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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 xynA 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  $endo-\beta-N$ -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.

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#### 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- $\beta$ -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].

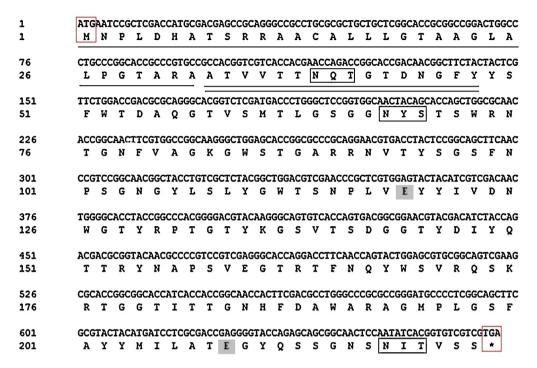
Pichia pastoris has many advantages that render it an attractive host for the expression and production of xylanase. Its advantages include ease of genetic manipulation, accurate eukaryotic post-translational modification, extracellular protein secretion [8], and low cost and high expression levels compared 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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Camaidicachias



**Fig. 1.** Nucleotide and deduced amino acid sequence of the *xynA* 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.

S.turgidiscabies	VCIMLLVAVAALTLPTTAQAATVVTTNQTGTNNGYYYSFWTDAPGTVS 4	8
S.ipomosas	HADTVITTNQTGTNNGYYYSFWTDAPGTVS 30	0
S.canus	TRRTVCGLLLATGATLALPGTAEAATVITTNQTGTNNGYYYSFWTDAQGTVS 5	2
S.sp. THW31	PGTARAGTVVTTNQEGTNNGYYYSFWTDSQGTVS 3	4
S.prunicolor	SVSGACALLLALLAMMTLPGTAGAATVITTNQTGTNNGYYYSFWTDSQGSVS 5	
S.sp. R1-NS-10	MTLPGTASAATVITINQTGTNNGYYYSFWTDSQGTVS 3	7
S.rameus	MNPLDHATSRRAACALLLGTAAGLALPGTARAATVVTTNQTGTDNGFYYSFWTDAQGTVS 60	0
S.olivacsoviridis	ATVITINQIGINNGFYYSFWIDGGGSVS 2	8
	**:*** **:**:***** *:**	
S.turgidiscabies	MTLNSGGNYSTSWSNTGNFVAGKGWSNGSRRTVRYSGSFNPSGNAYLALYGWTSNPLVEY 1	ne
S.ipomosas	MTLASGGNYRISWSNIGNFVAGKGWSIGSRRIVIYSGIFNPSGNAYLALYGWISNPLIEY 9	
S.canus	MTLNSGGNYSTSWRNTGNFVAGKGWSNGSRRTVSYSGSFNPSGNAYLALYGWTSNPLVEY 1	_
S.sp. THW31	MDMGSGGOYSTSWRNTGNFVAGKGWSNGGRRTVOYSGTFNPSGNAYLALYGWTSNPLVEY 9	
S.prunicolor	MNLGSGGNYSTTWSNTGNFVAGKGWSNGSRRSVTYSGSFNPSGNGYLALYGWTSNPLVEY 1	
S.sp. R1-NS-10	MTLNSGGNYGTTWRNTGNFVAGKGWSNGARRTVTYSGSFNPSGNGYLSLYGWTSNPLVEY 9	7
S.rameus	MTLGSGGNYSTSWRNTGNFVAGKGWSTGARRNVTYSGSFNPSGNGYLSLYGWTSNPLVEY 1	20
S. olivaceoviridis	MILNSGGNYSISWINCGNFAAGKGWSNGGRRNVQYSGSFYPSGNGYLALYGWISNPLVEY 8	8
	* : ***:* *:* * ***.*****.*.*.* ***:* ****.**:**	
S.turgidiscabies	YIVDNWGTYRPTGTYKGTVTSDGGTYDIYQTTRYNAPSVEGTRTFNQYWSVRQSKRTGGT 1	68
S.ipomosas	YIVDNWGTYRPTGTYKGTVTSDGGTYDIYKTTRYNAPSVEGTRTFDOYWSVROSKRTGGS 1	
S.canus	YIVDNWGTYRPTGTYKGTVTSDGGTYDIYOTTRYNAPSVEGNKTFNOYWSVROSRRTGGA 1	
S.sp. THW31	YIVDNWGTYRPTGEFKGTVTTDGGTYDIYKTTRYNAPSVEGTRTFDQYWSVRQSKRTGGT 1	
S.prunicolor	YIVDNWGTYRPTGTYKGTVTSDGGTYDIYKTTRYNAPSVEGTKTFDQYWSVRQSKRTGGT 1	
S.sp. R1-NS-10	YIVDNWGTYRPTGTYKGTVTSDGGTYDIYKTTRYNAPSVEGTKTFDQYWSVRQTKRTGGT 1	57
S.rameus	YIVDNWGTYRPTGTYKGSVTSDGGTYDIYQTTRYNAPSVEGTRTFNQYWSVRQSKRTGGT 18	03
S.olivaceoviridis	YIVDNWGNYRPIGIYKGIVISGGGIYDVYQITRYNAPSVEGIKIFNQYWSVRQSKRIGGI 1	48
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S.turgidiscabies	ITTGNHFDAWARAGMPMGSFNYYMILATEGYRSSGNSNITV 209	
S.ipomosas	ITTGNHFDAWARAGMPLGSFRYYMIMATEGYRSSGNSNITVA- 192	
S.canus	ITTGNHFDAWGRAGMPMGSFKYYMILATEGYOSSGSSNITVA- 214	
S.sp. THW31	ITTGNHFDAWSRAGMPLGSFSYYMIMATEGYQSSGSSDITV 195	
S.prunicolor	ITTGNHFDAWARAGMPLGSFSYYMILATEGYOSSGNSNITVS- 214	
S.sp. R1-NS-10	ITTGNHFDAWARAGMPLGSFSYYMILATEGYQSSGNSNITV 198	
S.rameus	ITTGNHFDAWARAGMPLGSFAYYMILATEGYQSSGNSNITVSS 223	
S.olivaceoviridis	ITTGNHFDAWARYGMQLGSFSYYMILATEGYQSSGSSNITVS- 190	
	********** ** :*** ****:****:***.*:***	4

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.

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