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Isolation and Identification of a Newly Isolated *Alternaria* sp. ND-16 and Characterization of Xylanase

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Abstract *Alternaria* sp. ND-16, a bacterium isolated from soil sample, was identified as a strain of *Alternaria mali* based on the morphology and comparison of internal transcribed spacer *rDNA* gene sequence studies. Furthermore, it is demonstrated that this strain has xylanase activity, and the activity can be optimized under suitable growing conditions where wheat bran and urea are the primary sources of carbon and nitrogen. Partially purified xylanase from *Alternaria* sp. ND-16 is shown to have an optimal pH of 6.0 and optimal temperature of 50 °C, making this enzyme potentially suitable for industrial

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applications. It is also demonstrated that Na^{+} and Mn^{2+} show strong inhibition of the xylanase while K^{+}, Li^{+}, Fe^{2+}, Cu^{2+}, and Zn^{2+} have no significant effect on the activity.

Keywords *Alternaria* sp. ND-16 · Isolation · Identification · Production · Xylanase

Introduction

Xylanases (EC 3.2.1.8) are glycosidases which catalyze the endohydrolysis of 1,4-β-D-xylosidic linkages in xylan. Xylanases derived from fungi and bacteria have garnered much attention in recent years from the biotechnology industry for various potential processes [1]. These enzymes have been produced on an industrial scale used as bleaching agents in the paper and pulp bleaching industry [2]. Xylanases, along with glucanase, pectinase, cellulase, proteases, amylases, phytase, and lipase, have been used in the degradation of arabinoxylans in feed ingredients to reduce the viscosity of the raw material and improve nutrition [3]. In food industry, they are used as food additives in wheat flour for improving dough handling and quality of baked products [4, 5]. The addition of exogenous xylanase to the mashing process has been effective in degrading arabinoxylans, improving the process for the brewing industry [6–8].

Xylanase production on an industrial scale is based on a microbial biosynthesis. Filamentous fungi, such as *Aspergillus* [9], *Penicillium* [10, 11], and *Trichoderma* [12], have been most extensively studied and have demonstrated a great capability for secreting a wide range of xylanase. There are two possibilities for cultivation of microbial xylanase producing strain, either submerged cultivation or solid-state. Currently, 80–90% of commercial xylanase are produced in submerged culture because it has a higher degree of processes intensification and a better level of automation [1].

In this paper, we isolated a new strain of *Alternaria* with xylanase producing ability. The strain was identified based on its morphology and internal transcribed spacer (ITS) *rDNA* gene sequence analysis. The optimum temperature and pH, as well as thermal stability of the partially purified xylanase was identified and determined to fit the standards for applications in the biotechnology industry.

Methods

Microorganism

Alternaria sp. ND-16 was isolated from soil and stored at 4 °C on potato dextrose agar (PDA) in our laboratory. Spore suspensions were made from seven-day cultures which had been grown on PDA slopes at 30 °C.

Isolation of Strains Producing Xylanase

Various strains including *Alternaria* sp. ND-16 were isolated based on their morphology from soil samples of apple garden (Hangzhou, Zhejiang, China) and the detailed procedure of isolation was performed according to the methods described by Abrusci et al. [13].

Culture Conditions

For submerged fermentation, the initial medium used for xylanase production was composed of: NH_4Cl, 9 g L^{-1}; KH_2PO_4, 1 g L^{-1}; $NaNO_3$, 1 g L^{-1}; $MgSO_4 \cdot 7\ H_2O$, 1 g L^{-1}; $CaCl_2 \cdot 2\ H_2O$, 0.3 g L^{-1}; yeast extract, 1 g L^{-1} [14]. The initial pH was adjusted to 8.0. Erlenmeyer flasks (250 mL) containing 75 mL of medium were inoculated with 1 mL of 1×10^6 spores mL^{-1} suspension prepared from 1-week-old PDA slants of *Alternaria* sp. ND-16, incubating at 30 °C on a rotary shaker (150 rpm). Samples were withdrawn at regular intervals and the mycelium was separated from the enzyme-containing broth by centrifugation at 10,000×*g* for 15 min at 4 °C to obtain the crude enzyme preparation.

DNA Extraction from Mycelia

Genomic DNA was extracted from fresh cultures using a modified protocol of Doyle and Doyle [15] and Lee and Taylor [16]. Fresh fungal mycelia (50 mg) was scraped from the surface of the agar plate and transferred into a 1.5-mL microcentrifuge tube with 700 μL of preheated (60 °C) 2× cetyltrimethylammonium bromide (CTAB) extraction buffer [2% (*w*/*v*) CTAB, 100 mM Tris-HCl, 1.4 M NaCl, 20 mM ethylene diamine tetraacetic acid (EDTA), pH 8.0], and 0.2 g sterilized quartz sand. Fungal mycelia was ground using a glass pestle for 5–10 min and then incubated in a 60 °C water bath for 30 min with occasional gentle swirling. 500 μL of phenol/chloroform (1:1) was added into each tube and mixed thoroughly to form an emulsion. The mixture was centrifuged at 12,000×*g* for 15 min in a microcentrifuge at room temperature; the aqueous phase was removed and transferred to a fresh 1.5-mL tube. The aqueous phase containing DNA was reextracted with phenol/isoamyl alcohol (24:1) until no interface was visible. 50 μL of 5 M potassium acetate was added into the aqueous phase followed by 400 μL of isopropanol and then inverted gently to mix. The genomic DNA was centrifuged at 9,000×*g* for 2 min. The DNA pellet was washed with 70% ethanol twice and dried at room temperature. The DNA pellet was then resuspended in 100 μL TE buffer (10 mM Tris-HCl and 1 mM EDTA).

PCR Amplification of the 5.8S Gene and Flanking ITS

For the sequence analysis, the ITS1–5.8S-ITS2 rDNA region of the fungi was amplified by polymerase chain reaction (PCR) using primer set pITS1 (5′-TCCGTAGGT GAACCTGCCG-3′) and pITS4 (5′-TCCTCCGCTT-ATTGATATGC-3′). The PCR mixture (50 μL) contained 37.5 μL of dd H_2O, 5 μL of PCR buffer, 4 μL of dNTP, 1 μL of each primer (10 pmol μL^{-1}), 1 μL of template DNA, and 0.5 μL Taq DNA polymerase (Takara, Kyoto, Japan). PCR amplification was performed in an automated thermal cycles (PTC-100, MJ researcher, USA) at the following conditions: predenaturation at 95 °C for 5 min; 30 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, and elongation at 72 °C for 1 min; before the extension at 72 °C for 10 min, final cooling to 4 °C. For each PCR reaction, 5 μL of PCR products were examined by electrophoresis at 70 V for 2 h in 0.8% (*w*/*v*) agarose gel in 1× tris-acetate–EDTA buffer (0.4 M Tris, 50 mM sodium acetate, 10 mM EDTA, pH 7.8) and visualized under ultraviolet light after staining with ethidium bromide (0.5 μg mL^{-1}).

DNA Sequencing

The PCR products were extracted and purified from the agarose gel using High Pure PCR Product Purification Kit (Roche, Germany). The resulting PCR fragment was ligated with pMD18-T (Takara, Japan) by using the T/A cloning procedure [17, 18]. The constructed vector was transferred to the competent cell *Escherichia coli* JM109, and then strains were spread on the LB plate containing the 5-Bromo-4-chloro-3-indolyl-β-D-galactoside (40 μg mL^{-1}), isopropyl-1-thio-β-D-galactoside (24 μg mL^{-1}), and ampicillin (50 μg mL^{-1}). Subsequently, a positive clone, designated *E. coli* JM109/pMD18-T-ITS, was obtained. DNA was sequenced on both strands with an Applied Biosystems Model 377 Bautomatic DNA sequencer, and a dye-labeled terminator sequencing kit (Applied Biosystems, Foster, CA, USA).

Sequence Data Analysis

The sequences obtained were compiled and compared with sequences in the GenBank databases using Basic Local Alignment Search Tool program. Sequence analysis was performed using SEQBOOT, NEIGHBOR-JOIN and DNASENSE of Phylips (version 3.572) softwares and FITCH, DRAWGRAM, and the alignment match was then used to construct the neighbor-joining phylogenetic tree. Evaluation of the MicroSeq database system and comparison to other frequently used systems are from DDBJ/EMBL/GenBank.

Xylanase Activity Assays

Xylanase activity was determined by Dinitrosalicylic Acid method, which measures the release of reducing sugars from oat spelt xylan (1%, *w*/*v*; lot. X0627, Sigma–Aldrich, St. Louis, MO, USA) at 615 nm [19]. Reaction mixture containing 1 mL of a solution of 1% oat spelt xylan in citrate buffer 50 mM, pH 5.0 plus 1 mL of the diluted crude enzyme, was incubated at 50 °C for 30 min. One unit of xylanase was defined as the amount of enzyme required to release 1 μmoL of xylose from xylan in 1 min under the assay condition.

Effect of Different Carbon and Nitrogen Sources on Xylanase Production

To investigate the effect of carbon sources on xylanase production, different carbon sources including oat spelt xylan, wheat bran, corn cob, glucose, starch, fructose, xylose, sorbitol, and maltose were supplemented into medium at a variety of concentration. Various organic nitrogen and inorganic nitrogen sources, including $(NH_4)_2SO_4$, NH_4Cl, $NaNO_3$, yeast extract, tryptone, urea, and casein hydrolysate, were added to the medium at fixed level (0.1%, *w*/*v*) to test the effect of nitrogen sources on xylanase production from *Alternaria* sp. ND-16.

Partial Purification of Xylanases by Ammonium Sulphate Fractionation

One liter of culture medium was centrifuged at 8,000×*g* for 20 min at 4 °C, the supernatant were reserved for ammonium sulfate precipitation. Solid ammonium sulfate was added to achieve 40%, 10 °C, with constant stirring. The sample was then centrifuged at 8,000×*g* at 4 °C for 20 min. The precipitate was discarded, and the supernatant was subsequently adjusted to 70% saturation of ammonium sulfate. The sample was again centrifuged at

8,000×*g* at 4 °C for 20 min. The supernatant was discarded, and the pellet was dissolved in a small volume of citrate buffer (50 mM, pH 5.0). The enzyme solution was then dialyzed for about 18–24 h at 10 °C against 50 mM citrate buffer (pH 5.0) fortified with 100 mg L^{-1} sodium azide, with three intermittent changes of the buffer. Xylanase activity and protein estimation were carried out as well.

Effect of Temperature and pH on Partially Purified Xylanase Activity and Thermostability

To evaluate the effect of temperature on the enzyme activity, the enzymes were incubated at various temperatures ranging from 30 to 80 °C in a preheated water bath. The thermal stability was determined at the temperature 50, 55, 60, and 70 °C after incubation of suitable diluted enzyme samples in absence of substrate for 0, 30, 60, 90, and 120 min. The optimum pH was determined using buffers ranging from 3.0 to 9.0. Acetate buffer, phosphate buffer, and Tris buffer were used for pH 3.0–5.0, 6.0–7.0, and 8.0–9.0, respectively.

Effect of Metal Ions on the Activity of Enzyme

The effects of various metal ions on the activity of the partially purified xylanase were assessed by the induction of the appropriate metal ion salt in the standard assay (1 mM final concentration). The xylanase activity was measured in the presence of various metal ions K^+, Na^+, Li^+, Fe^{2+}, Ca^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Zn^{2+}, and Hg^{2+}.

Statistical Analyses

Each experiment was carried out in duplicate and the mean values were given. One-way analysis of variance and *t* test were used to interpret the difference in means at the 95% confidence level in PC SAS (Cary, NC, USA).

Sequence Submission

The nucleotide sequence reported in this paper has been deposited in the GenBank database under accession number DQ912836.

Results

Identification of Strain *Alternaria* sp. ND-16

The identification of *Alternaria* sp. ND-16 was based on standard morphological and nucleotide sequence analysis of enzymatically amplified ITS rDNA. The colony morphology of *Alternaria* sp. ND-16 after growth at 30 °C for 7 days on PDA is shown in Fig. 1. From the *ITS rDNA* gene sequence analysis (GenBank accession no. DQ912836), this strain was found to be similar to *Alternaria mali* (AY154683; homology, 99.8%/571 bps, based on ITS rDNA). Through the alignment and cladistic analysis of homologous nucleotide sequences of known bacteria, phylogenetic relationships could be inferred and the approximate phylogenetic position of the strain is shown in Fig. 2. According to the

Fig. 1 Colony morphology of *Alternaria* sp. ND-16 after growth at 30 °C for 7 days on PDA

morphology and comparison of *ITS rDNA* gene sequence, *Alternaria* sp. ND-16 was identified as a strain of *A. mali* and named *A. mali* ND-16.

Time Course of Xylanase Production

Time course analysis of xylanase accumulation by *A. mali* ND-16 in submerged fermentation is presented in Fig. 3. The highest xylanase activities were 19.5 and 20.8 U mL^{-1}

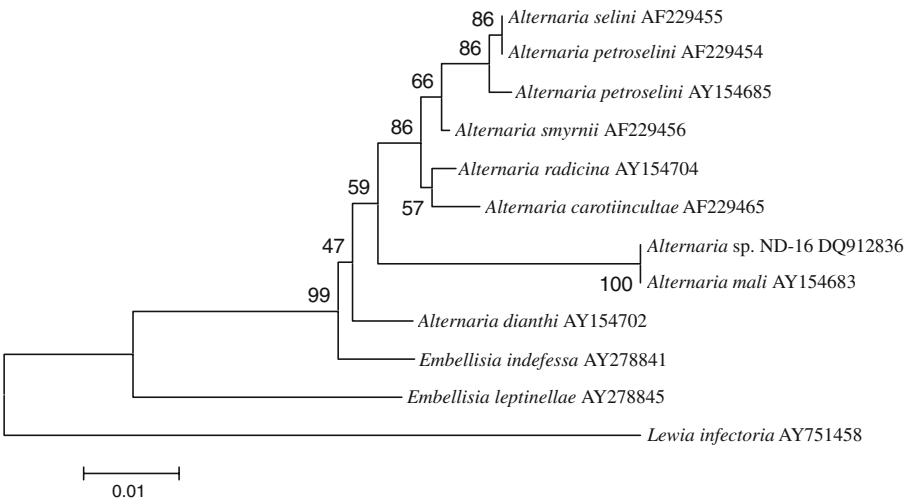

Fig. 2 The phylogenetic dendrogram for *Alternaria* sp. ND-16 and related strains based on the ITS rDNA sequence. *Numbers in parentheses* are accession numbers of published sequences. Bootstrap values were based on 1,000 replicates. *Lewia infectoria* was used as the outgroup

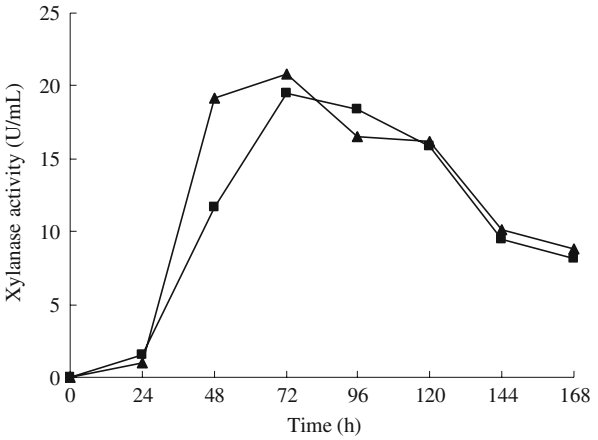

Fig. 3 Time course of xylanase production by *Alternaria mali* ND-16 on oat spelt xylan (*squares*) and wheat bran (*triangles*) used as substrates under submerged fermentation at 30 °C

for oat spelt xylan and wheat bran used as substrates after 72 h cultivation, respectively. There is a slight decrease in xylanase production was found for both oat spelt xylan medium and wheat bran medium when prolonging the cultivation time. This phenomenon can be explained by that the autolysis of biomass resulted in a release of intracellular material, including protease, into the fermentation broth.

Effect of Carbon Sources on Xylanase Production

To select a suitable carbon source for the xylanase production, *Alternaria mali* ND-16 was cultivated in the basal medium (NH_4Cl, 9 g L^{-1}; KH_2PO_4, 1 g L^{-1}; $NaNO_3$, 1 g L^{-1}; $MgSO_4 \cdot 7\ H_2O$, 1 g L^{-1}; $CaCl_2 \cdot 2\ H_2O$, 0.3 g L^{-1}; yeast extract, 1 g L^{-1}) containing various carbon sources (Table 1). The results indicated that the xylanase production was highest (19.1 U mL^{-1}) when 1% wheat bran was added as the carbon source. However, xylanase production only reached 5.7 and 11.7 U mL^{-1} when using pure xylan (oat spelt xylan) as the carbon source at 0.5% and 1% level, respectively. The xylanase activity was negligible when starch, glucose, fructose, sorbitol, or maltose was used as carbon source. Interestingly,

Table 1 Effect of carbon source on xylanase production by *Alternaria mali* ND-16 at 30 °C under submerged fermentation for 48 h.

Carbon source	Xylanase activity (U mL^{-1})
0.5% Oat spelt xylan	5.7
1% Oat spelt xylan	11.7
0.5% Wheat bran	8.4
1% Wheat bran	19.1
0.5% Corn cob	0.2
1% Corn cob	0.4
5% Corn cob	0.4
1% Glucose	2.7
1% Starch	1.4
1% Fructose	1.8
1% Xylose	15.8
1% Sorbitol	1.5
1% Maltose	2.3

Table 2 Effect of nitrogen source on xylanase production by *Alternaria mali* ND-16 at 30 °C under submerged fermentation for 48 h.

Nitrogen source	Xylanase activity (U mL^{-1})
0.1% $(NH_4)_2SO_4$	9.7
0.1% NH_4Cl	10.5
0.1% $NaNO_3$	11.9
0.1% Yeast extract	6.2
0.1% Tryptone	6.5
0.1% Urea	14.2
0.1% Casein hydrolysate	5.3

relative high xylanase production (15.8 U mL^{-1}) was observed when xylose was selected as carbon source at 1% level.

Effect of Nitrogen Source on Xylanase Production

Nitrogen source has a dramatic effect on the production of xylanase by *Alternaria mali* ND-16. The composition of basal medium was as follows: KH_2PO_4, 1 g L^{-1}; $MgSO_4 \cdot 7\ H_2O$, 1 g L^{-1}; $CaCl_2 \cdot 2\ H_2O$, 0.3 g L^{-1}. The effect of inorganic and organic nitrogen sources on xylanase production is shown in Table 2. The xylanase production follows a urea >$NaNO_3$ > NH_4Cl> $(NH_4)_2SO_4$ > tryptone > yeast extract > casein hydrolysate sequence. Maximum xylanase activity (14.2 U mL^{-1}) was evident when 0.1% urea was added.

Effect of pH on Partially Purified Xylanase Activity

The favorable pH range for xylanase activity of *A. mali* ND-16 was 5.8–6.2, with optimal pH of 6.0 (Fig. 4). A sharp decrease of xylanase activity was observed below at pH below 5.8 (98.1%) and above 6.2 (94.7%) and negligible xylanase activity was found at pH 3.0

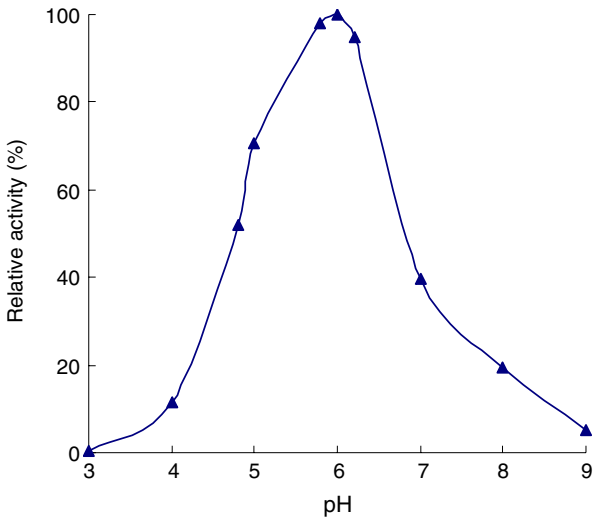

Fig. 4 Influence of pH on the activity of xylanase from *Alternaria mali* ND-16

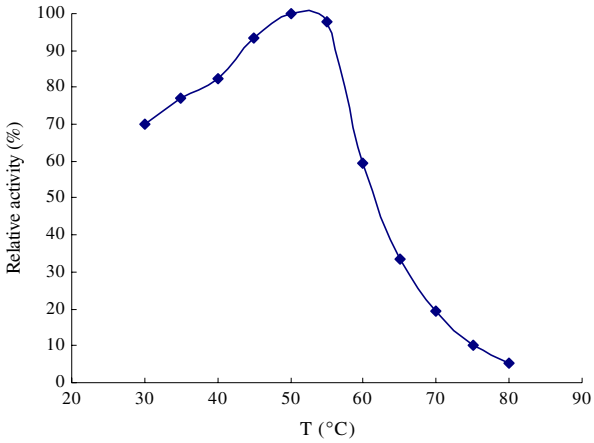

Fig. 5 Influence of temperature on the activity of xylanase from *Alternaria mali* ND-16

(0.2%). However, relative xylanase activity was gradually decreased when pH was above 7.0. It is shown that xylanase activity at pH 7, 8, and 9 were 39.8%, 19.6%, and 5.3%, respectively.

Effect of Temperature on Partially Purified Xylanase Activity and Stability

The effect of temperature on the xylanase activity from *A. mali* ND-16 is shown in Fig. 5. For 30 min reaction, the optimum temperature for the xylanase was 50 °C. A slight decrease in xylanase activity was observed between 45 °C (93.4%) and 55 °C (97.7%). Relative enzyme activity was rapidly reduced to 59.3% when the temperature was at 60 °C, and there was negligible activity (5.4%) above 80 °C.

Thermal stability tests were carried out by preincubating xylanase up to 120 min in the range of 50–70 °C (Fig. 6). There was no significant difference in xylanase activity during 120 min between incubation at 50 and 55 °C. There was more than 80% relative xylanase activity left after 60-min incubation at 50 and 55 °C, but relative xylanase activities still remained 74.0% and 62.8% for 50 and 55 °C after 120 min incubation, respectively. The enzyme was sensitive at 60 °C, retaining 58.1% activity after 30 min exposure and only 3.6% after 120 min. At 70 °C the residual xylanase activity was negligible (1.6%) after the first 30 min incubation. These results clearly indicated that the suitable temperature range for industrial application of xylanase from *Alternaria mali* ND-16 was 50–55 °C.

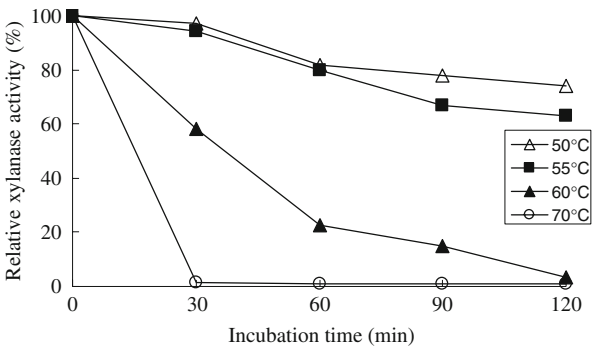

Fig. 6 Themostability of xylanase from *Alternaria mali* ND-16

Table 3 Effect of metal ions (1 mM) on xylanase activity from *Alternaria mali* ND-16.

Metal ion	Relative xylanase activity (%)
Control	100
K^+	94.8
Na^+	33.2
Li^+	105.8
Fe^{2+}	105.4
Ca^{2+}	112.2
Mn^{2+}	80.5
Co^{2+}	132.8
Cu^{2+}	103.2
Zn^{2+}	96.9
Mg^{2+}	118.9

Effect of Metal Ions on Partially Purified Xylanase Activity

The effect of metal ions on xylanase activity is shown in Table 3. K^+, Li^+, Fe^{2+}, Cu^{2+}, and Zn^{2+} showed no significant effect on xylanase activity. However, it is shown that Na^+ and Mn^{2+} strongly inhibited 33.2% and 80.5% of xylanase activity, respectively. This result agrees with the report in which the addition of Mn^{2+} resulted in the inhibition of xylanase from *Streptomyces sp.* [20]. A slight but still significant effect of stimulation of xylanase activity was observed with Ca^{2+}, Co^{2+}, and Mg^{2+}, indicating their possible role as a metal ion cofactor in the enzyme-substrate reaction and its ability to stabilize various enzymes [21].

Discussion

The aim of this study was to isolate and identify novel strains with xylanase activity. Generally, microorganisms are mainly identified by using morphological characteristics. However, these methods of identification are often problematic, as there can be different morpho/biotypes within a single species. They are also time-consuming, and require a great deal of skills. DNA sequence analysis methods are objective, reproducible, and rapid means of identification, and have been widely used [22]. For the molecular approach, our strategy was to identify these mycelia sterilia by rDNA sequence comparison (i.e. assessment of percentage nucleotide similarity with reference sequences), as well as phylogenetic analysis conducted in several stages of varying taxonomic resolutions. The analysis of the ribosomal region via PCR has been widely employed for characterizing different fungal species [23, 24]. In both vegetal and fungal organisms the structure of the ribosomal region comprises repeated clusters of coding regions [25]. The ITS regions separating genes *17 S* and *25 S* can be amplified by specific primers anchored in these two units. Because the ITS region is highly conserved intraspecifically, it is variable between different species. ITS region polymorphism is often used in taxonomy; but now, it is being well recognized for identifying fungi species [26]. In this study, we combined the morphological and physiological method with DNA sequence analysis method to identify the genetic position of the isolated strain producing xylanase.

To the best of our knowledge, this is the first report on xylanase production by the species *A. mali*. *A. mali* belongs to the *Alternaria alternata* (E.M. Fries) Keissler group and

is distinguished mainly by its pathogenicity towards apple (*Malus* sp.). The fungus causes apple leaf spots, which enlarge in zonate circular or crescent-shaped rings. The fungus also infects the fruit, causing a soft rot, particularly where the skin is already damaged through other causes. *A. mali* is spread by means of conidia and its dispersal is particularly favored by rainfall. It can infect up to 85% of leaves on susceptible cultivars, compared with less than 1% on resistant cultivars. In the USA, the disease is only important where Asian *A. mali* has been introduced (North Carolina) [27]. However, the mechanism of biological infection is not clear until now. The new isolated xylanase, as an extracellular enzyme detected by *A. mali* growth, would facilitate plant pathologists to better understand the role of xylanase in pathogenesis and system disease symptoms of apple leaf spots.

Carbon source is the most important element during the period of growth and metabolic process of microorganisms. This indicated that the choice of an appropriate carbon source is of great importance for the successful production of xylanases. Because the cost of the substrate plays a crucial role in the economics of xylanase production process, another approach to reduce the cost of xylanase production is the use of lignocellulosic materials as carbon sources rather than choosing the expensive pure xylans. Recently, researchers focused on using several inexpensive carbon sources, including oat husk, corn cobs [28], wheat bran [29], wheat straw [30], and rice straw [31] for xylanase production. In this study, result showed that wheat bran is a suitable carbon source for xylanase production from *A. mali* ND-16. Sermanni et al. [32] and Cai et al. [33] did not detect cellulolytic enzyme activities when the fungi were grown in submerged fermentation condition using corn cob as carbon source. Their results were identical to our data indicating that xylanase activity was negligible (0.4 U mL^{-1}) when using corn cob as carbon source. Xylose, which can be easily obtained from the xylan portion of lignocellulosic material, has been described as an effective inducer of xylanase activity in few organisms. It has been utilized not only as an inducing carbon source for the production of xylanase in *Aureobasidium pullulans* [34], *Fusarium oxysporum* [35], and *Trichosporon cutaneum* [36] but also as an inhibitor for xylanase production from *Aspergillus kawachii* [37]. Interestingly, xylose was found to be a suitable carbon source or inducer for xylanase production from *A. mali* ND-16 in this study.

Generally, the mechanisms which govern the formation of extracellular xylanase are influenced by the availability of precursors for protein synthesis [38]. High xylanase production could be obtained when peptones or yeast extract were used in the production medium. Relatively cheaper complex nitrogen supplements that have been successfully used in certain organisms include corn steep powder, soybean meal, or potato protein [39]. The most suitable nitrogen source for xylanase production from *A. mali* ND-16 was urea, which was similar to the recent report of Dobrev et al. [40] that urea favored the xylanase production by *Aspergillus niger* B03.

The optimal pH and temperature for xylanase production from *A. mali* ND-16 were pH 6.0 and 50 °C, respectively, which was consistent with most of fungal xylanases (optimal pH 5–6 and temperature at 45–55 °C) according to the report of Polizeli et al. [1]. Experience from industry has shown that it is more difficult and costly to adjust process temperature than pH. Therefore, the most important criteria for xylanase in biotechnology sense, is thermal stability. Our results showed that the enzyme had good thermal stability at 50 and 55 °C but still had more than 50% enzyme activity left after 30 min incubation at 60 °C. Xylanases that are thermostable and have optimum pH in the acidic range are considered to have good potential application in the brewing industry because the use of such enzyme is expected to reduce the need for costly pH and temperature readjustment before enzyme addition. According to our pervious study, optimal pH for degradation of

arabinoxylans by xylanase in mashing was pH 5.5 [6] and enzymatic degradation of these polymers occurred significantly during the first step mashing process (45 °C) [41]. Therefore, xylanase from *A. mali* ND-16 would be a promising enzyme for solving arabinoxylans problems in the brewing industry. In order to improve the stability and activity of *A. mali* xylanase, the gene encoding xylanase will be cloned, and the xylanase could be modified by rational design of proteins or by directed molecular evolution of proteins [42, 43]. Meanwhile, overexpression and modification of this kind of xylanase would facilitate to better understand the structure of enzyme, and could result in the production of large amounts of efficient xylanase biocatalyst for industrial purpose, which are undergoing in our further studies.

Conclusions

In this study, we isolated and characterized a strain of *Alternaria* capable of producing xylanase. According to the morphology and comparison of ITS rDNA gene sequence, *Alternaria* sp. ND-16 was identified as a strain of *A. mali* and named *A. mali* ND-16. The time course for xylanase accumulation by *A. mali* ND-16 in submerged fermentation showed that the highest xylanase activities reached 19.5 and 20.8 U mL^{-1} for oat spelt xylan and wheat bran used as substrates after 72 h cultivation, respectively. Wheat bran and urea were the most suitable carbon and nitrogen source for xylanase production from *A. mali* ND-16, respectively. The optimal pH and temperature for partial purified xylanase were pH 6.0 and 50 °C, respectively. The result of thermal stability tests clearly suggested that the suitable temperature range for industrial application of xylanase from *A. mali* ND-16 was 50–55 °C.

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