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Production and characterization of a thermostable endo-type β-xylanase produced by a newly-isolated *Streptomyces thermocarboxydus* subspecies MW8 strain from Jeju Island

Won-Jae Chi, Ju Hyeon Lim, Da Yeon Park, Jae Seon Park, Soon-Kwang Hong*

Department of Biological Science, Myongji University, Gyeonggido 449-728, Republic of Korea

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ABSTRACT

A xylanase-producing, Gram-positive, aerobic, and spore-forming bacterium was isolated from a soil sample collected from Jeju Island and was classified as a novel subspecies of *Streptomyces thermocarboxydus* on the basis of 16S rRNA gene sequence similarity, the results of DNA–DNA hybridization analysis, and phenotypic characteristics. The novel strain was named as *S. thermocarboxydus* subsp. MW8 (=KCTC29013 = DSM52054). This strain produced extracellular xylanase. Xylanase from the strain was purified to homogeneity and had an apparent molecular weight of 52 kDa. The NH_2-terminal sequence (Ala-Glu-Ile-Arg-Leu) was distinct from those of previously reported xylanases. The purified xylanase produced xylobiose as the end-product of birchwood xylan hydrolysis. The K_m and V_{max} values of the purified xylanase on birchwood xylan were 1.71 mg/ml and 357.14 U/mg, respectively. The optimum pH and temperature for the enzyme were found to be 7.0 and 50 °C, respectively, and the enzyme exhibited significant heat stability. In addition, the enzyme was active over broad pH ranges: 84% of the maximum activity at pH 5.0, 84–88% at pH 6.0, 88% at pH 8.0, and 75–81% (pH 9.0). These enzymatic properties may be very useful for use in bio-industrial applications.

1. Introduction

Members of the genus *Streptomyces* (family *Streptomycetaceae*) are exploited for the production of commercially significant and bioactive compounds such as antitumor agents, enzymes, enzyme inhibitors, and antibiotics [1]. The genus *Streptomyces*, first proposed by Waksman and Henrici [2], is composed of more than 540 published species [3] of Gram-positive, aerobic, and spore-forming bacteria with high DNA G + C content. They are widespread in nature, but are found mostly in the soil.

A subset of *Streptomyces* can hydrolyze xylan, as shown by several studies [4–9]. Xylan, composed of β-1,4-linked xylosyl residues, is the second most abundant polysaccharide in plants after cellulose [10]. In general, the xylose unit is substituted with acetyl, arabinosyl, and methyl glucuronosyl residues. Xylan is a major component of the cell walls of terrestrial plants as well as marine algae. Xylanase (EC 3.2.1.8) is an enzyme that hydrolyzes xylan into xylo-oligosaccharides and D-xylose of various lengths. Synergic effects of β-arabinofuranosidase (EC 3.2.1.55), β-D-glucuronidase (EC 3.2.1.1), and acetyl xylan esterase (EC 3.2.11.6) are required to release the side chains of xylan; however, 2 enzymes, namely β-1,4-endoxylanases (EC 3.2.1.8) and β-xylosidase (EC 3.2.1.37), are sufficient to degrade the xylan backbone [10].

Xylanase is used in industrial applications such as the pulp-prebleaching process to remove hemicelluloses, utilization of hemicellulosic biomass for production of biofuels, food and feed additives, bakery processing, and xylitol production. Xylo-oligosaccharides, the hydrolysis products of xylan, exhibit various biological activities including prebiotic, antioxidative, and antibacterial activities. Thus, xylo-oligosaccharides can also be used in the pharmaceutical and cosmetic industries [11]. Methods to promote more efficient degradation of xylan are required to take advantage of hemicellulose, which is plentiful in nature. Thus, screening for novel microorganisms producing xylanase is an important goal of xylanase research.

In this study, we describe the isolation of a novel xylanase-producing bacterial subspecies of *Streptomyces thermocarboxydus*, and the purification and characterization of xylanase from the strain.

* Corresponding author at: Department of Biological Science, Myongji University, Yongin, Gyeonggido 449-728, Republic of Korea. Tel.: +82 31 330 6198; fax: +82 31 335 8249.
E-mail addresses: skhong@mju.ac.kr, skhong1030@hanafos.com (S.-K. Hong).

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2. Materials and methods

2.1. Materials

Medium ingredients such as soytone were purchased from Difco (Becton, Dickinson and Company, USA), and other chemicals were purchased from Sigma–Aldrich Co. (St. Louis, USA), unless specified otherwise.

2.2. Isolation of strain MW8 and detection of xylanase activity

Soil sample was collected from Jeju Island. Ten milligrams of the soil sample was suspended in 1 ml of sterilized water with vortexing. The suspended sample was lightly centrifuged to sediment the soil particles. The supernatant was serially diluted from 10^{-1} to 10^{-5} in sterilized water. Two hundred microliters of diluted solution was spread on TNX agar plates consisting 1% (w/v) Bacto tryptone, 1% (w/v) NaCl, and 0.3% (w/v) birchwood xylan. The plates were incubated at 40 °C for 48 h to isolate thermophilic soil bacteria which could be useful for production of thermostable enzymes. The colonies were replicated from the source plate to 2 replica plates containing the same media as in the source plate. The replica plates were incubated at 40 °C for 48 h and then were stained with 2% (w/v) Congo red solution, followed by destaining with 1 M NaCl solution. Xylanase activity was detected from a clear zone around the colonies. Xylanase-positive colonies were transferred onto a fresh TNX agar plate. To obtain pure isolates, several serial dilutions and plating techniques were applied. The colonies were classified into 4 groups on the basis of morphological differences. Among the selected colonies, the bacterial strain with the largest clear zone as determined using Congo red staining was selected for further study. The isolate, designated as strain MW8, was selected and routinely cultured in TNX medium at 40 °C and maintained as 20% (w/v) glycerol stock culture at −80 °C. The shape of the isolated strain was observed by transmission electron microscopy (TEM) after negative staining with 1% (w/v) phosphotungstate of cell cultured at 40 °C for 5 days.

2.3. Phenotypic and biochemical characterization

Gram staining was performed on cells cultured at 40 °C for 48 h by using a Gram staining kit (BD Biosciences, USA) according to the manufacturer's instruction. Growth at different temperatures (25 °C, 37 °C, 40 °C, 45 °C, 50 °C, and 55 °C) and at different NaCl concentrations (0–10%, w/v, with increment of 1% in each medium) was investigated after incubation on LB plates for 2 days at 40 °C. Carbohydrate metabolism and enzyme activities were observed using API ZYM and API Staph strips (bioMérieux, France) according to the manufacturer's instructions. For the tests of API strips, cells on LB plates were suspended in the supplied medium supplemented with 0.2% (w/v) final concentration of trace element solution [12]. Cell suspension with a turbidity equivalent to 0.5 McFarland was immediately poured in microtubes containing dehydrated substrates. The strips were incubated at 40 °C for 48 h.

2.4. Phylogenetic analysis based on 16S rRNA gene sequence

Genomic DNA of strain MW8 was extracted using a Genomic DNA Extraction Kit (DyneBio, Korea) for use as the template for polymerase chain reaction (PCR). The 16S rRNA gene was amplified using the bacterial universal primers (27F; 5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R; 5′-TACCTTGTTACGACTT-3′) [13]. The amplified DNA fragment was cloned into pGEM-T easy vector and sequenced. The nucleotide sequencing was performed using an Applied Biosystems 3730xl DNA Analyzer. The nearly complete 16S rRNA gene sequence (1403 bp) of strain MW8 was registered as JN578484 in GenBank. The 16S rRNA gene sequences of related type strains were obtained from the EzTaxon server [14]. The phylogenetic tree was constructed using a Neighbor-joining (NJ) method in the PHYLIP suit program [15]. Sequence alignments were performed using the ClustalW software [16], and the gaps at the 5′- and 3′-ends were edited using the BioEdit program [17]. Bootstrap value was calculated with data restructured close to 1000 times and marked into branching point. The evolutionary distance matrix was estimated according to the Kimura two-parameter model [18].

2.5. DNA–DNA hybridization

Genomic DNAs from strain MW8 and its phylogenetically-related type strains *S. thermocarboxydus* DSM44293^T [19], *Streptomyces indiaensis* NBRC13964^T, *Streptomyces massasporeus* NBRC12796^T, *Streptomyces longispororuber* NBRC13488^T were prepared from the cells grown on LB plates by using the Genomic DNA Extraction Kit (DyneBio, Korea). *Escherichia coli* KCCM12119^T was used as negative control. Probe DNA preparation and hybridization reactions were performed using the DIG High Prime DNA Labeling and Detection Starter Kit II (Roche Applied Science, Germany) according to the manufacturer's instructions. The resulting hybridization signals were measured using the Quantity One Program (Bio-rad, USA). The signal from strain MW8 was taken to be 100%. The mean values from 3 replicate experiments were used for comparison.

2.6. Chemotaxonomic analysis

The chemotaxonomic characteristics were determined using cells grown on LB medium at 40 °C for 48 h. Cellular fatty acids were prepared according to the standard protocol of the Microbial Identification System (MIDI) and identified via gas chromatography (GC) by using the Microbial Identification system [20], which consisted of an Agilent Technologies 6890 GC fitted with an HP-1 capillary column (cross-linked methyl siloxane, 30 m × 0.320 mm × 0.25 μm). The fatty acid identities and percentages were calculated using the Sherlock MIS Standard Software. The DNA G + C content was determined using a high performance liquid chromatography (HPLC) system equipped with a reverse-phase column [21].

2.7. Medium optimization for xylanase production

To choose the optimal culture media for xylanase production, we investigated different nitrogen and carbon sources of media. Four different nitrogen sources (soytone, meat extract, bacto peptone, and bacto tryptone) were added at a final concentration of 1% (w/v) to a medium containing 1% (w/v) NaCl and 0.3% (w/v) birchwood xylan. Eight different carbon sources (xylan, xylose, maltose, fructose, lactose, glucose, carboxyl methyl cellulose [CMC], and dextrin) were added at a final concentration of 0.3% (w/v) to medium containing 1% (w/v) NaCl and 1% (w/v) soytone as a sole nitrogen source. In addition, xylanase production was investigated at different concentrations (0.1%, 0.3%, 0.5%, and 1%) of the carbon source. The bacterial culture broth (2.58×10^5 cells/ml) was inoculated into each medium with an inoculum size of 0.1% (v/v). Fermented broths were sampled at regular intervals (24 h) and centrifuged at 10,000 rpm for 20 min to obtain cell-free culture supernatant, which was used as the source of xylanase. The optimized medium consisting of 1% (w/v) soytone, 1% (w/v) NaCl, and 0.5% (w/v) xylan was designated as SNX in this study.