiment showed that the weight loss rate of filter paper on the 15th days could reach 30.69%. A morphologic and ITS gene sequence analysis suggested that CF-C1 was *Cladosporium*. We mainly studied the effects of culture time, inoculation amount, initial pH and different sources of carbon, nitrogen and inorganic salt on the cellulase production of strain WR-C1. Under optimum cultural condition, the highest value of WR-C1 enzyme production and filter paper enzyme were 3.27 U·mL^{-1} and 0.51 U·mL^{-1}.

Key words: low temperature, cellulose-degrading fungi, screening, enzyme activity

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Introduction

Straw is the largest yield of biomass energy and provides a variety of nutrient elements to enrich soil for corn growth. Cellulose is the most important element in the physical composition of straw and also one of the most abundant organic compounds on the earth (Catherine and Vanitha, 2012; Irfan *et al*., 2012). Photosynthesis can produce more than 10 billion tons of dry plant materials per year, more than half of which are cellulose and hemicellulose (Leschine, 1995).

Cellulase whose optimum reaction temperature is 45-65℃ is important to the conversion of cellulose, and its chemical properties are unstable at moderate low temperature. Yet ideal reaction temperature for low temperature cellulose is as low as 20℃ (Akila and Chandra, 2003), where it can react efficiently, and inactivating enzymes through heat treatments during production process at lower temperature can save energy and cost, making it a focus of relevant researches and development (Dong *et al*., 2011). Shang *et al*. (2012) isolated *Pseudomonas* sp B6-15 bacterium strain from humus soil samples, which had the highest enzyme activity at 24.94 U·L^{-1}, and kept 54.43% of enzyme activity at 10℃. Fu *et al*. (2010) isolated *Bacillus* sp BMZ-14 strain from mud-flat sediment, which achieved 65% of maximum enzyme activity at 5℃. Ren *et al*. (2013) isolated the strain cellulose degradation *Bacillus subtili*. This study showed that M7 could produce CMCase to be maximized at 20℃.

This study isolated a strain from litter layer at low

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temperature owning a high enzyme activity, whose morphological features, growth characteristics and enzyme production were also studied, which built up a good basis for exploring straw degradation at low temperature in cold region.

Materials and Methods

Materials

Strains

This strain type was isolated at low temperature environment from litter layer of a children's garden in Harbin, Heilongjiang Province. This region has monsoon-influenced continental climate, with an average annual temperature of 5℃, and an average minimum temperature of –13.5℃. Average soil temperature is 0℃.

Medium

Red cellulose medium: K_2HPO_4 0.5 g, microcystalline cellulose 1.88 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, gelation 2 g, Congo red 0.2 g, agar 18 g, and distilled water 1 000 mL (Tan *et al*., 2006).

Fungi screening medium: glucose 10 g, peptone 5 g, K_2HPO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, rose Bengal 33.4 mg, penicillin 1 000 mL, streptomycin 100 ug·L^{-1}, and distilled water 1 000 mL (Ye, 1997).

Hector and Johnson inorganic salt medium: K_2HPO_4 1 g, NaCl 0.1 g, $MgSO_4 \cdot 7H_2O$ 0.3 g, $NaNO_3$ 2.5 g, $FeCl_3$ 0.01 g, $CaCl_2$ 0.1 g, and distilled water 1 000 mL.

Enzyme production fermentation medium: CMC-Na 5 g, peptone 5 g, yeast powder 1 g, K_2HPO_4 0.5 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, and distilled water 1 000 mL (Hu, 2008).

PDB medium: PDA 26 g and distilled water 1 000 mL.

PDA medium: PDA 26 g, agar 18 g and distilled water 1 000 mL.

Experimental methods

Preliminary screening

An accurate measure of 5 g deadwood (rotten leaves) was collected at low temperature, and placed in 95 mL sterile water flasks. Ten mL of solution was inoculated into the fungal isolation medium, cultivated and enriched for 10 days in fungal isolation medium under 10℃. The inoculum was diluted to 10^{-3}, 10^{-4} and 10^{-5} times, and then coated in a 0.1 mL dilution in red cellulose medium, respectively. First, large bacterial colonies with fast growth and a rich red transparent circle were lined and purified continuously, then a single colony on PDA slant was picked and saved at 10℃.

Secondary screening

The initial screening strains were inoculated in 50 mL potato glucose liquid medium culturing for 10 days at 10℃, coated with diluents bacterial fluid in potato glucose agar medium culturing for 7 days, and then inoculated in 50 mL Hektoen and Johnson inorganic salt media. Then added an appropriate filter paper and cultured for 15 days at 10℃, after which the filter paper was dried and weighed loss.

Morphology identification of strain

The screened strains were inoculated into PDA medium and the morphology of fungi was identified using the fungal identification manual.

Scanning electron microscopy of strain

The samples were fixed, washed, dehydrated, and dried for scanning by electron microscope to observe the bacterium morphology.

DNA extraction and amplification in conserved sequence of ITS

The sequence was: first, genomic DNA was extracted according to the reference documents (Li, 2002); second, ITS regions of rDNA sequences were determined, and universal primers ITS-ITS4 (ITS1: TCCGTAGGTGAACCTGCGG, ITS4: TCCTCCGC TTATTGATATGC) were used in PCR amplification of these sequences, then synthesized by Shenzhen Huada Gene Technology Co., Ltd. At the same time, amplification conditions consisted of an initial denaturation step of 94℃ for 3 min, followed by 30 cycles of 94℃ for 30 s, 55℃ for 30 s and 72℃ for 1 min and a final elongation step at 72℃ for 5 min.

Enzyme producing conditions of strain

Culture time: four pieces of bread of diameter 0.7 cm