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Improvement of the catalytic performance of a hyperthermostable GH10 xylanase from *Talaromyces leycettanus* JCM12802

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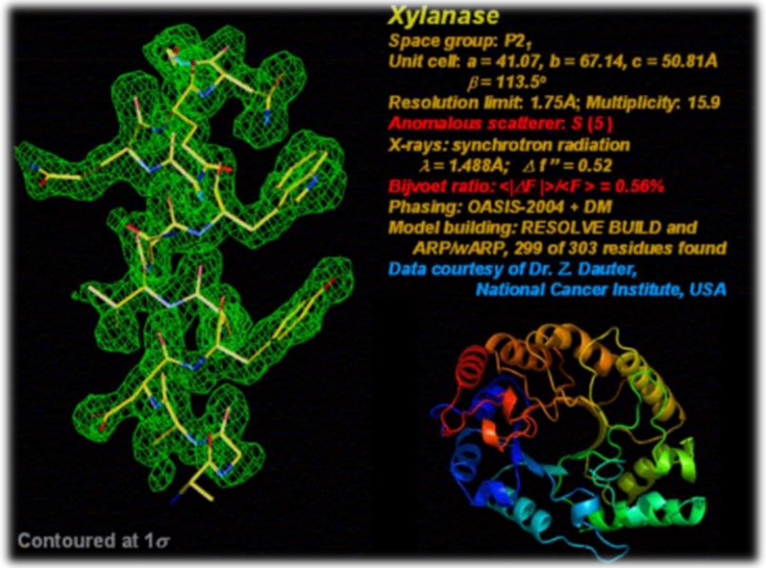
提高产自*Talaromyces leycettanus* JCM12802的超热稳定GH10木聚糖酶的催化性能。

CONTENT

- Introduction
- Materials and Methods
- Results and Discussion
- Conclusion

Introduction

Introduction



木聚糖酶(蛋白质晶体结构)

木聚糖(xylan)：植物半纤维素(hemicellose)

比例：约占15%～35%（植物细胞干重）

自然界第二大丰富的多糖

大多数木聚糖酶（xylanse）分为糖苷水解酶（GH）10和11家族，其他少数群体属于5,8和30家族。

微生物：细菌，酵母和真菌

Introduction

酶的基本性质，如比活性，热稳定性及对阳离子和化学品的耐受性，是决定其潜在应用的重要因素。

碱性木聚糖酶用于纸浆漂白工业（Ma and Yang, 2015; Techapun *et al*., 2003）；

酸性木聚糖酶可作为饲料和食品添加剂(Collins *et al*., 2005; Du *et al*., 2013; Zhao *et al*., 2013)；

冷适应性木聚糖酶用于洗涤剂和纺织工业(Chen *et al*., 2013; Wang *et al*., 2011, 2012a)。

Introduction

在生物转化过程中，通常在酶处理之前或同时使用热水，蒸汽爆破和酸性预处理(Mielenz, 2001; Saha, 2003)。

在酿造工业上，酸性热稳定性木聚糖酶需要在70°C下 进行更长时间的水解 (Kunze, 1999)。

在饲料工业中，木聚糖酶必须承受造粒过程温度（通常为70-90°C）并适应消化道（pH 4.8）(van Campenhout *et al*., 2003)。

Introduction

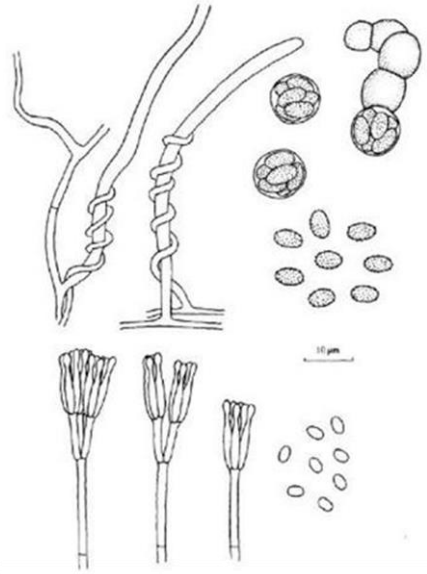
热稳定性 ↑

催化性能 ↑

→ 成本 ↓

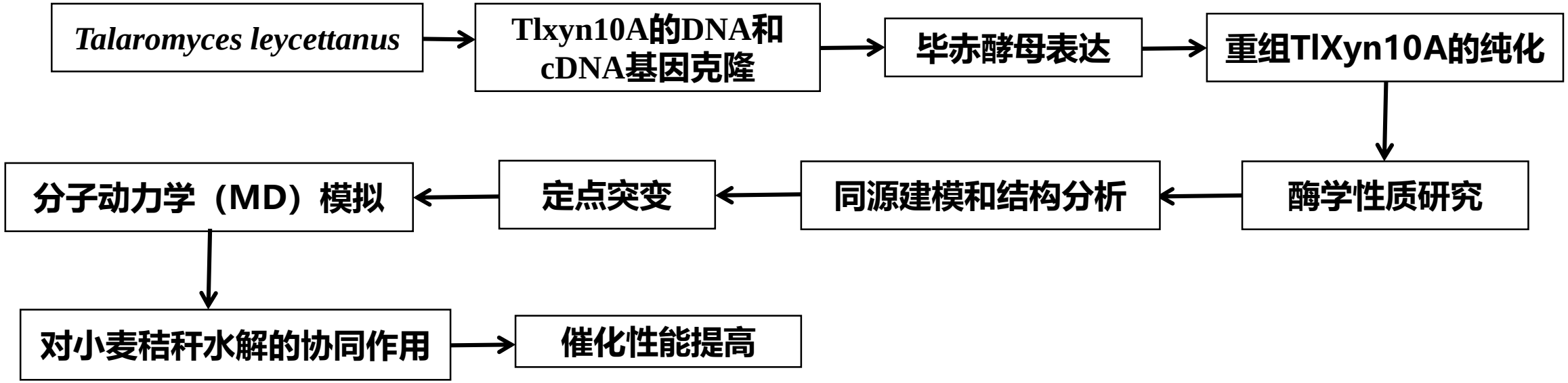
Gene mining and Protein engineering

在本项研究中，一种具有高度热稳定性的酸性木聚糖酶TlXyn10A，在*Talaromyces leycettanus* JCM 12802中鉴定并在毕赤酵母GS115中成功表达。出于商业目的，通过定点诱变进一步修饰该热稳定性木聚糖酶，提高其催化性能。



Materials and Methods

技术路线


Talaromyces leycettanus
Tlxyn10A的DNA和cDNA基因克隆
毕赤酵母表达
重组TlXyn10A的纯化
酶学性质研究
同源建模和结构分析
定点突变
分子动力学（MD）模拟
对小麦秸秆水解的协同作用
催化性能提高

Results and Discussion

重组蛋白的表达和纯化

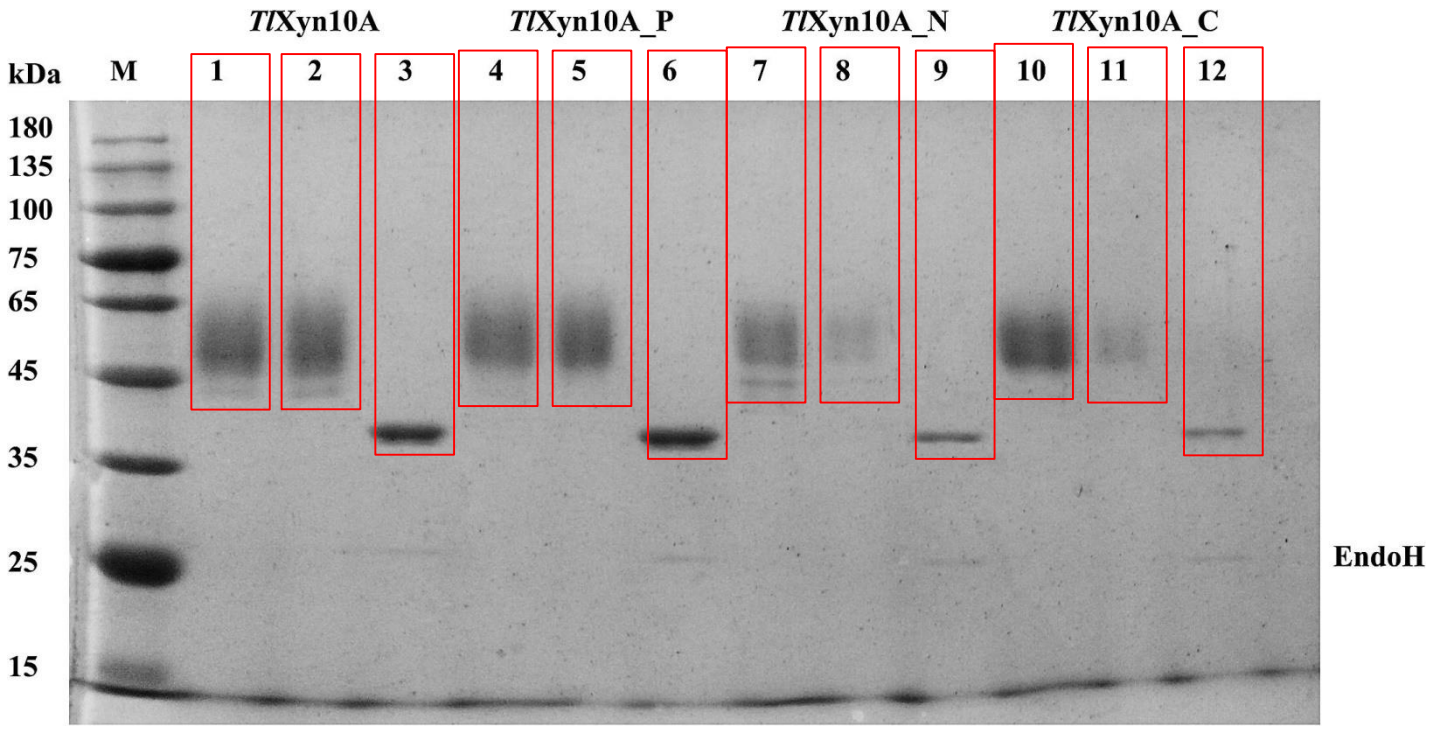


Fig. 1. SDS-PAGE analysis of TlXyn10A and its mutants. Lanes: M, the molecular mass standards; 1, 4, 7 and 10, the crude enzymes; 2, 5, 8, and 11, the purified recombinant enzymes; 3, 6, 9, and 12, the deglycosylated enzymes with Endo H treatment.

比较野生型TlXyn10A和突变酶的酶学性质

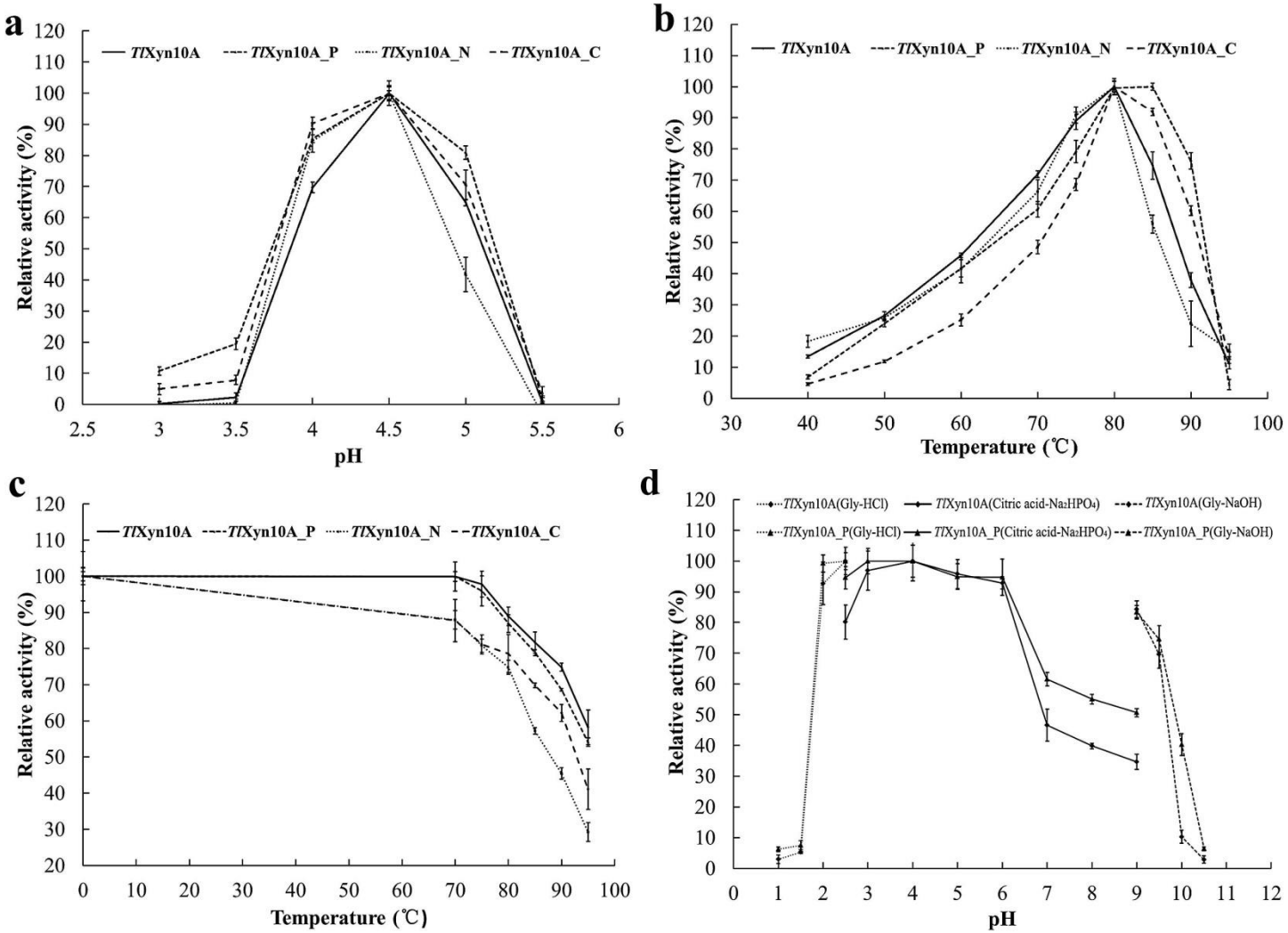


Fig. 2. Characterization of the purified recombinant TlXyn10A and its mutants with and without N-deglycosylation. (a) Effect of pH on the xylanase activities. (b) Effect of temperature on the xylanase activities. (c) Thermostability of the xylanase activities. (d) pH stability of TlXyn10A and TlXyn10A_P.

比较野生型TlXyn10A和突变酶的酶学性质

Table 1
The specific activities and kinetic values of purified *Tl*Xyn10A and its mutants.[a]

Sample	Specific activity (U/mg)	V_{max} (U/mg)	K_m (mg/mL)	k_{cat}/K_m (mL/s/mg)
*Tl*Xyn10A	2240 ± 34	2542 ± 22	1.01 ± 0.05	1626 ± 81
*Tl*Xyn10A_P	3232 ± 76	3852 ± 55	1.49 ± 0.06	1665 ± 61
*Tl*Xyn10A_N	1327 ± 32	1520 ± 24	1.09 ± 0.06	901 ± 50
*Tl*Xyn10A_C	1769 ± 16	2015 ± 24	1.11 ± 0.04	1175 ± 42

[a] Data are shown as mean ± standard deviation (n = 3).

Table 2
Effect of metal ions and chemical reagents (5 mM) on the activity of purified recombinant *Tl*Xyn10A.

Chemicals	Relative activity (%)[a]	Chemicals	Relative activity (%)
Control	100.0 ± 1.6	Ca^{2+}	102.8 ± 7.6
Na^{+}	122.3 ± 1.9	Ag^{+}	98.5 ± 3.2
Mg^{2+}	115.5 ± 4.5	Fe^{3+}	95.8 ± 1.0
Mn^{2+}	115.0 ± 1.6	Cu^{2+}	95.1 ± 6.3
K^{+}	111.3 ± 5.1	Pb^{2+}	93.2 ± 1.2
Ni^{2+}	110.6 ± 9.4	β-Mercaptoethanol	164.1 ± 4.6
Cr^{3+}	107.0 ± 5.9	EDTA	102.2 ± 1.7
Zn^{2+}	104.0 ± 1.0	SDS	0.9 ± 0.4

[a] Values represent the mean ± standard deviation (n = 3) relative to the untreated control samples.

差示扫描量热法（DSC）分析

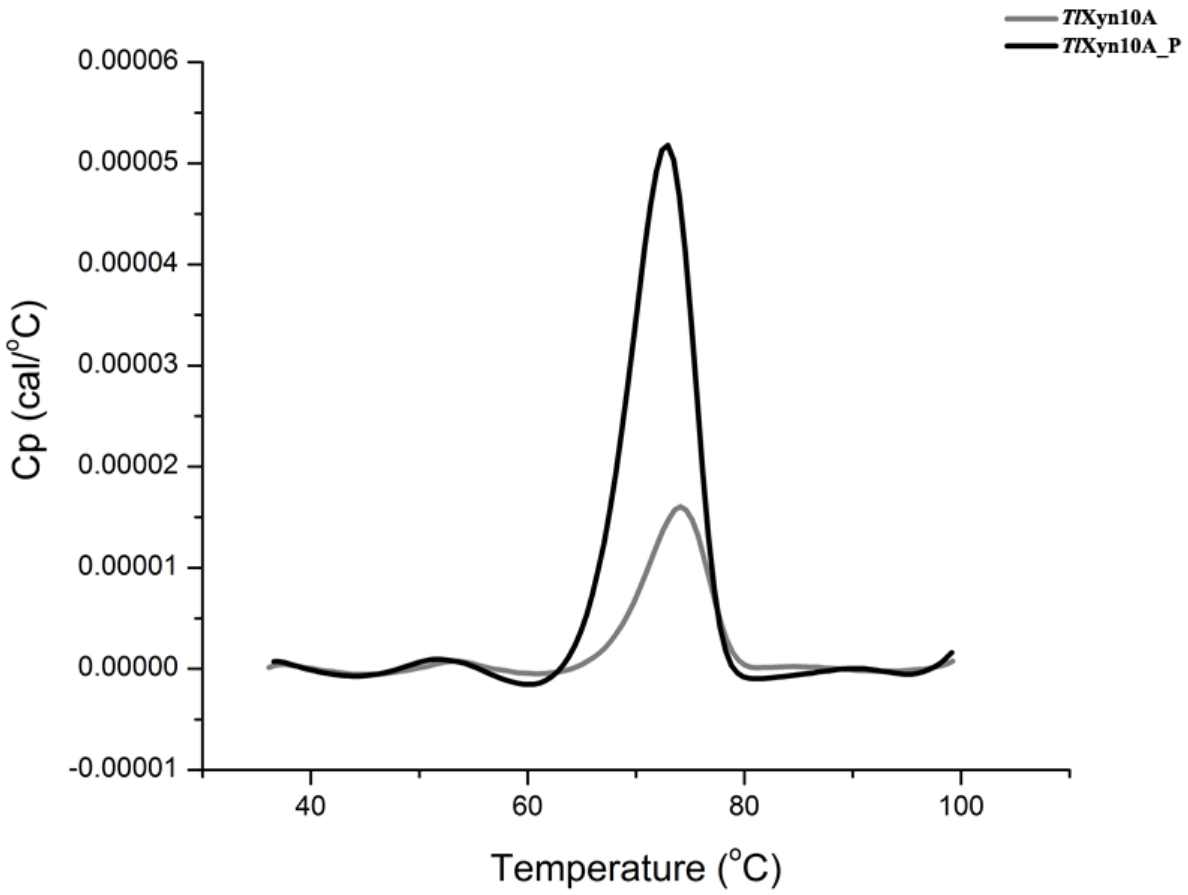


Fig. S3. The DSC results of TlXyn10A and TlXyn10A_P.

同源建模和模型结构分析

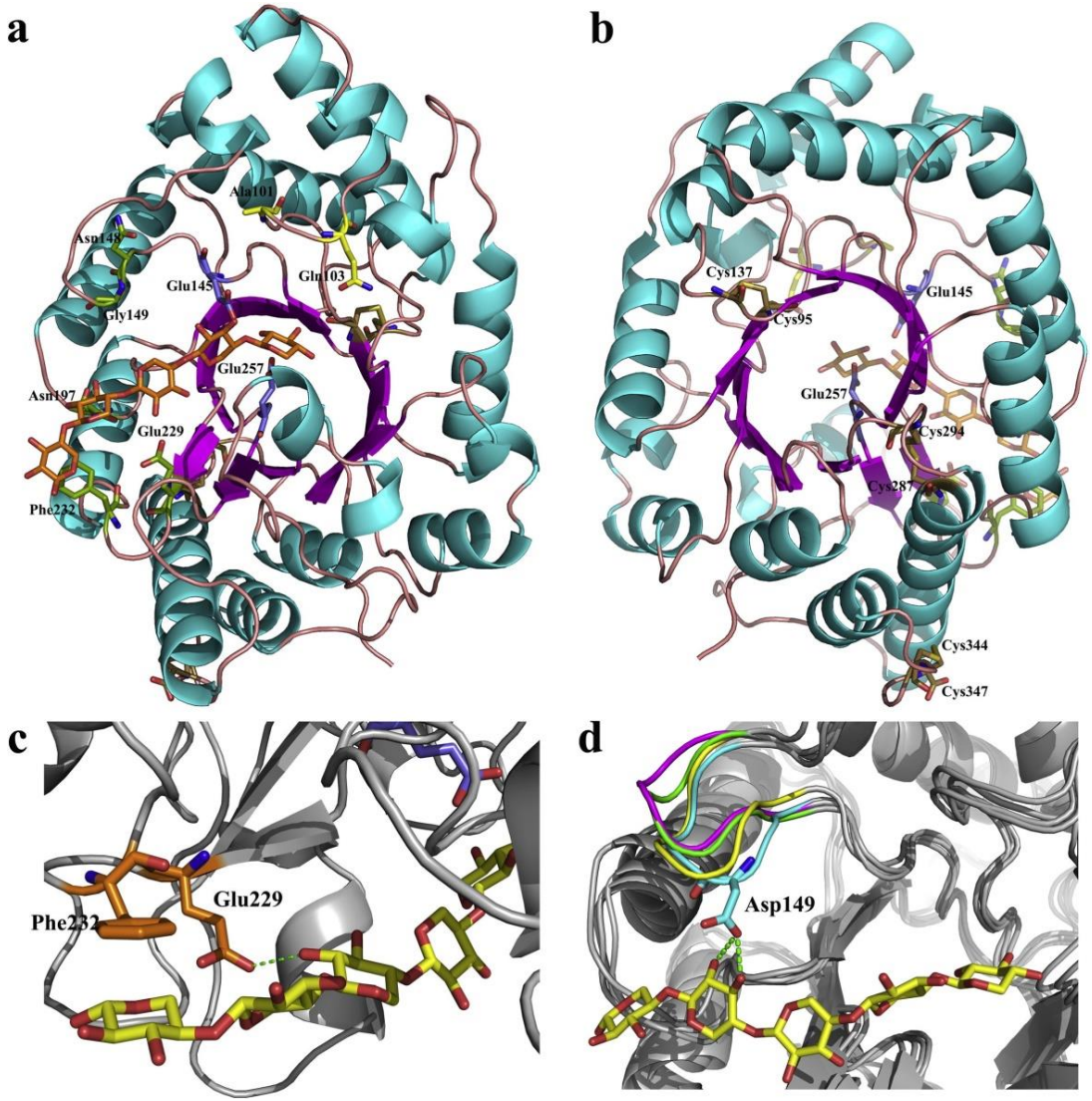


Fig. 3. Structure analysis of modeled TlXyn10A.

Xyn10A含有三个二硫键

二硫键在维持蛋白质热稳定性方面至关重要(Hattori et al., 2015; Wang et al., 2012b; Yin et al., 2015)。

Tl Xyn10A_P和纤维素酶对小麦秸秆水解的协同作用

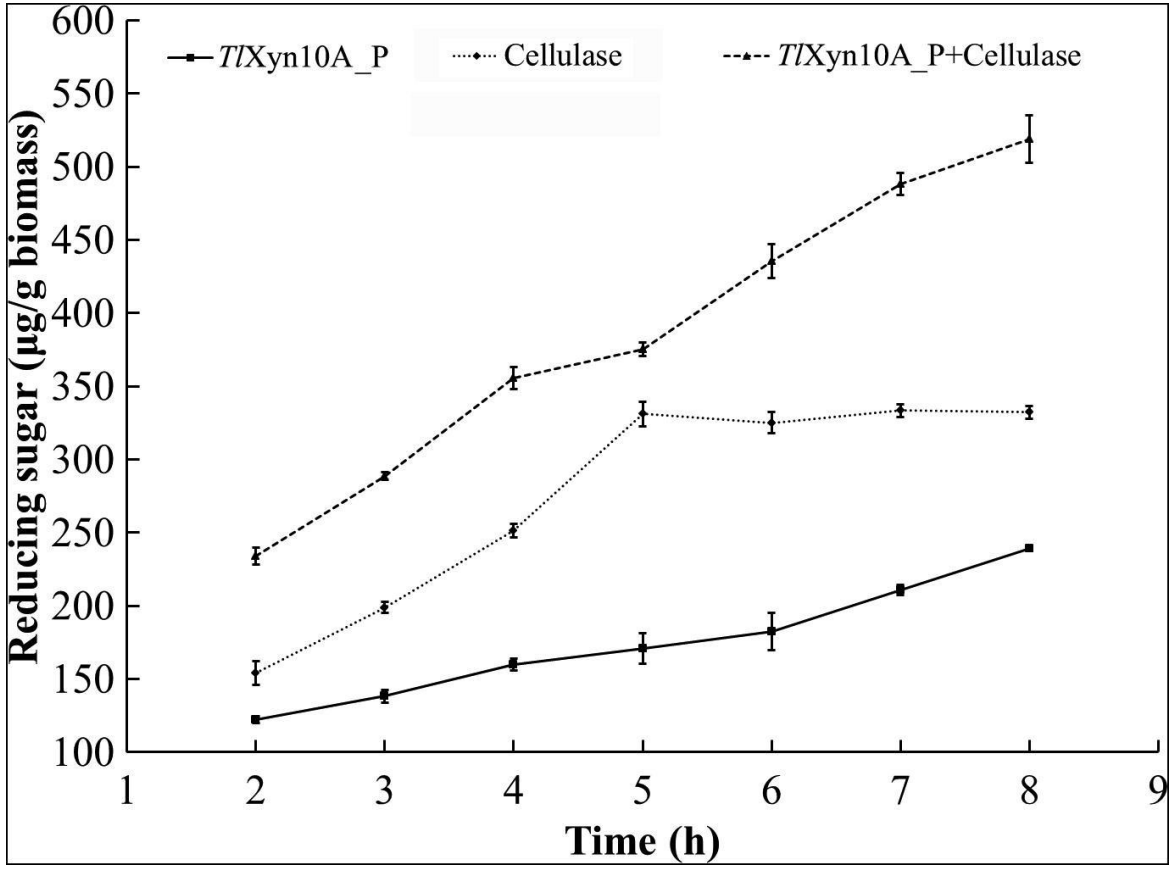


Fig. 5. The reducing sugar yields of different experiment groups over 8 h.

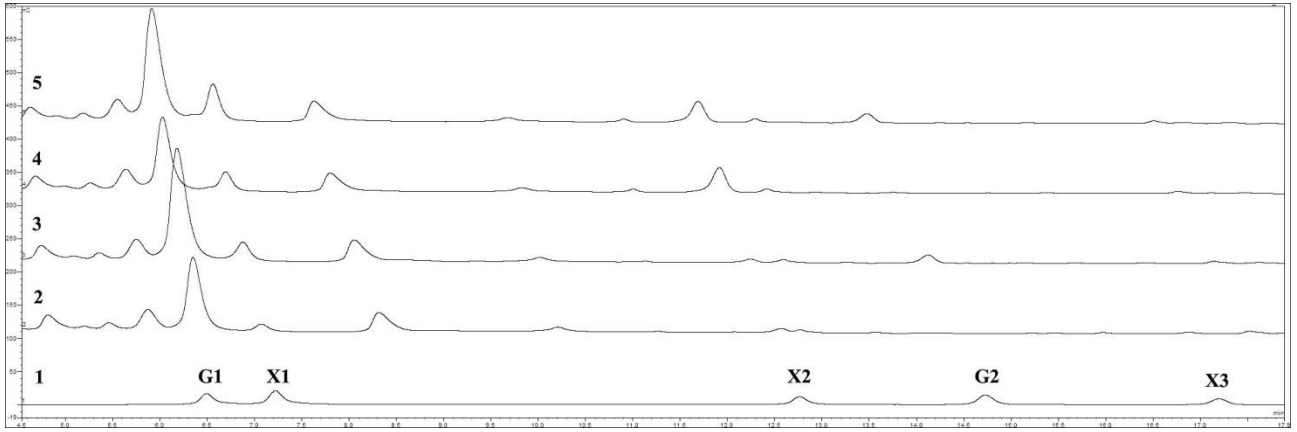


Fig. 6. HPAEC analysis of the hydrolysis products of wheat straw. 1, the xylooligosaccharide and cellooligosaccharide standards; 2, the control without enzyme addition; 3–5, the hydrolysis products released from cellulase group, TlXyn10A_P group, and TlXyn10A_P and cellulase group, respectively. G1, glucose; G2, cellobiose; X1, xylose; X2; xylobiose; and X3, xylotriose.

Conclusion

Conclusion

01 高度热稳定的木聚糖酶TlXyn10A在*T.leycettanus* 中鉴定并在巴斯德毕赤酵母中成功表达。

02 重组TlXyn10A表现出优异的热稳定性和对所有测试的金属离子的抗性。

03 除了改善了催化性能外，突变体TlXyn10A_P的pH稳定性也得到提高，并证明可持续水解小麦秸秆长达8小时。

请各位老师同学批评指导!