读书报告

吉伟利　　2018-04-14

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BIOTECHNOLOGICALLY RELEVANT ENZYMES AND PROTEINS

Synergistic hydrolysis of xylan using novel xylanases, β-xylosidases, and an α-L-arabinofuranosidase from *Geobacillus thermodenitrificans* NG80-2

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使用来自*Geobacillus thermodenitrificans* NG80-2的新木聚糖酶，

β-木糖苷酶和α-L-阿拉伯呋喃糖苷酶协同水解木聚糖

IF：3.67

Introduction

甘蔗渣

麦麸

玉米芯

米糠

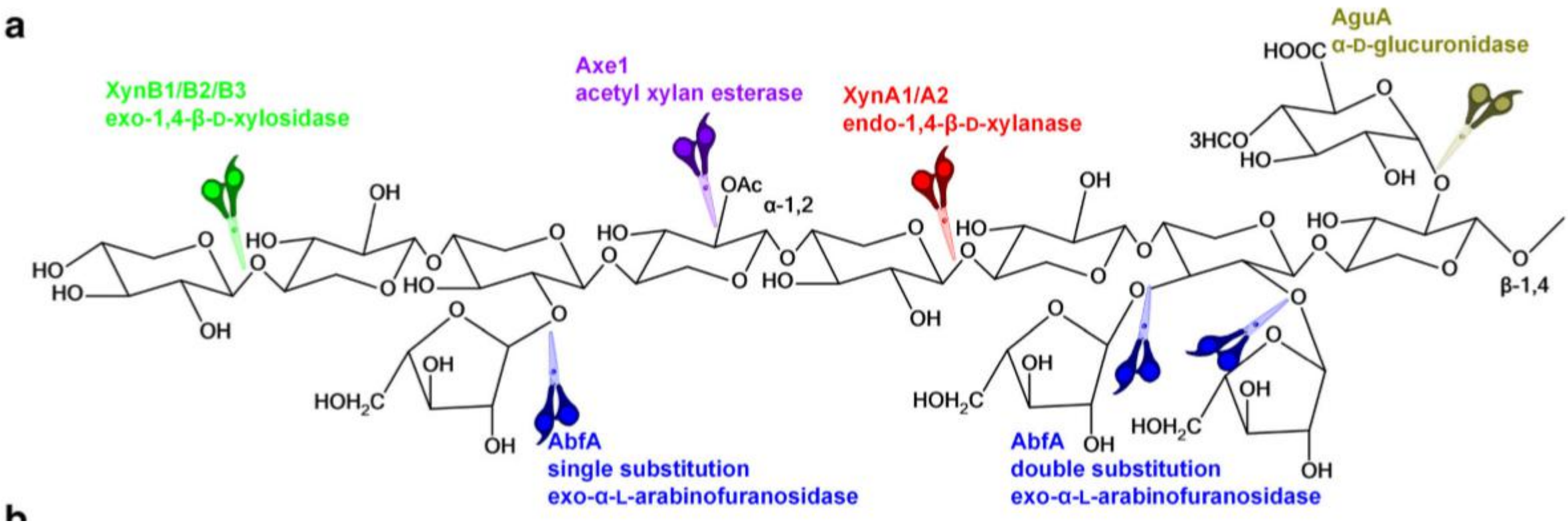
Introduction

Xylan
木聚糖

chemical method

enzyme hydrolysis approach

Introduction



木聚糖由被L-阿拉伯呋喃糖，甲基-D-葡萄糖醛酸和乙酰基侧链取代的β-1,4-连接的吡喃木糖组成。

Introduction

Xylanase and β-xylosidase have been reported in many microorganisms

Halorhabdus utahensis

Geobacillus stearothermophilus

Bacillus thermantarcticus

Thermomonospora fusca

Humicola insolens

mesophilic(嗜温)?

neutrophilic(中性)?

55-70℃

碱性pH

Introduction

Geobacillus thermodenitrificans NG80-2

来源：deep oil reservoir in Northern China

生长温度：45~73°C之间

可以有效地将木聚糖用作其唯一的碳源和能源

two xylanases：XynA1 (*GTNG_1761*) 、XynA2 (*GTNG_1774*)

three β-xylosidases：XynB1(*GTNG_1769*)、XynB2 (*GTNG_1775*) 、XynB3 (*GTNG_1758*)

one α-L-arabinofuranosidase gene：AbfA (*GTNG_1791*)

Materials and methods

Materials

primers

DNA purification kits

DNA Polymerase（聚合酶）

restriction endonucleases

T4 DNA ligase

Xylan from corn cob, oat spelt, birchwood, beechwood, and barley β-glucan, CMC-Na, xylose, pNP-Xyl, pNP-Gal, pNP-Glc, pNP-Araf, pNP, PMSF

Sugar beet arabinan, water-soluble wheat arabinoxylan, debranched arabinan, xylobiose, xylotriose, xylotetraose, and xylopentose were purchased from Megazyme

Materials and methods

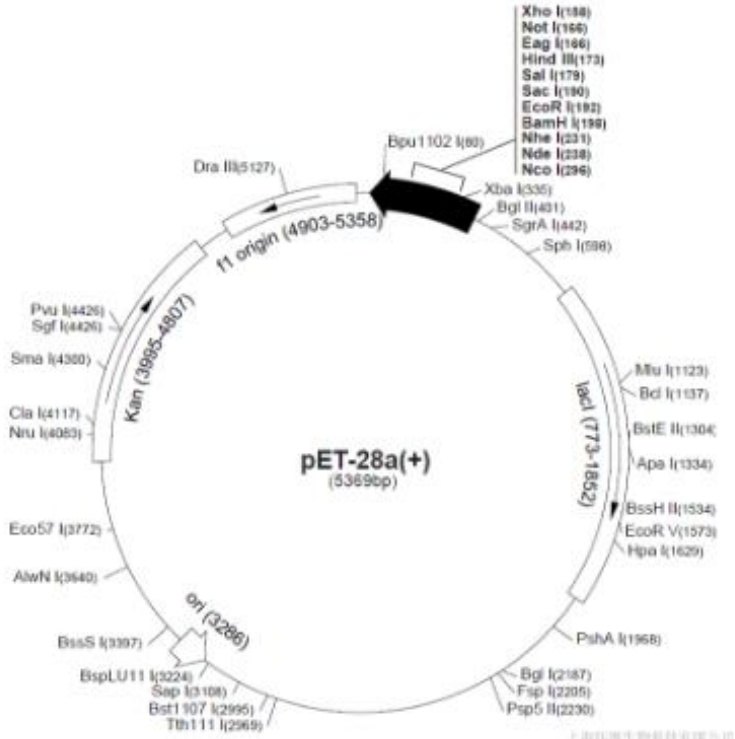
Bacterial strains, plasmids, and growth conditions

Bacteria: *G. thermodenitrificans* NG80-2 60℃

E.coli DH5α and BL21(DE3) 37℃

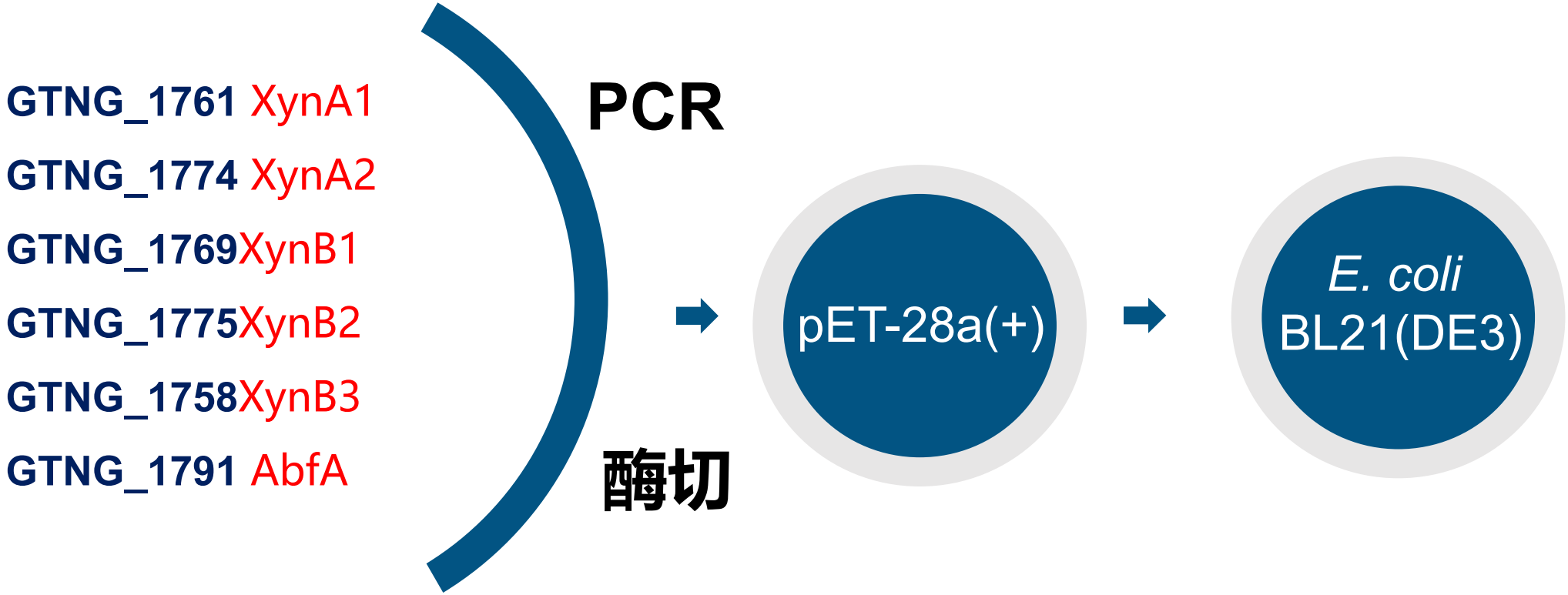
plasmids : pET-28a(+)

Kanamycin (50 mg/mL)



Materials and methods

Construction of recombinant plasmids



Materials and methods

Protein expression and purification

1、OD_{600} reached 0.6

0.1mM isopropylthio-β-D-galactopyranoside(半乳糖) for 4h at 37°C

2、10,000×g for 10 min at 4°C

washed with binding buffer (50 mM Tris–HCl, pH 8.0, 300 mM NaCl, 10 mM imidazole)

resuspended in the binding buffer (1 mM PMSF and 1 mg/mL of lysozyme)

3、ultrasonic processor（超声破碎）

10 mg/L RNase A and 5 mg/L DNase I and centrifuged at 18,000×g for 30min

4、Fast Flow column　　eluted

20 mM imidazole　　250 mM imidazole

5、SDS–PAGE and native–PAGE

Materials and methods

Enzyme activity assay DNS

100 μL total volume

for 10 min at 60 °C

150 μL of DNS solution

to a 100°C water bath for10 min

and then cooled to ambient temperature

UV-2550UV–Vis spectrophotometer

540nm

performed separately in triplicate

Materials and methods

Biochemical properties of the enzymes

Optimum temperature

Temperature range 25 to 100 °C at pH 7.0 for 20 min

Optimum pH

for 20 min at pH values from 3.0 to 12.0 in universal buffer at the optimal temperature

Thermostability

50 to 85°C

various metals

(5 mM Na+,K +, Cu2+, Ca2+, Co2+, Ni2+, Mn2+, Mg2+, Zn2+, Fe3+, or Al3+)

inhibitors

(5mM DTT, βME, EDTA)

surfactants (0.5% SDS orTween40) organic solvents (5% alkane or alcohol)

Materials and methods

Tolerance to xylose by β-xylosidase（β-木糖苷酶对木糖的耐受性）

The influence of various xylose concentrations on the β-xylosidase activities of XynB1, XynB2, and XynB3 was assessed

The substratep NP-Xyl, appropriate amounts of enzymes, and various concentrations of xylose (50–1000 mM) were mixed together and incubated at 50 °C for 10 min.

Materials and methods

Activity of xylanase, β-xylosidase, and α-L-arabinofuranosidase combinations

Synergetic activity of xylanase, β-xylosidase, and α-L-arabinofuranosidase

on various substrates,

including birchwood xylan, beechwood xylan, and oat spelt xylan, was tested as described previously

0.25U XynA1 or XynA2, 1.0 U XynB1 or XynB2 or XynB3, and/or 5.0 U AbfA)

and 900μL of 0.5%(w/v) substratein 100mM citrate– sodium citrate buffer (pH 6.0),

were incubated at 50 °C for 12 h

The reactions were terminated in a 100 °C water bath for10min and cooledto roomtemperature

Materials and methods

Analysis of sugar compositions by HPLC

at 10,000 rpm, 4 °C for 10 min

HPLC

Bioinformatics

利用生物信息学 对基因组进行对比分析
氨基酸分析
信号肽预测等

Results

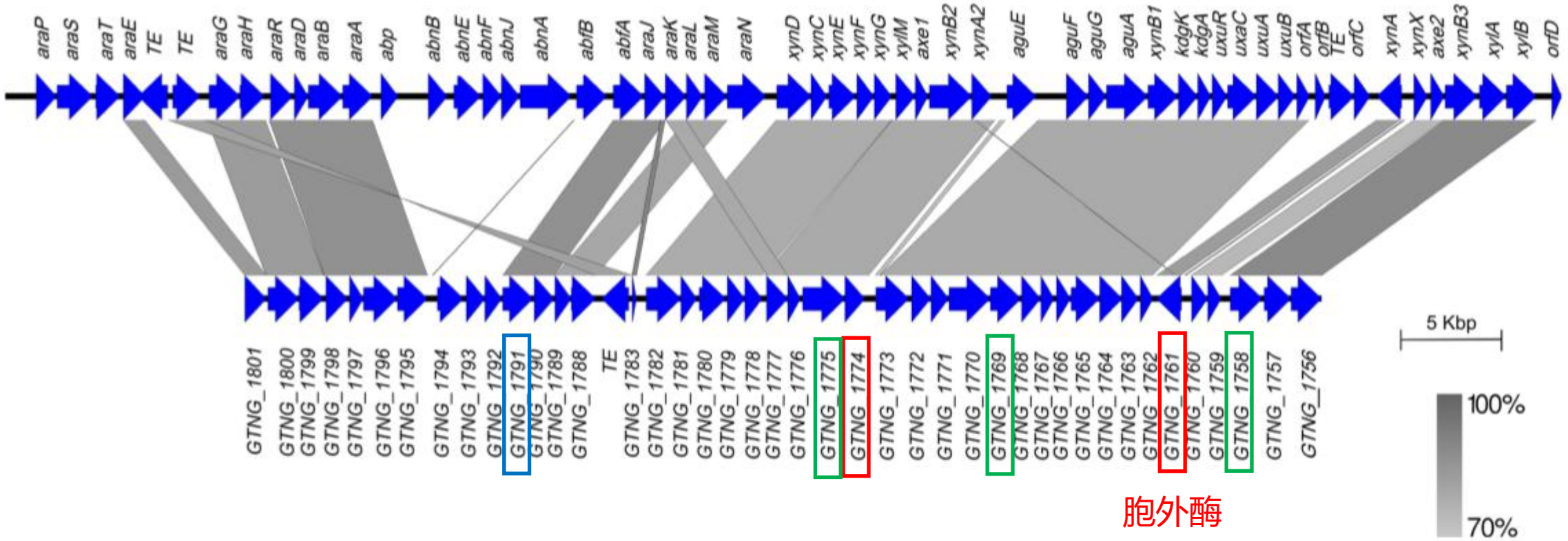


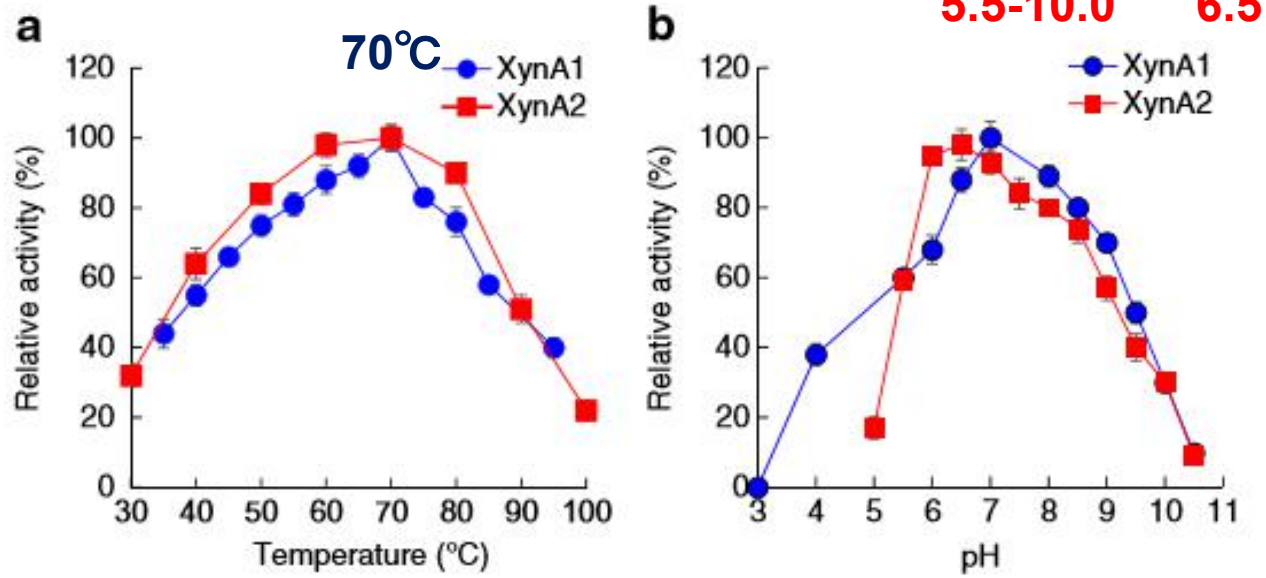
Fig. 2 Comparison of the genes involved in xylan hydrolysis in *G. thermodenitrificans* NG80-2 and *G. stearothermophilus* T-6. Comparisons were made using tBLASTx, and similarities with E values lower than 0.01 are plotted as *gray lines*, as indicated by the figure legend

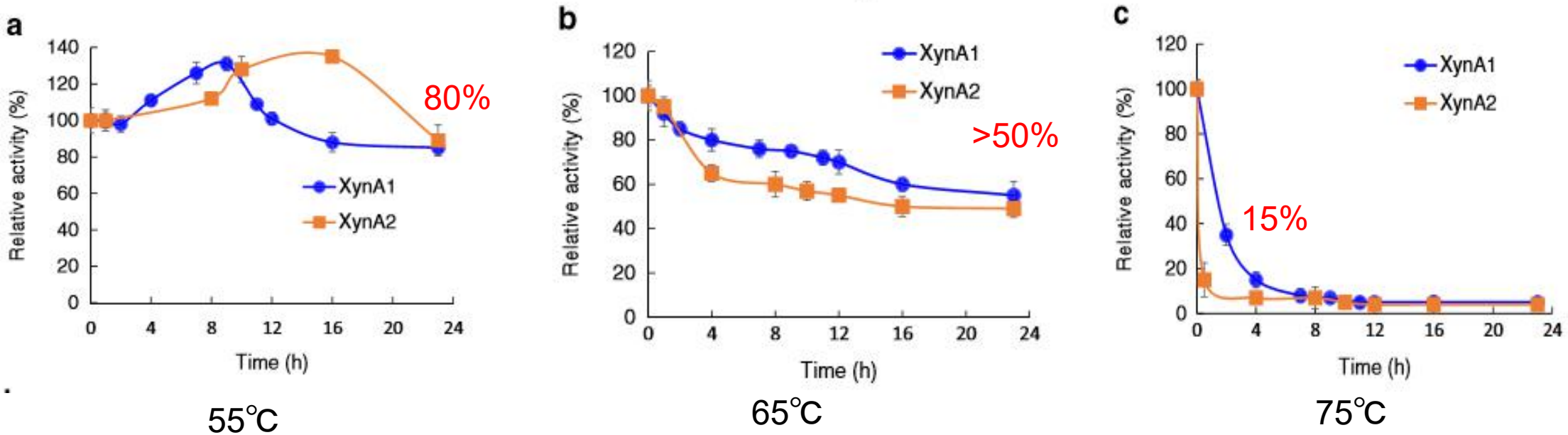
Results

XynA1	48kDa	GH10
XynA2	40kDa	
XynB1	62kDa	GH39
XynB2	83kDa	GH52
XynB3	64kDa	GH43
AbfA	59kDa	GH51

Results

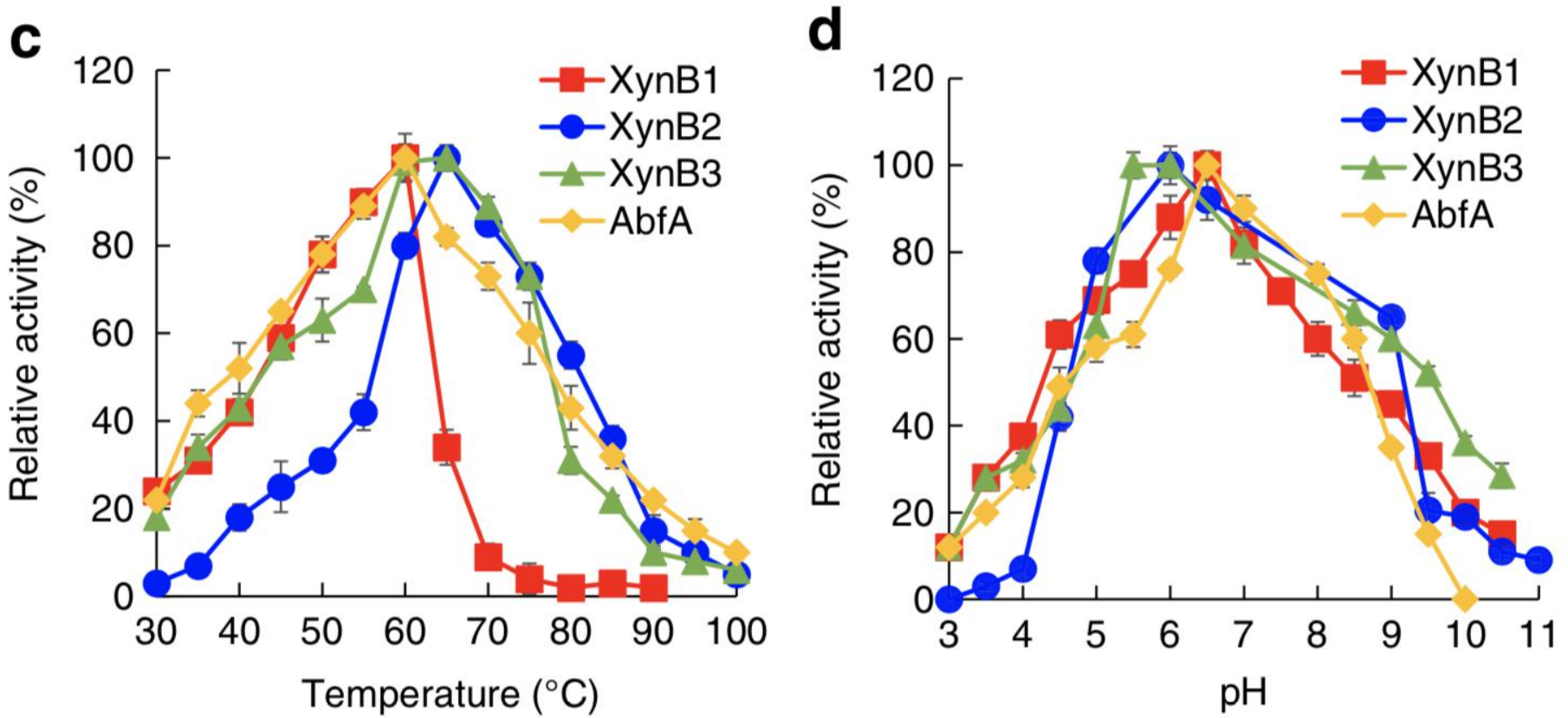
4.0-10.5 7.6
5.5-10.0 6.5





55℃ 65℃ 75℃

Results

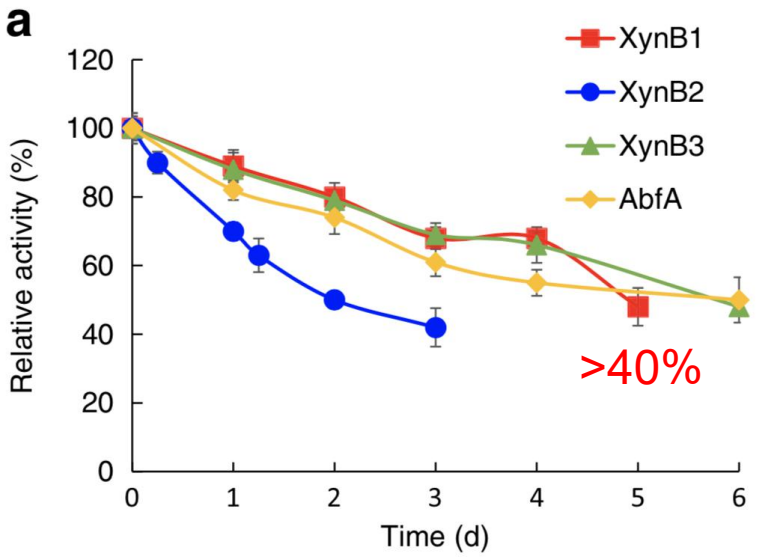


XynB1	60℃	
XynB2	65℃	
XynB3	65℃	
AbfA	60℃	

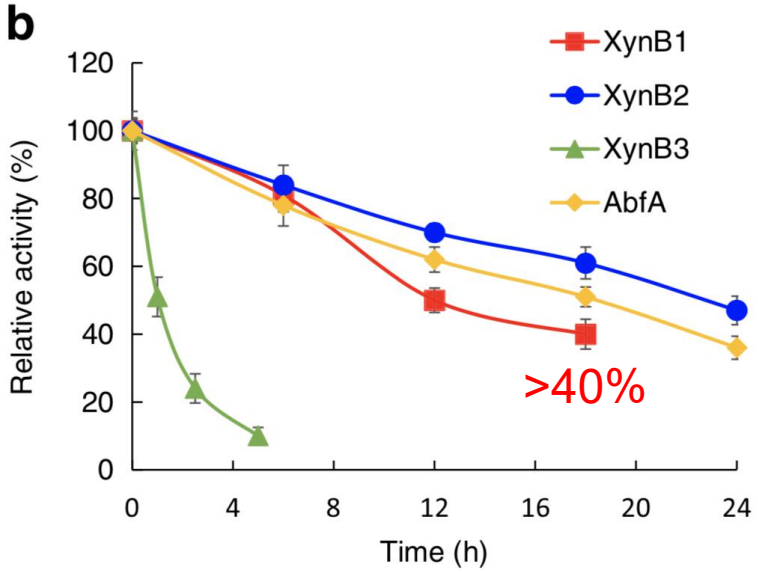
XynB2	4.5-10.4	6.0
XynB1	3.0-10.5	6.5
XynB3	3.0-10.5	5.5
AbfA	3.5-9.5	6.5

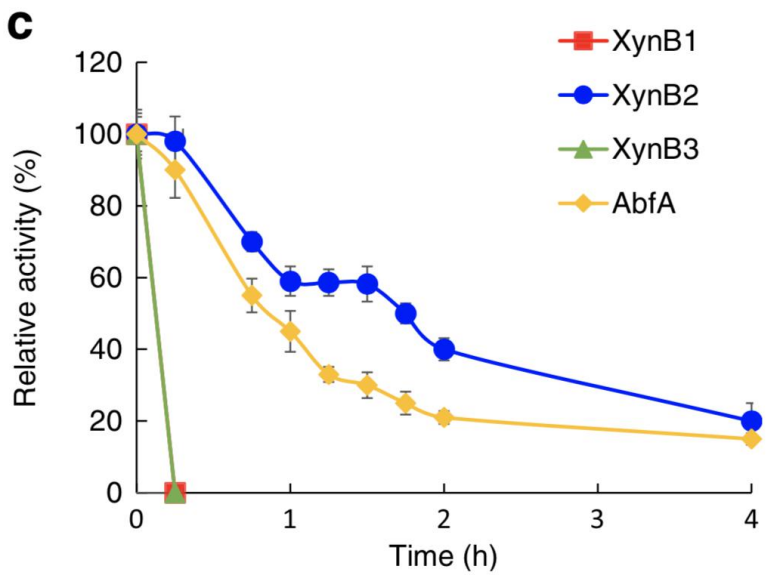
Results

75℃



80℃





85℃

15%

Fig. 5 Thermostability of *XynB1*, *XynB2*, *XynB3*, and *AbfA*. **a** The residual activities when *XynB1*, *XynB2*, *XynB3*, and *AbfA* were individually incubated at 75 °C. **b** The residual activities when *XynB1*, *XynB2*, *XynB3*, and *AbfA* were individually incubated at 80 °C. **c** The residual activities when XynB1, XynB2, XynB3, and AbfA were individually incubated at 85 °C

<10min 无活性损失

80°C 6h (XynB1) 8h (XynB2) 30min (XynB3) 80%

5h XynB3 10%

Results

Table 1 Effects of reagents on XynA1, XynA2, XynB1, XynB2, XynB3, and AbfA activities

Reagents	Relative activity (%)					
	XynA1	XynA2	XynB1	XynB2	XynB3	AbfA
None	100.0 ± 7.9	100.0 ± 3.1	100.0 ± 9.2	100.0 ± 5.6	100.0 ± 4.1	100.0 ± 6.8
Al^{3+} [5 mM]	92.3 ± 5.6	86.5 ± 4.1	98.9 ± 1.1	122.3 ± 10.2	106.6 ± 6.8	96.5 ± 1.6
Ca^{2+} [5 mM]	165.3 ± 2.0	116.6 ± 8.1	168.8 ± 4.4	125.4 ± 8.4	138.8 ± 3.3	112.5 ± 8.8
Co^{2+} [5 mM]	20.3 ± 3.5	0	40.8 ± 7.3	96.5 ± 4.8	50.8 ± 1.4	99.8 ± 2.1
Cu^{2+} [5 mM]	32.2 ± 5.5	0	88.6 ± 3.5	93.2 ± 3.5	69.2 ± 5.9	91.1 ± 3.6
Fe^{3+} [5 mM]	25.8 ± 6.8	20.1 ± 4.5	89.9 ± 2.1	146.4 ± 6.5	98.9 ± 3.3	92.3 ± 5.0
K^{+} [5 mM]	145.4 ± 10.1	108.4 ± 3.4	137.7 ± 3.8	102.5 ± 12.9	99.6 ± 12.1	98.8 ± 6.5
Mg^{2+} [5 mM]	102.2 ± 3.5	106.5 ± 10.6	87.6 ± 6.7	114.7 ± 2.3	86.4 ± 6.8	105.1 ± 3.9
Mn^{2+} [5 mM]	42.5 ± 1.8	16.9 ± 10.6	92.5 ± 3.8	144.2 ± 6.5	84.9 ± 3.1	106.6 ± 8.8
Na^{+} [5 mM]	126.6 ± 0.1	105.5 ± 1.8	109.9 ± 1.3	97.9 ± 5.7	96.4 ± 1.6	114.4 ± 10.5
Ni^{2+} [5 mM]	0	101.8 ± 5.1	17.9 ± 0.1	87.6 ± 7.7	40.8 ± 0.5	88.4 ± 1.1
Zn^{2+} [5 mM]	140.7 ± 7.8	0	14.8 ± 0.1	65.6 ± 5.3	38.2 ± 0.8	70.3 ± 1.5
DTT [5 mM]	65.5 ± 2.9	85.9 ± 0.6	105.5 ± 1.8	95.6 ± 4.7	92.3 ± 5.8	80.5 ± 2.2
βME [5 mM]	90.3 ± 0.5	73.3 ± 3.3	105.9 ± 5.1	98.8 ± 2.2	92.8 ± 1.1	87.7 ± 2.6
EDTA [5 mM]	103.3 ± 3.5	98.5 ± 1.5	95.6 ± 6.8	104.5 ± 3.7	95.6 ± 8.9	93.3 ± 3.0
SDS [0.5% (*w*/*v*)]	0	0	63.6 ± 3.8	77.8 ± 12.6	0	70.3 ± 5.8
Tween 40 [0.5% (*v*/*v*)]	0	0	50.6 ± 5.5	87.6 ± 7.7	0	87.9 ± 11.2
Pentane [5% (*v*/*v*)]	98.5 ± 2.2	85.9 ± 1.7	100.5 ± 3.3	93.6 ± 2.5	97.8 ± 8.5	90.6 ± 7.4
Decane [5% (*v*/*v*)]	82.6 ± 3.9	77.3 ± 6.5	94.3 ± 5.5	90.5 ± 5.0	88.9 ± 4.5	80.9 ± 6.6
Pentadecane [5% (*v*/*v*)]	90.3 ± 0.8	88.0 ± 1.2	96.8 ± 3.6	98.8 ± 3.4	90.9 ± 6.5	92.5 ± 6.9
Acetone [5% (*v*/*v*)]	90.6 ± 6.6	94.4 ± 2.9	85.9 ± 1.7	81.5 ± 5.6	90.9 ± 3.3	97.8 ± 4.6
Methanol [5% (*v*/*v*)]	92.2 ± 1.5	80.4 ± 2.7	89.5 ± 0.9	92.9 ± 6.5	93.6 ± 5.1	85.5 ± 7.7
Ethanol [5% (*v*/*v*)]	82.5 ± 8.8	80.3 ± 1.7	85.5 ± 2.5	78.9 ± 3.6	81.0 ± 5.5	86.6 ± 4.2
n-Propanol [5% (*v*/*v*)]	85.5 ± 1.8	70.9 ± 2.9	75.5 ± 3.8	83.6 ± 12.5	85.9 ± 8.4	80.6 ± 5.1
n-Butanol [5% (*v*/*v*)]	72.7 ± 5.9	80.5 ± 5.7	77.7 ± 7.1	81.3 ± 5.5	77.5 ± 4.9	79.1 ± 7.0

Results

Table 1 Effects of reagents on XynA1, XynA2, XynB1, XynB2, XynB3, and AbfA activities

Reagents	Relative activity (%)					
	XynA1	XynA2	XynB1	XynB2	XynB3	AbfA
None	100.0 ± 7.9	100.0 ± 3.1	100.0 ± 9.2	100.0 ± 5.6	100.0 ± 4.1	100.0 ± 6.8
Al^{3+} [5 mM]	92.3 ± 5.6	86.5 ± 4.1	98.9 ± 1.1	122.3 ± 10.2	106.6 ± 6.8	96.5 ± 1.6
Ca^{2+} [5 mM]	165.3 ± 2.0	116.6 ± 8.1	168.8 ± 4.4	125.4 ± 8.4	138.8 ± 3.3	112.5 ± 8.8
Co^{2+} [5 mM]	20.3 ± 3.5	0	40.8 ± 7.3	96.5 ± 4.8	50.8 ± 1.4	99.8 ± 2.1
Cu^{2+} [5 mM]	32.2 ± 5.5	0	88.6 ± 3.5	93.2 ± 3.5	69.2 ± 5.9	91.1 ± 3.6
Fe^{3+} [5 mM]	25.8 ± 6.8	20.1 ± 4.5	89.9 ± 2.1	146.4 ± 6.5	98.9 ± 3.3	92.3 ± 5.0
K^{+} [5 mM]	145.4 ± 10.1	108.4 ± 3.4	137.7 ± 3.8	102.5 ± 12.9	99.6 ± 12.1	98.8 ± 6.5
Mg^{2+} [5 mM]	102.2 ± 3.5	106.5 ± 10.6	87.6 ± 6.7	114.7 ± 2.3	86.4 ± 6.8	105.1 ± 3.9
Mn^{2+} [5 mM]	42.5 ± 1.8	16.9 ± 10.6	92.5 ± 3.8	144.2 ± 6.5	84.9 ± 3.1	106.6 ± 8.8
Na^{+} [5 mM]	126.6 ± 0.1	105.5 ± 1.8	109.9 ± 1.3	97.9 ± 5.7	96.4 ± 1.6	114.4 ± 10.5
Ni^{2+} [5 mM]	0	101.8 ± 5.1	17.9 ± 0.1	87.6 ± 7.7	40.8 ± 0.5	88.4 ± 1.1
Zn^{2+} [5 mM]	140.7 ± 7.8	0	14.8 ± 0.1	65.6 ± 5.3	38.2 ± 0.8	70.3 ± 1.5
DTT [5 mM]	65.5 ± 2.9	85.9 ± 0.6	105.5 ± 1.8	95.6 ± 4.7	92.3 ± 5.8	80.5 ± 2.2
βME [5 mM]	90.3 ± 0.5	73.3 ± 3.3	105.9 ± 5.1	98.8 ± 2.2	92.8 ± 1.1	87.7 ± 2.6
EDTA [5 mM]	103.3 ± 3.5	98.5 ± 1.5	95.6 ± 6.8	104.5 ± 3.7	95.6 ± 8.9	93.3 ± 3.0
SDS [0.5% (*w*/*v*)]	0	0	63.6 ± 3.8	77.8 ± 12.6	0	70.3 ± 5.8
Tween 40 [0.5% (*v*/*v*)]	0	0	50.6 ± 5.5	87.6 ± 7.7	0	87.9 ± 11.2
Pentane [5% (*v*/*v*)]	98.5 ± 2.2	85.9 ± 1.7	100.5 ± 3.3	93.6 ± 2.5	97.8 ± 8.5	90.6 ± 7.4
Decane [5% (*v*/*v*)]	82.6 ± 3.9	77.3 ± 6.5	94.3 ± 5.5	90.5 ± 5.0	88.9 ± 4.5	80.9 ± 6.6
Pentadecane [5% (*v*/*v*)]	90.3 ± 0.8	88.0 ± 1.2	96.8 ± 3.6	98.8 ± 3.4	90.9 ± 6.5	92.5 ± 6.9
Acetone [5% (*v*/*v*)]	90.6 ± 6.6	94.4 ± 2.9	85.9 ± 1.7	81.5 ± 5.6	90.9 ± 3.3	97.8 ± 4.6
Methanol [5% (*v*/*v*)]	92.2 ± 1.5	80.4 ± 2.7	89.5 ± 0.9	92.9 ± 6.5	93.6 ± 5.1	85.5 ± 7.7
Ethanol [5% (*v*/*v*)]	82.5 ± 8.8	80.3 ± 1.7	85.5 ± 2.5	78.9 ± 3.6	81.0 ± 5.5	86.6 ± 4.2
n-Propanol [5% (*v*/*v*)]	85.5 ± 1.8	70.9 ± 2.9	75.5 ± 3.8	83.6 ± 12.5	85.9 ± 8.4	80.6 ± 5.1
n-Butanol [5% (*v*/*v*)]	72.7 ± 5.9	80.5 ± 5.7	77.7 ± 7.1	81.3 ± 5.5	77.5 ± 4.9	79.1 ± 7.0

Results

XynA1　木三糖　木四糖
XynA2　木二糖　木三糖

Table 2 Substrate specificities of XynA1, XynA2, XynB1, XynB2, XynB3, and AbfA

Substrates[a]	Relative activity (%)					
	XynA1	XynA2	XynB1	XynB2	XynB3	AbfA
Beechwood xylan	100.0 ± 2.5	100.0 ± 5.1	5.5 ± 1.3	8.4 ± 1.1	6.2 ± 0.5	–
Birchwood xylan	78.3 ± 4.4	66.5 ± 2.6	4.2 ± 0.1	6.9 ± 1.6	5.5 ± 0.8	–
Oat spelt xylan	65.3 ± 5.8	70.3 ± 11.2	3.6 ± 0.8	4.8 ± 0.9	1.5 ± 0.5	4.5 ± 1.2
Corn cob xylan	42.8 ± 4.4	50.2 ± 5.5	1.1 ± 0.5	0.8 ± 0.2	1.5 ± 0.4	1.2 ± 0.5
Water-soluble wheat arabinoxylan	54.9 ± 2.6	50.8 ± 4.5	3.1 ± 0.6	3.1 ± 0.6	3.1 ± 0.6	6.5 ± 2.2
Sugar beet arabinan	10.8 ± 6.9	12.5 ± 4.4	5.6 ± 1.0	5.6 ± 1.0	5.6 ± 1.0	7.8 ± 0.9
Debranched arabinan	6.9 ± 5.1	8.3 ± 3.9	–	–	–	–
*p*NP-Xyl	–	–	100.0 ± 2.6	100.0 ± 6.2	100.0 ± 4.4	2.5 ± 1.0
*p*NP-Araf	–	–	26.6 ± 3.2	–	4.1 ± 0.5	100.0 ± 3.9
*p*NP-Gal	–	–	–	–	–	–
*p*NP-Glc	–	–	–	12.6 ± 2.5	–	–
*p*NP-Arap	–	–	–	–	–	–

– not detected

[a] Substrate specificities were examined under standard assay conditions with 1% (*w*/*v*) beechwood xylan, birchwood xylan, oat spelt xylan, corn cob xylan, water-soluble wheat arabinoxylan, sugar beet arabinan, debranched arabinan, and 1 mM of *p*NP-Xyl, *p*NP-Araf, *p*NP-Gal, *p*NP-Glc, and *p*NP-Arap

Results

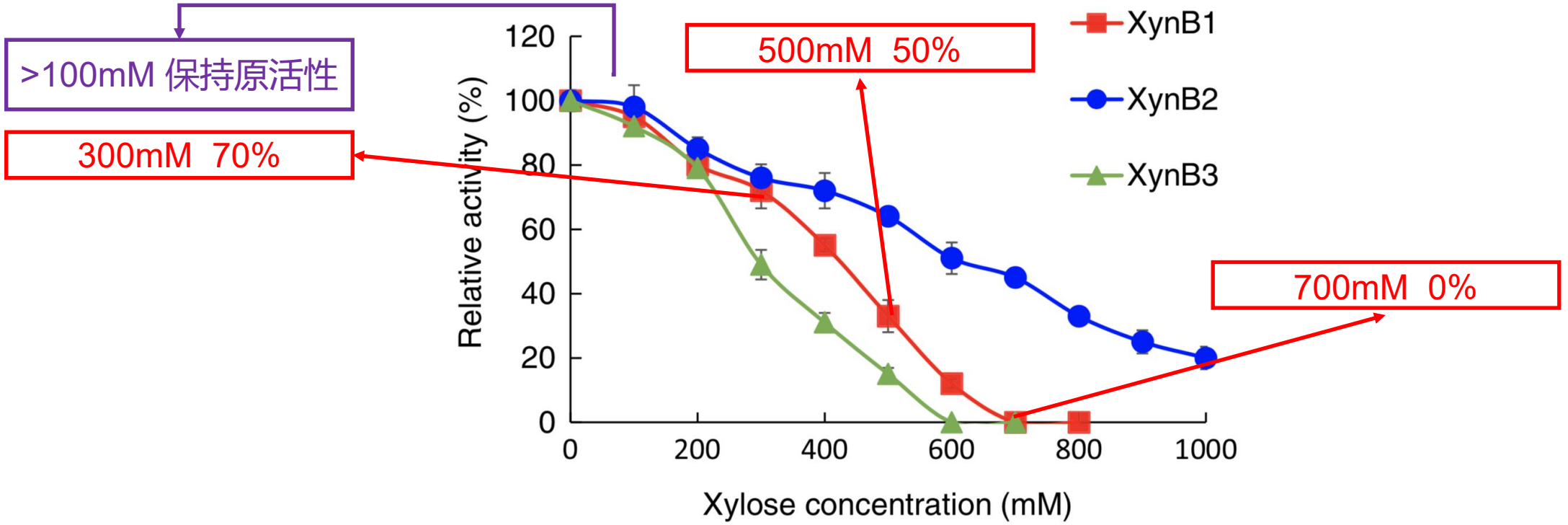


Fig. 6 Effect of xylose on *XynB1*, *XynB2*, and *XynB3* activities. Residual activities were determined when *XynB1*, *XynB2*, and *XynB3* were individually incubated in the presence of different concentrations of xylose

Results

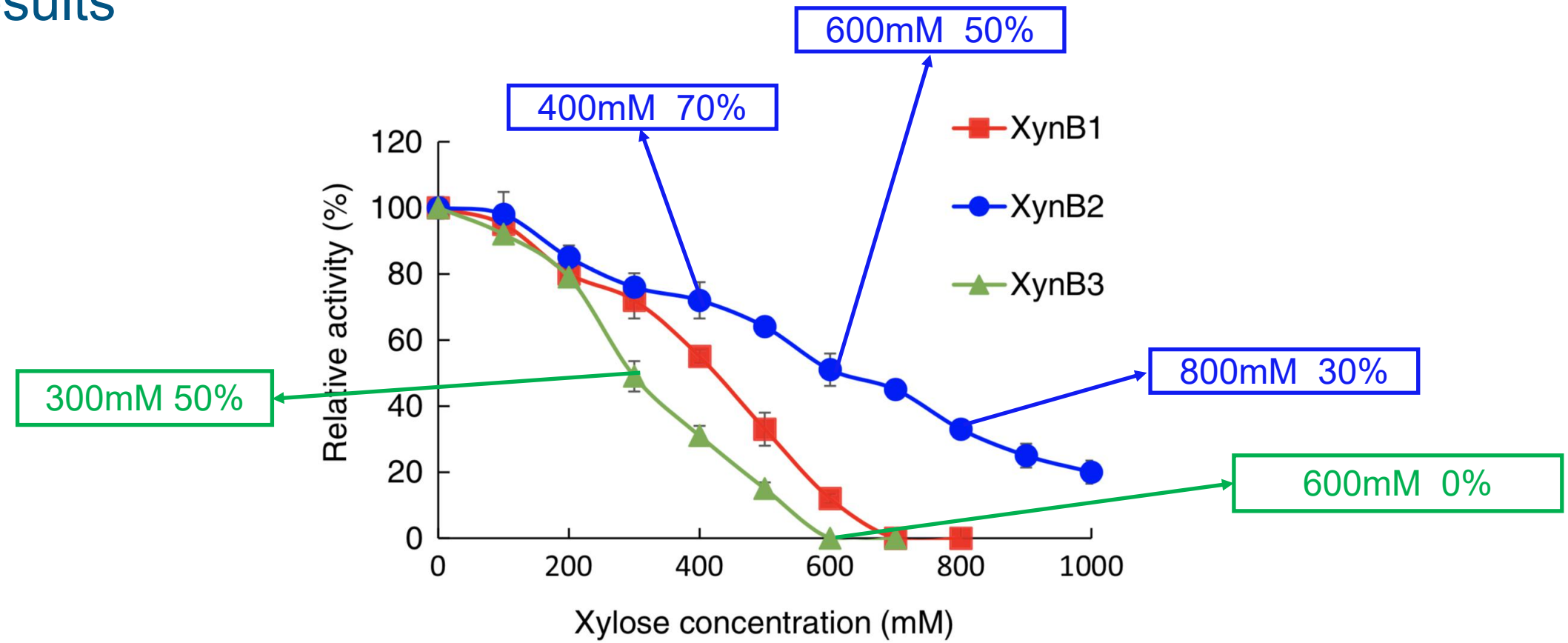


Fig. 6 Effect of xylose on *XynB1*, *XynB2*, and *XynB3* activities. Residual activities were determined when *XynB1*, *XynB2*, and *XynB3* were individually incubated in the presence of different concentrations of xylose

Results

Table 3 Synergistic action of xylanases, β-xylosidases, and α-L-arabinofuranosidase on reducing sugar yields

Enzyme preparations	Oat spelt xylan		Birchwood xylan		Beechwood xylan	
	Reducing sugar yields (mM)	Degree of synergy[a]	Reducing sugar yields (mM)	Degree of synergy	Reducing sugar yields (mM)	Degree of synergy
XynA1	3.359 ± 0.108		6.885 ± 0.133		12.544 ± 0.121	
XynA2	5.514 ± 0.075		5.033 ± 0.093		8.841 ± 0.055	
XynB1	0.036 ± 0.008		0.041 ± 0.011		0.059 ± 0.003	
XynB2	0.065 ± 0.011		0.078 ± 0.007		0.088 ± 0.008	
XynB3	0.022 ± 0.005		0.049 ± 0.008		0.062 ± 0.012	
AbfA	0.068 ± 0.013		–		–	
XynA1 + XynA2	9.368 ± 0.123	1.06	12.968 ± 0.083	1.09*	22.658 ± 0.155	1.06
XynA1 + XynB1	3.674 ± 0.052	1.08*	6.971 ± 0.103	1.01	13.451 ± 0.191	1.06*
XynA1 + XynB2	4.543 ± 0.108	1.33*	10.095 ± 0.029	1.45*	16.039 ± 0.023	1.27*
XynA1 + XynB3	4.024 ± 0.034	1.19*	8.044 ± 0.015	1.16*	14.092 ± 0.016	1.12*
XynA1 + AbfA	4.461 ± 0.009	1.30*	9.561 ± 0.031	1.39*	15.602 ± 0.105	1.24*
XynA2 + XynB1	10.561 ± 0.015	1.90*	6.638 ± 0.073	1.31*	10.015 ± 0.016	1.13*
XynA2 + XynB2	11.104 ± 0.054	1.99*	9.672 ± 0.057	1.89*	9.942 ± 0.020	1.11*
XynA2 + XynB3	10.069 ± 0.024	1.82*	10.066 ± 0.010	1.98*	10.106 ± 0.131	1.14*
XynA2 + AbfA	8.833 ± 0.035	1.58*	7.985 ± 0.031	1.59*	12.311 ± 0.014	1.39*
XynA1 + XynB1 + AbfA	6.589 ± 0.023	1.90*	9.223 ± 0.061	1.33*	18.156 ± 0.033	1.44*
XynA1 + XynB2 + AbfA	7.331 ± 0.055	2.10*	11.316 ± 0.092	1.63*	16.882 ± 0.051	1.34*
XynA1 + XynB3 + AbfA	6.665 ± 0.059	1.93*	9.881 ± 0.012	1.43*	16.536 ± 0.033	1.31*
XynA2 + XynB1 + AbfA	13.991 ± 0.031	2.49*	8.855 ± 0.066	1.75*	14.451 ± 0.011	1.62*
XynA2 + XynB2 + AbfA	12.578 ± 0.022	2.23*	11.744 ± 0.034	2.30*	11.514 ± 0.038	1.29*
XynA2 + XynB3 + AbfA	11.855 ± 0.044	2.12*	12.221 ± 0.019	2.40*	13.150 ± 0.024	1.48*
XynA1 + XynB1 + XynB2 + AbfA	7.085 ± 0.014	2.01*	11.253 ± 0.105	1.61*	19.655 ± 0.083	1.55*
XynA1 + XynB1 + XynB2 + XynB3 + AbfA	6.989 ± 0.029	1.97*	10.386 ± 0.064	1.47*	18.059 ± 0.033	1.42*
XynA2 + XynB1 + XynB2 + AbfA	10.016 ± 0.045	1.76*	9.008 ± 0.038	1.75*	9.896 ± 0.151	1.10*
XynA2 + XynB1 + XynB2 + XynB3 + AbfA	9.124 ± 0.014	1.60*	8.325 ± 0.055	1.60*	9.988 ± 0.104	1.10*

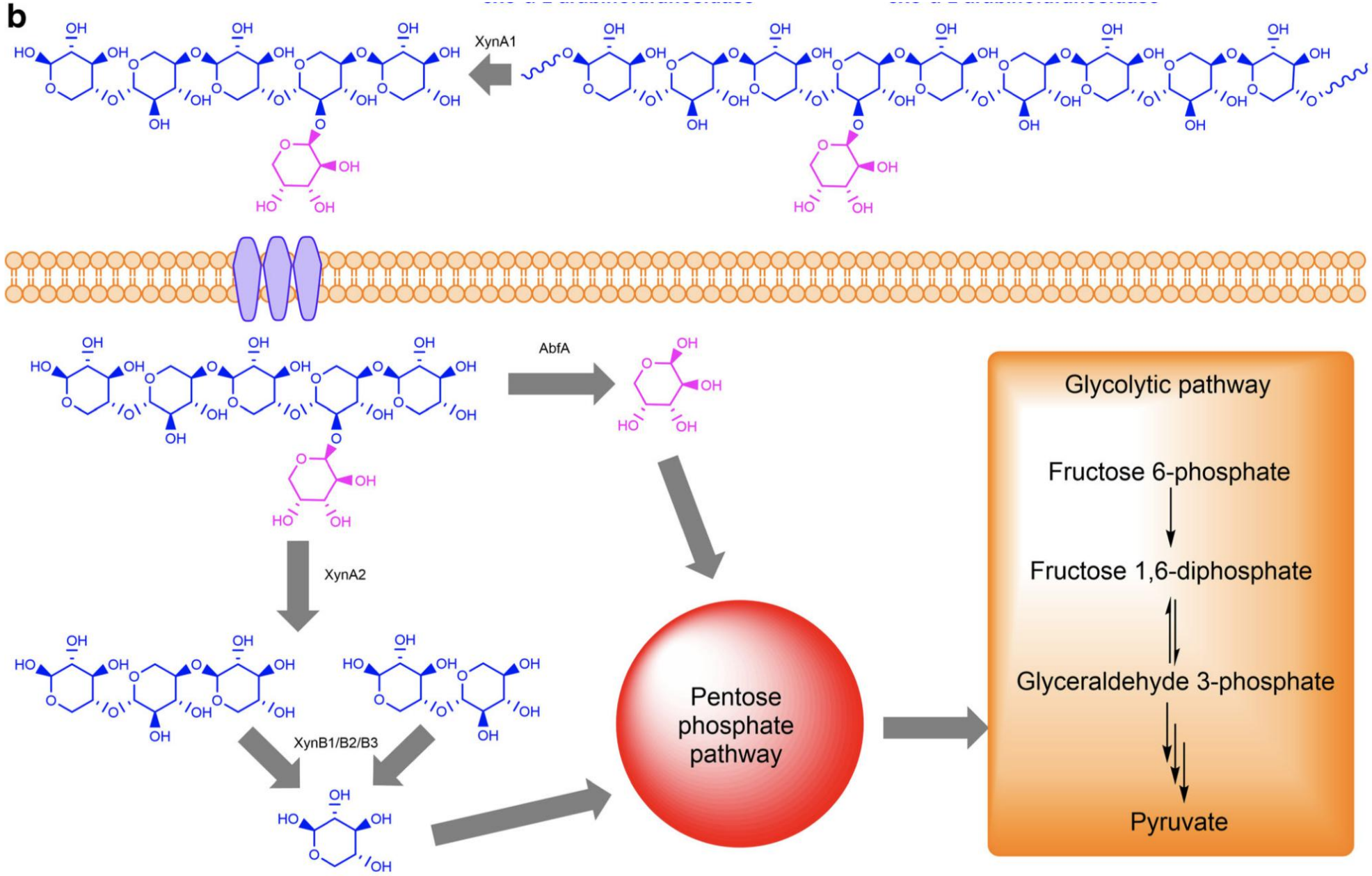
Samples of xylan were incubated in the presence of either single or a combination of endo-xylanase (0.25 U XynA1 or XynA2), β-xylosidase (1.0 U XynB1, XynB2, or XynB3), and α-L-arabinofuranosidase (1.0 U AbfA)

– not detected

*The enzymatic reaction is significantly synergistic at $P < 0.05$ (Tukey's test by OriginPro 8)

[a] The degree of synergy is defined as the ratio of xylose equivalents from enzyme reactions to the sum of the xylose equivalents released by the individual enzymes

Discussion


b
XynA1
AbfA
XynA2
XynB1/B2/B3
Pentose phosphate pathway
Glycolytic pathway
Fructose 6-phosphate
Fructose 1,6-diphosphate
Glyceraldehyde 3-phosphate
Pyruvate

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