Optimizing xylanase production by a newly isolated strain CAU44 of the thermophile Thermomyces lanuginosus

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Summary

Thermomyces lanuginosus CAU44, a newly isolated thermophilic fungus strain, was used for the production of extracellular xylanase on various lignocellulosic materials under shake flask conditions. High-level production of xylanase by the strain was enhanced by optimizing the type of carbon sources, substrate concentration, particle size and surfactants in the culture medium. The titre of xylanase activity obtained of up to 4156 U ml\(^{-1}\) was the highest ever reported.

Introduction

Xylanases (EC 3.2.1.8) have many different industrial applications, including biodegradation of lignocelluloses in animal feed, foods, and textiles, as well as biopulping in the paper and pulp industry (Wong et al. 1988). Although xylanases are produced by many microorganisms such as bacteria, fungi and actinomycetes, fungi have received great attention due to their ability to secrete high level of xylanases into the growth medium. The majority of commercial xylanases are extracellular enzymes produced by mesophilic fungi (Haltrich et al. 1996). Since the use of xylanases is related to industrial processes operating at high temperatures, application of thermostable enzymes produced by thermophilic fungi appears to be advantageous (Maheshwari et al. 2000). Among thermophilic fungi, Thermomyces lanuginosus is one of the best producers of thermostable xylanases from the industrial point of view, mainly due to the fact that it excretes a high level of xylanase into the medium (Singh et al. 2003). Some work has already been carried out to study the efficient production of xylanases from this fungus (Gomes et al. 1993a, b; Alam et al. 1994; Hoq & Deckwer 1995; Puchart et al. 1999; Singh et al. 2000), and different surfactants (TWEEN-80) or fatty acids are frequently added to the medium to enhance the yields of xylanases (Gomes et al. 1993b; Haltrich et al. 1996; Kuhad et al. 1998; Ding et al. 2004).

T. lanuginosus is reported to be among the best producers of thermostable xylanase in nature. However, differences in xylanase production amongst strains from diverse geographical origin do exist (Hoq & Deckwer 1995; Puchart et al. 1999; Singh et al. 2003). Hence, the main purpose of the present study was to enhance and accelerate the high-level xylanase production by strain CAU44 of T. lanuginosus newly isolated in China. In the present investigation, a successful attempt was made to enhance the xylanase yield from T. lanuginosus CAU44 by optimizing the culture conditions. We have achieved a two-fold increase in xylanase production, which is the highest titre of xylanase activity ever reported.

Materials and methods

Materials

Birchwood xylan, beechwood xylan, oat-spelt xylan and carboxymethylcellulose (low viscosity) were purchased from Sigma Chemical Company, St. Louis, MO, USA. The lignocellulosic materials, namely corncobs, corn straw, wheat straw, wheat bran, sugar cane bagasse, rice straw and rice husk, were obtained locally. Corncob xylan was prepared as described previously (Ding et al. 2004). Tryptone and yeast extract were products from Oxoid (Basingstoke, Hampshire, England). All other chemicals used were analytical grade reagents unless otherwise stated.

Fungal strains and growth conditions

Thermomyces lanuginosus CAU44 was isolated from the soil samples obtained from earth under decaying tree
fibre layers in Sinkiang Province, China and was identified by the Institute of Microbiology of the Chinese Academy of Sciences (IMCAS). Stock cultures were maintained on potato dextrose-agar (PDA) slopes stored at 4 °C.

For xylanase production, the basal medium of flask culture contained (g l⁻¹): corn cob particles, 30; yeast extract, 10; tryptone, 10; MgSO₄·7H₂O, 0.3; FeSO₄·6H₂O, 0.3; CaCl₂·2H₂O, 0.3. The initial pH of the medium was adjusted to 6.0 and was not further controlled. The medium was then sterilized at 121 °C for 15 min. An agar block (1.0 cm³) of an actively growing 5-day-old culture of the strain was used to inoculate the growth medium (100 ml) in 300 ml Erlenmeyer flasks. Triplicate cultures were shaken at 200 rev min⁻¹ at 50 °C. After 4 days of cultivation, each culture broth was centrifuged (10,000 × g) for 15 min, and the supernatants were examined for xylanase activity.

**Enzyme assay and protein determination**

Xylanase activity was assayed according to the method of Bailey *et al.* (1992). Reaction mixture containing 0.9 ml of 1.0% (w/v) birchwood xylan and 0.1 ml of a suitably diluted enzyme solution was incubated in 0.05 mol l⁻¹, pH 6.0 citrate-phosphate buffer for 10 min at 50 °C. The reaction was stopped by adding 1 ml DNS (dinitrosalicylic acid). The amount of reducing sugar liberated was determined by the DNS method using xylose (Sigma) as the standard. All assays were conducted in triplicate from three independent samples.

**SDS-polyacrylamide gel electrophoresis (SDS-PAGE)**

SDS-PAGE was performed using 12.5% (w/v) acrylamide in gels as described by Laemmli (1970). Protein bands were visualized by Coomassie brilliant blue R-250 staining. The molecular weight standard used was the low molecular weight calibration kit (Amersham): phosphorylase b (97.0 kDa), albumin (66.0 kDa), ovalbumin (45.0 kDa), carbonic anhydrase (30.0 kDa), trypsin inhibitor (20.1 kDa), z-lactalbumin (14.4 kDa).

**Selection of substrates for xylanase production**

To investigate the effect of various substrates on the production of xylanases, *T. lanuginosus* CAU44 was cultivated for 4 days on different media with 1.5% of each xylan or 3.0% of each lignocellulosic material as the sole substrate. The initial pH of the medium was adjusted to pH 6.0. Various lignocellulosic materials were chopped in a laboratory hammer mill to a particle size smaller than 0.45 mm (40 mesh), and were used at the same particle size in the experiment.

**Effect of substrate (corn cob) concentration and particle size on xylanase production**

Corncobs were chopped by a chopper into small pieces, dried and ground in a hammer mill. This ground material was then separated by sieves into particles of different sizes and the fraction that passed through the 0.45 mm sieve was used in the medium. Different concentrations of the corncob particles ranging from 0.5 to 6.0% (w/v) were used as corncob substitutents in the basal enzyme production medium. Corncob particles were divided into six fragments by sieving. The 0.45–0.9 mm fragment consisted of particles that passed through the 0.9 mm sieve, but not through the 0.45 mm sieve. The same procedure was used to sort out the <0.125, 0.125–0.3, 0.3–0.45, 0.45–0.9, 0.9–2, and >2 mm fragments.

**Effect of surfactants and fatty acids**

The effect of different surfactants (Tween-80 and Triton X-100) and fatty acid (olive oil) on xylanase production by *T. lanuginosus* CAU44 was investigated. Different Tween-80 contents ranging from 0 to 3.0% (v/v) were added to media and the enzyme production was monitored.

**Results**

**Xylanase production with different xylans and lignocellulosic materials**

The newly isolated *T. lanuginosus* CAU44 was grown on several xylans or lignocellulosic materials to determine the effects of these substrates on the production of xylanases (Table 1). Among the xylans used, corn cob was the best carbon source for the xylanase production, and its activity reached the highest value of 3260 U ml⁻¹. Other xylans also resulted in high levels of xylanase activities, which were more than 1500 U ml⁻¹. The use of purified xylan as a substrate for xylanase production is costly and, therefore, the current impetus is focused on the utilization of the more cost-effective lignocellulosic materials. Among the lignocellulosic materials tested as carbon sources, the highest activity (2156 U ml⁻¹) of xylanase was produced on corn cobs, whereas much lower levels (25–153 U ml⁻¹) of xylanase activities were produced with corn straw, wheat straw, wheat bran, sugar cane bagasse, rice straw and rice husk (Table 1). Since corn cob was by far the most effective for xylanase production, it was thus selected as the substrate for enzyme production in the following experiments.