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Cloning, overexpression and characterization of a xylanase gene from a novel *Streptomyces rameus* L2001 in *Pichia pastoris*

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ABSTRACT

A novel GC-rich xylanase gene named *xyn*A from *Streptomyces rameus* L2001 was cloned by high-efficiency thermal asymmetric interlaced PCR. The open reading frame of the cloned gene contained 672 bp and encoded 223 amino acid residues with a calculated molecular mass of 24.5 kDa. The recombinant xylanase (XynA) was scaled up in a 2.5 L fermenter using BSM and BMMY medium; the highest enzyme activity, 13626 U/mL, was obtained from BSM. This is the first report of high-cell-density production of a xylanase from *S. rameus* in *Pichia pastoris*. The xylanase showed a single band at about 25.0 kDa on SDS-PAGE after being treated with *endo*-β-*N*-acetylglucosaminidase H. Studies of enzymatic properties showed that the optimal temperature and pH of XynA were 55 °C and 4.5, respectively. XynA was very stable in a broad pH range (3.0–11.0), and the residual activity was >70% after incubation at 50 °C for 30 min. Thin-layer chromatography analysis showed that the recombinant xylanase was able to digest xylan, forming xylotriose and xylobiose as the main products; hence XynA from *S. rameus* L2001 could potentially be useful for preparation of xylooligosaccharides.

1. Introduction

Xylanases are the key enzymes capable of exploiting xylan, the second most abundant polysaccharide in nature [1]. In recent years, xylanases have been widely used in the animal feed, pulp and paper industries to generate commercial value [2]. In addition, xylanases effectively hydrolyze internal 1-4-β-D-xylose units and convert xylan feedstocks to oligosaccharides and D-xylose for biotechnological applications. Thus, the enzymes have also been used widely in the food and drink industries, for example for bread making and as food additives [3].

Many forms of xylanases have been isolated and characterized from various microorganisms including bacteria and fungi [4,5]. However, production of xylanases isolated from their original hosts is limited and the cost of enzymes is one of the main factors determining the economics of a commercial process. Meanwhile, many enzymes co-secreted with xylanase can cause problems. For instance, the presence of cellulase can be a problem for many industrial applications where xylanase is used. A production system is needed that can minimize this contamination and can easily produce the specific xylanolytic enzyme required in a pure form. Recombinant DNA procedures allow the design of a production route for specific xylanase and the xylanase will be produced with high yield and robustness under industrial conditions [6,7].

Pichia pastoris has many advantages that render it an attractive host for the expression and production of xylanase. Its advantages include ease of genetic manipulation, accurate eukaryotic post-translational modification, extracellular protein secretion [8], and low cost and high expression levels compared with baculovirus or mammalian expression systems [6]. In bioreactor cultures, *P. pastoris* can grow to high cell densities to gain high level protein production. Three phases in fed batch cultures as a common strategy led to high cell densities. The first phase corresponds to a batch culture with glycerol/glucose, the biomass concentration increased rapidly, but protein expression is repressed [9]. Secondly, fed-batch control is initiated by feeding concentrated glycerol until a certain cell density is reached [10]. The third phase, methanol as the carbon source is added to induce heterologous protein production.

In our previous work, a crude xylanase was purified from *Streptomyces rameus* L2001 [11] and its enzymatic properties appeared

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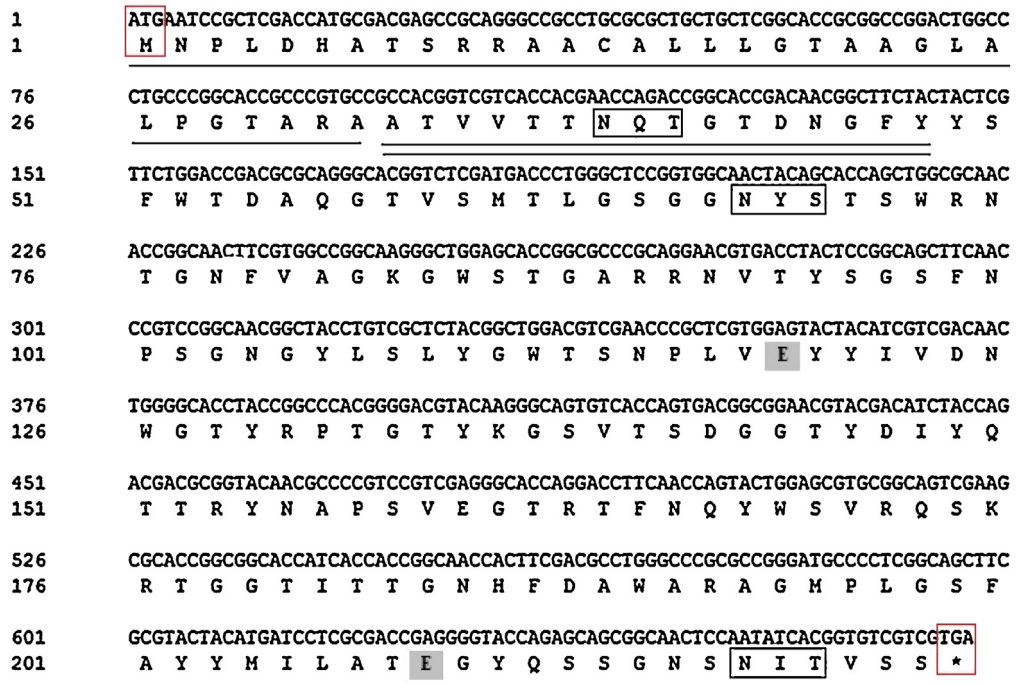

Fig. 1. Nucleotide and deduced amino acid sequence of the *xyn*A gene from *S. rameus* L2001. Sequence of the signal peptide is underlined; N-terminal amino acid sequence is double underlined; N-glycosylation sites are boxed with black; initiation and stop codon are boxed with red; conversed catalytic residues of glutamic acid are shown in shadow.

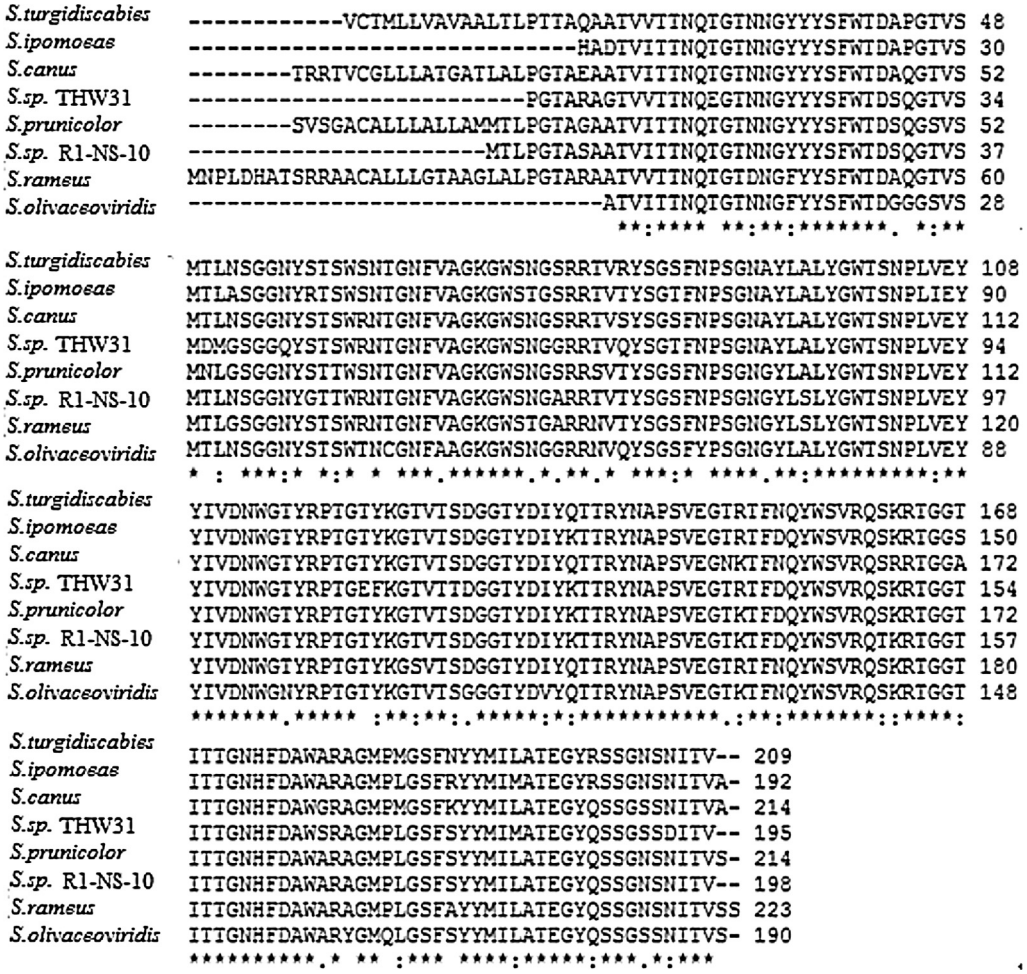

Fig. 2. Multiple sequence alignment of *S. rameus* L2001 xylanase with other *Streptomyces* xylanases. Multiple alignment was obtained using ClustalW on line. The amino acid residues are numbered; '*' — invariant residues; ':'— similar amino acids; '.'— less similar amino acids.