读书报告

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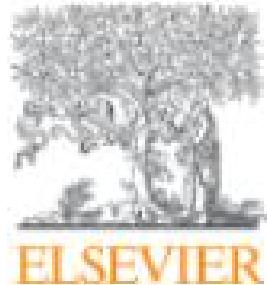
Heterologous expression and characterization of a xylanase and xylosidase from white rot fungi and their application in synergistic hydrolysis of lignocellulose

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白腐真菌木聚糖酶和木糖苷酶的异源表达和鉴定及其在木质纤维素协同水解中的应用

CONTENT

Introduction
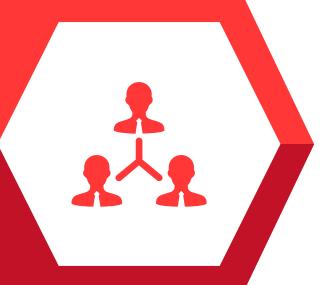
木质纤维素

　　木质纤维素是一种巨大且无休止的可再生资源，是生物燃料生产的可持续来源，因为它含有大约75％的多糖(Marriott et al., 2016)。

　　木质纤维素的主要成分是碳水化合物，如纤维素和半纤维素，以及木质素，而纤维素和半纤维素是地球上最多和第二丰富的多糖(Marriott et al., 2016)。

　　（1）作为最丰富的碳水化合物之一，半纤维素的有效利用将提高理论产量并改善木质纤维素转化的经济性(Merino and Cherry, 2007)。

　　（2）半纤维素的降解将进一步促进纤维素酶进入纤维素，从而更有效地利用纤维素，因为纤维素嵌入在半纤维素，果胶和木质素的基质中 (Van Dyk and Pletschke, 2012)。

半纤维素包括异构聚合物，如木聚糖，甘露聚糖，半乳聚糖和阿拉伯聚糖，其中，木聚糖是最丰富的成分，代表了自然界中最多的半纤维素(Beg et al., 2001)。

内切-1,4-木聚糖酶和β-木糖苷酶在木聚糖的降解中起重要作用，并且被认为是半纤维素水解的核心酶。

对于天然木质纤维素底物，纤维素酶和木聚糖酶显示出显着的水解协同效应(Song et al., 2016)。

　　白腐真菌*Pleurotus ostreatus* HAUCC 162和*Irpex lacteus* CD$_2$具有降解木质纤维素的强大能力(Qin et al., 2017b; Zhuo et al., 2017, 2018)。

　　在本次研究中，分别从白腐真菌*Pleurotus ostreatus* HAUCC 162和*Irpex lacteus* CD$_2$克隆了新的内切-1,4-木聚糖酶和β-木糖苷酶基因。 这些内切-1,4-木聚糖酶和β-木糖苷酶被异源表达和表征。 然后研究了内切-1,4-木聚糖酶和β-木糖苷酶对木聚糖和氢氧化钠预处理玉米秸秆（SHPC）水解的协同作用，以及它们与商业纤维素酶对SHPC降解的协同作用。

Materials and Methods

第二部分
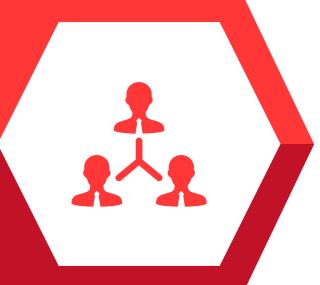
Materials and Methods

菌株培养

endo-1,4-xylanases 和 β-xylosidase 基因的克隆

构建表达载体，异源表达和Xyn162和XylCD$_2$的纯化

内切-1,4-木聚糖酶和β-木糖苷酶活性的测定

pH和温度对rXyn162和rXylCD$_2$活性的影响

金属化合物和不同表面活性剂对rXyn162活性的影响

协同度的计算

玉米秸秆预处理

通过rXyn162，rXylCD$_2$和商业纤维素酶协同水解氢氧化钠预处理的玉米杆

水解产物的MALDI-TOF-MS和HPLC分析

Results and Discussion
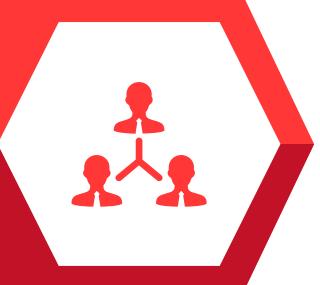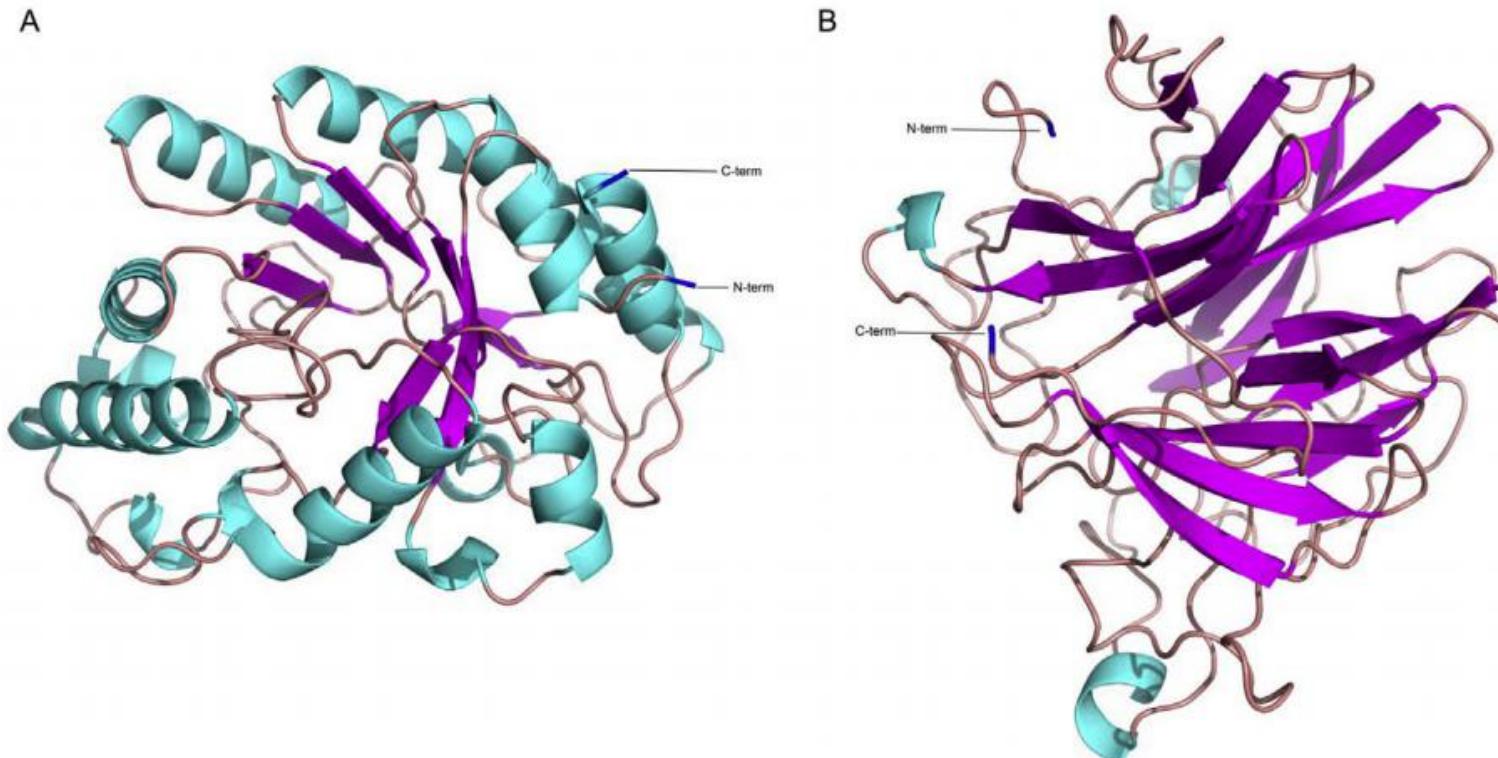
Fig. 1. Overall structures of the (A) Xyn 162 and (B) XylCD2 proteins. Ribbon representation of the structure with secondary structure elements. Alpha helices were colored in azure, Beta sheets were colored in purple. The N− and C-termini are labeled. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)
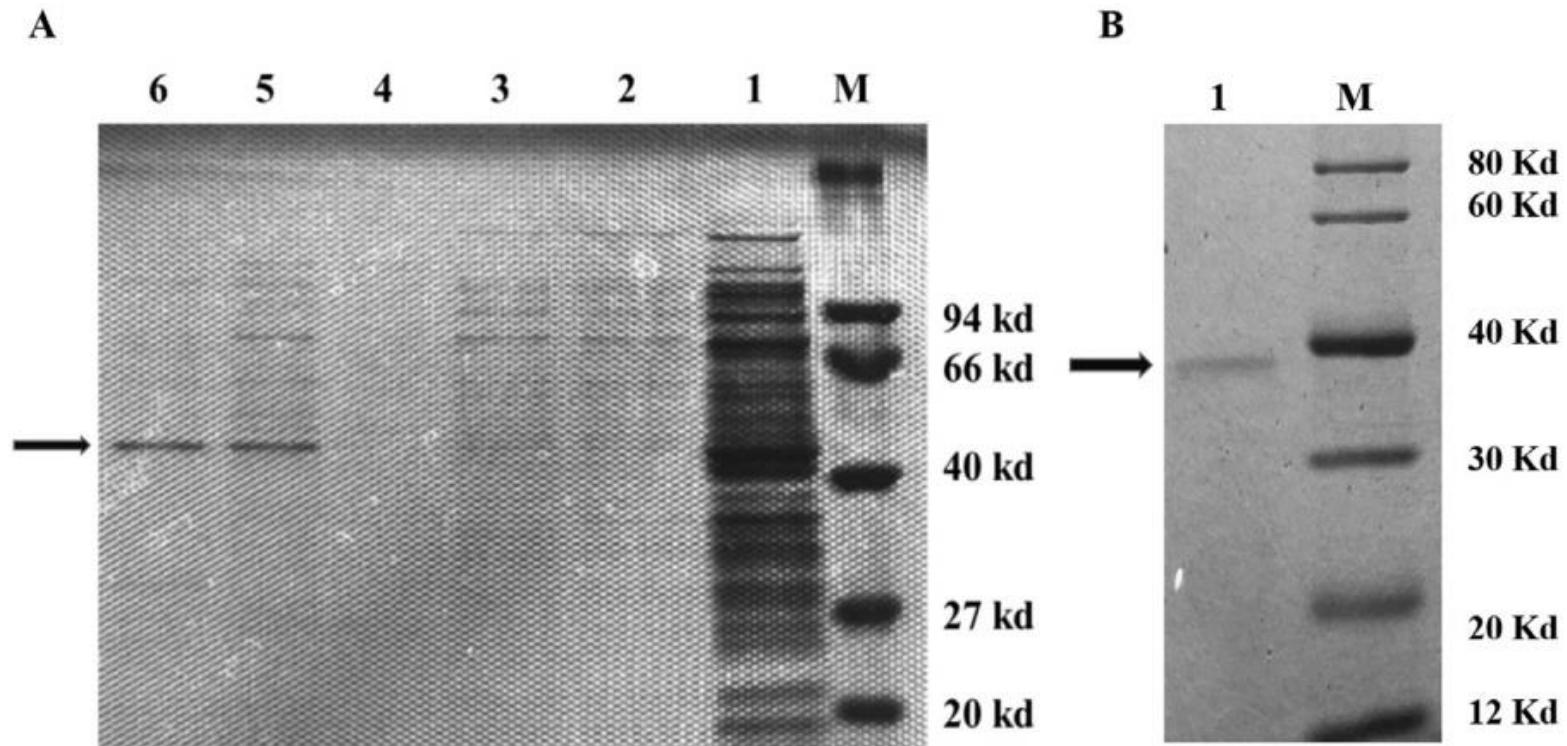
Fig. 2. SDS-PAGE of purified recombinant proteins. (A) rXyn162, M: Protein marker, Lane 1: extracellular protein, Lane 2: 10 mM imidazole effluent, Lane 3: 40 mM imidazole effluent, Lane 4: 80 mM imidazole effluent, Lane 5: 120 mM imidazole effluent, Lane 6: 160 mM imidazole effluent, purified rXyn162. (B) rXylCD2, M: Protein marker, Lane 1: purified rXylCD2 after ion exchange chromatography.
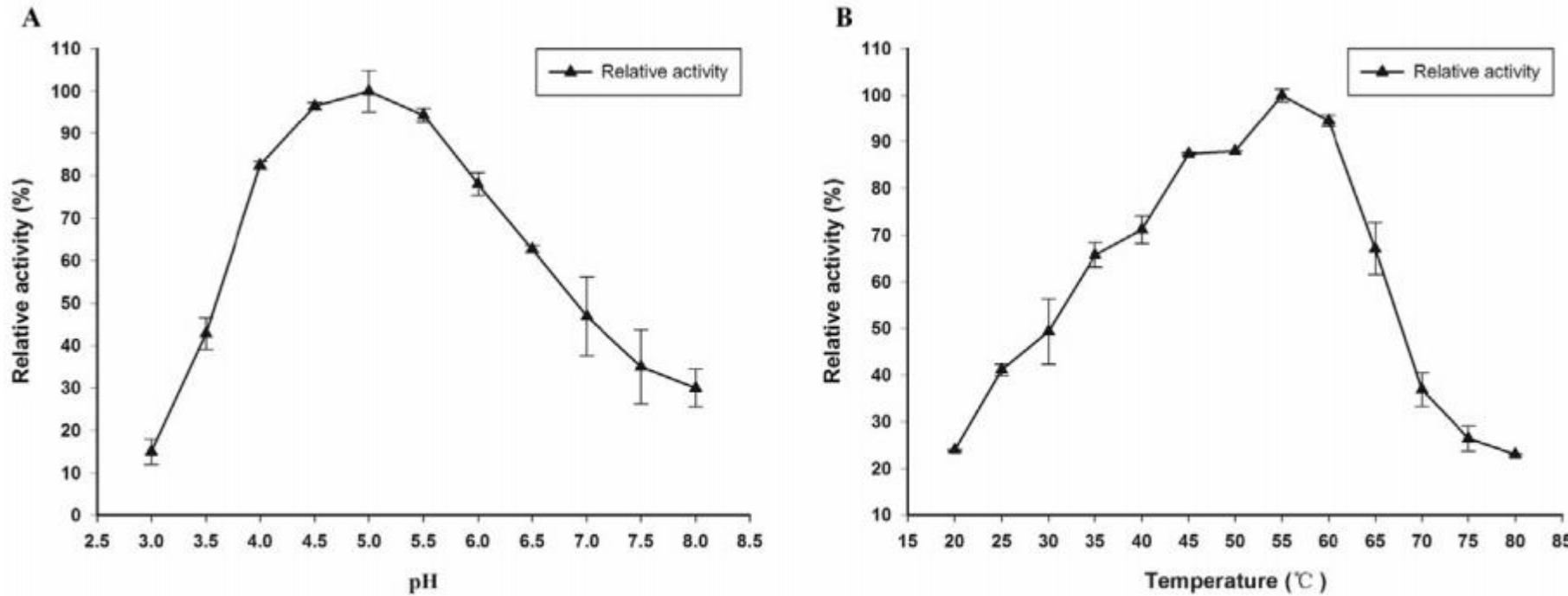
Fig. 3. Effects of (A) pH and (B) temperature on the rXyn162 activity.

Table 2

The effects of metal ions and different surfactants on rXyn162 activity.

Metal compounds	Relative activity (%)	Surfactants	Relative activity (%)
Control	100 ± 4.06	Control	100 ± 4.06
LiCl	60.64 ± 3.90	1% Triton X-100	129.43 ± 3.65
KCl	67.45 ± 5.64	4% Triton X-100	132.21 ± 3.46
NaCl	93.98 ± 4.51	1% Tween 20	140.21 ± 8.24
$NiCl_2$	56.02 ± 6.27	4% Tween 20	166.36 ± 7.19
$ZnCl_2$	104.05 ± 0.76	0.5% SDS	64.57 ± 4.20
$CrCl_3$	128.28 ± 8.60	2% SDS	40.19 ± 1.34
$MgCl_2$	86.89 ± 3.71	1% Tween 80	133.29 ± 4.20
$CaCl_2$	126.38 ± 8.35	4% Tween 80	138.55 ± 1.41
$CdCl_2$	63.21 ± 3.12	1% PEG 200	82.34 ± 7.61
$AlCl_3$	92.39 ± 7.51	4% PEG 200	54.42 ± 7.98
$CuCl_2$	3.43 ± 0.27		
$FeCl_3$	63.45 ± 3.92		

PEG 200 and SDS acted as enzyme inhibitor for rXyn162. (Wang et al., 2016).

Table 3
The kinetic parameters of rXylCD2.

| Substrate	Km (mM)	Vmax (umol*min^{-1}*mg^{-1})	kcat (s^{-1})	kcat/Km (mM^{-1}*s^{-1})
pNPX	1.15	15.44	9.40	8.18
pNPAf	1.31	4.62	2.81	2.14
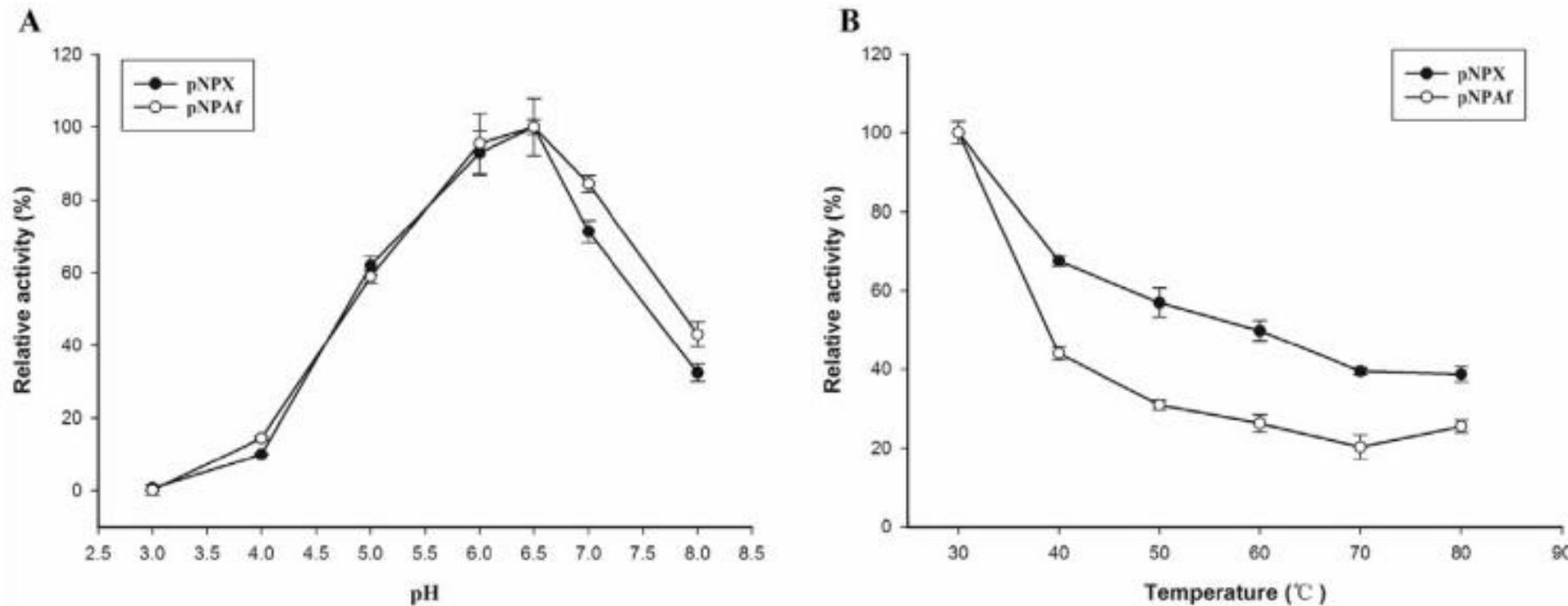
Fig. 4. Effects of (A) pH and (B) temperature on the rXylCD2 activity.
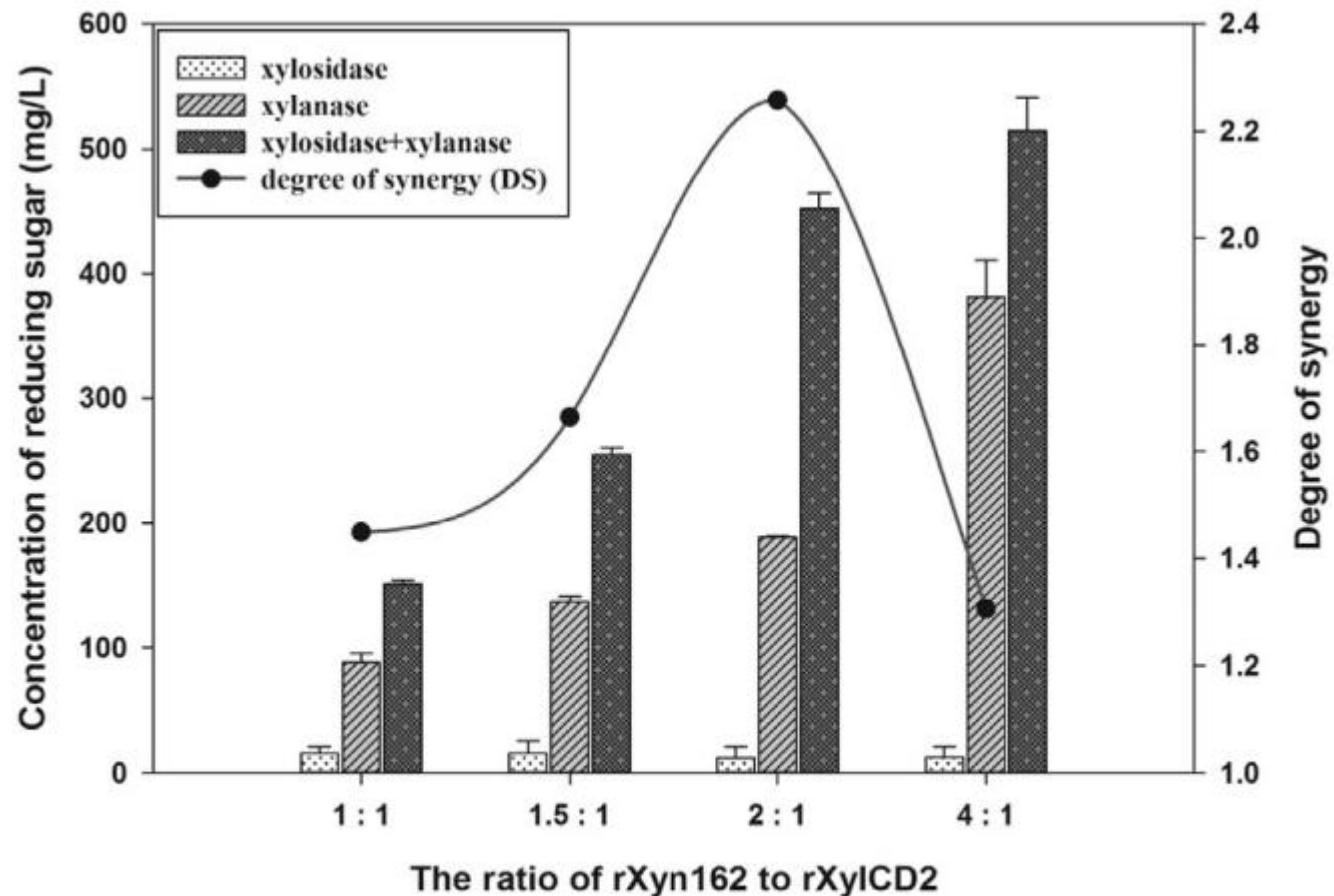
Fig. 5. Synergistic hydrolysis of oat spelt xylan by rXyn162 and rXylCD2.
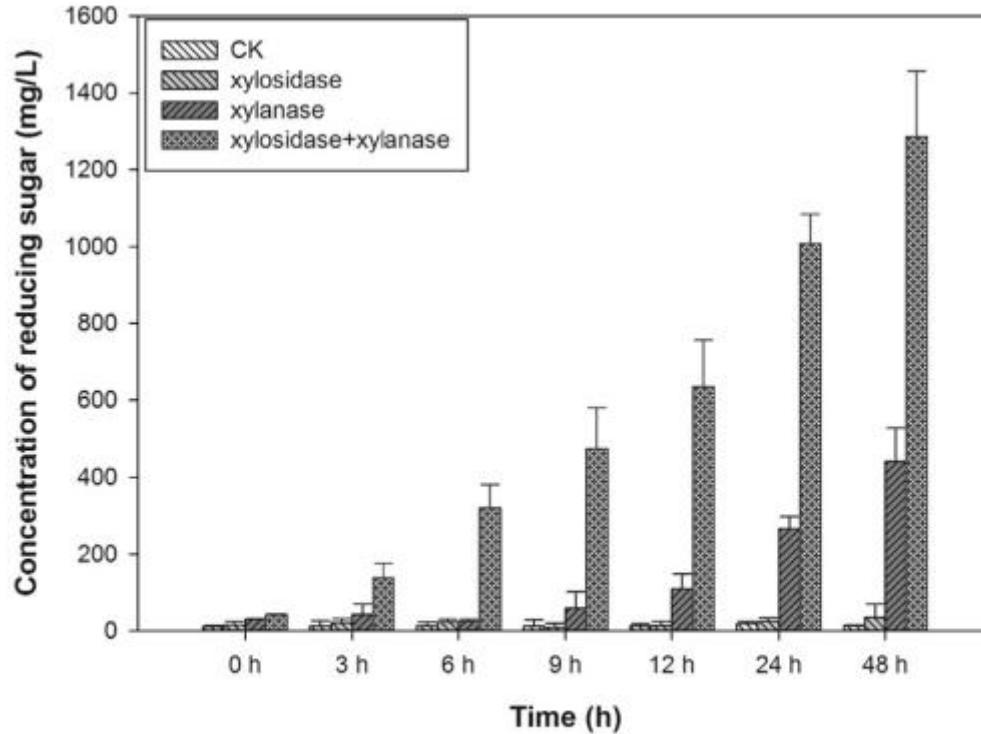
Fig. 7. Hydrolysis of sodium hydroxide pretreated cornstalk (SHPC) with rXyn162, rXylCD2, and combination of rXyn162 and rXylCD2.
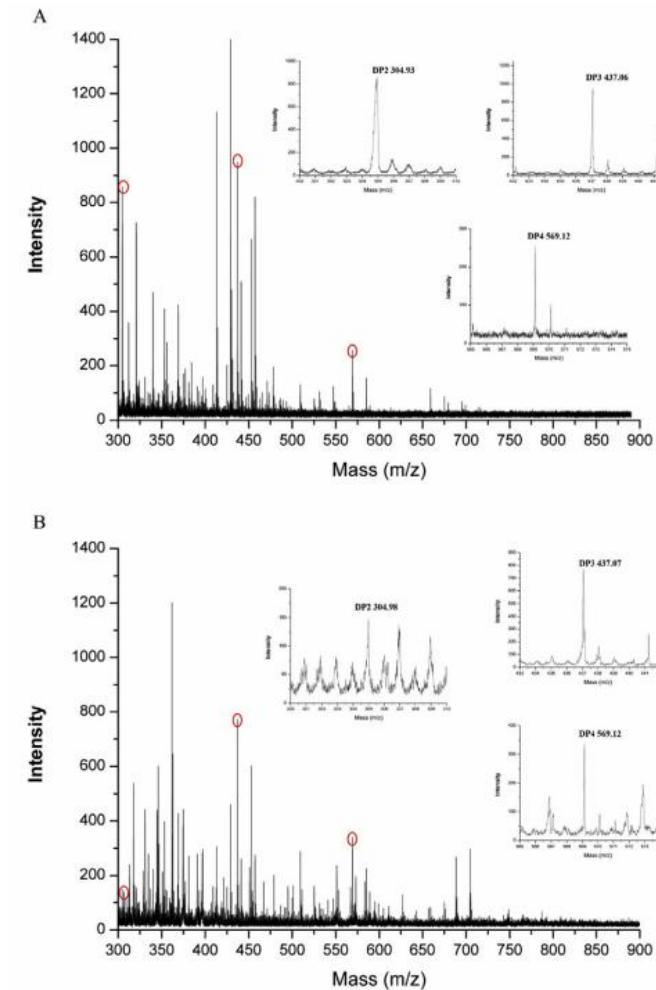

Fig. 8. MALDI-TOF-MS analysis of the hydrolyzate of sodium hydroxide pretreated cornstalk (SHPC) by (A) individual rXyn162 and (B) combination of rXyn162 and rXylCD2.
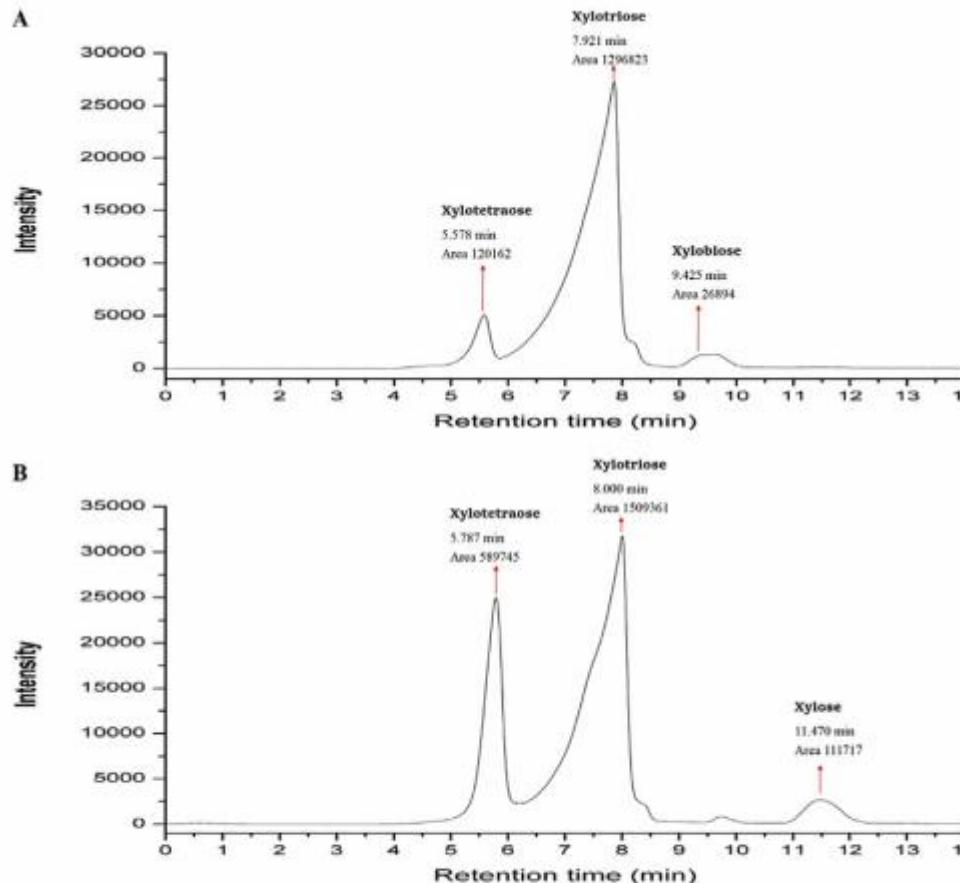
Fig. 9. HPLC analysis of the hydrolyzate of sodium hydroxide pretreated cornstalk (SHPC) by (A) individual rXyn162 and (B) combination of rXyn162 and rXylCD2. The peak time and peak area are annotated.
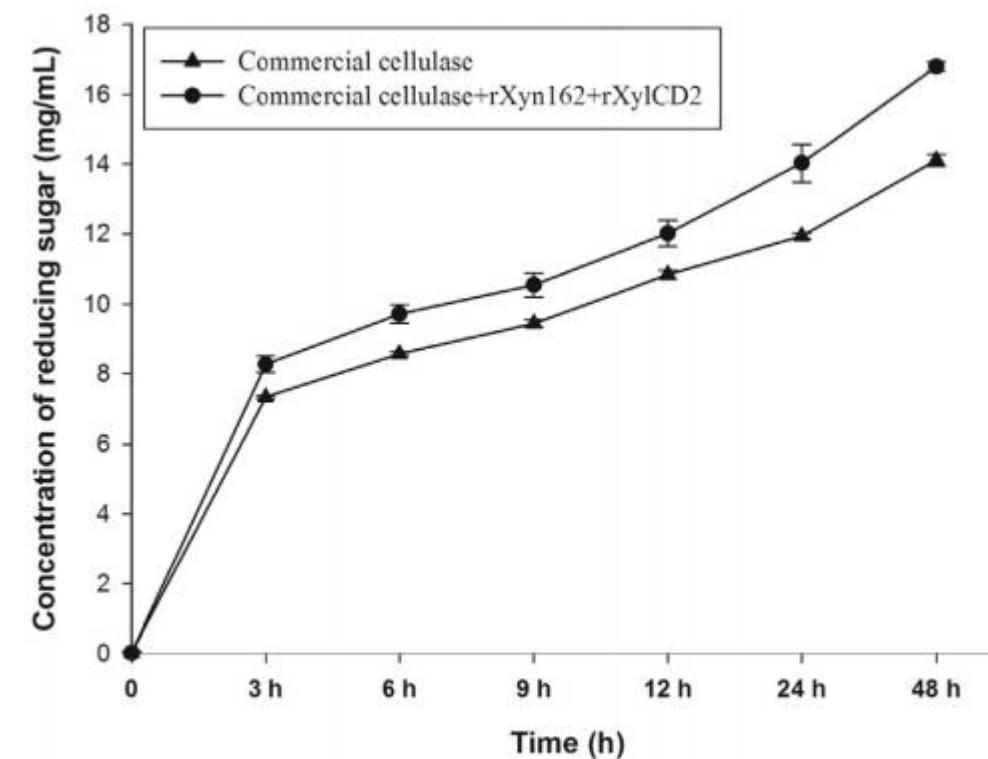
Fig. 10. Synergistic hydrolysis of SHPC by commercial cellulase (▲), and combination of xylanase and β-xylosidase with commercial cellulase (●).
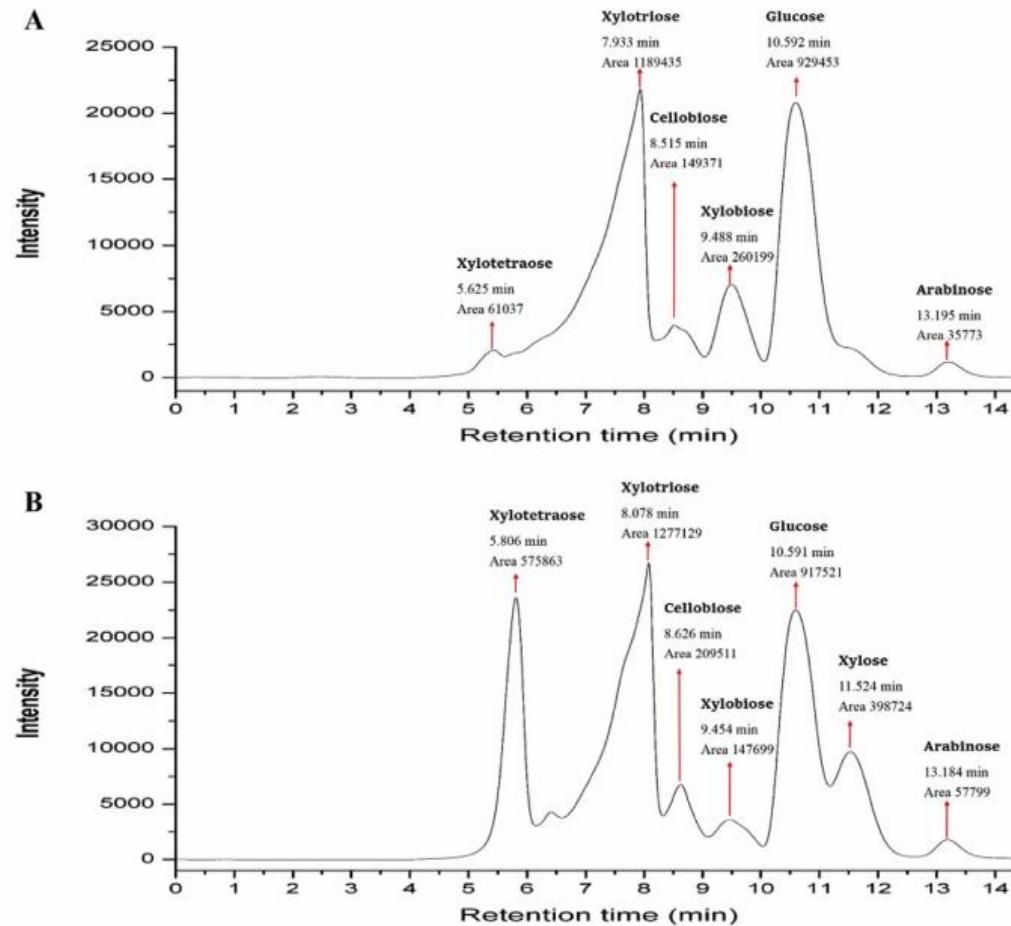
Fig. 11. HPLC analysis of the hydrolyzate of sodium hydroxide pretreated cornstalk (SHPC) by (A) commercial cellulase, and (B) combination of xylanase and β-xylosidase with commercial cellulase. The peak time and peak area are annotated.

Conclusion

结论

01   该研究有助于木质纤维素的有效酶糖化，并表明在生物质预处理领域中使用rXyn162和rXylCD$_2$的巨大潜力。

02   此外，本研究中使用的rXyn162和rXylCD2是从不同的白腐真菌中分离出来的，表明可以将不同的白腐真菌结合用于有效的木质纤维素预处理。

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