Result:

1. Proved that empty vector transformed E. *coli* osmotic shock supernatant is not able to degrade xylan at 65℃
2. Proved that osmotic shock supernatant of E.*coli* transformed by either pET22b(+)_x2 or pET22b(+)_ArfB can degrade xylan from beech wood.
3. Proved that osmotic shock supernatant of E.*coli* transformed by pET22b(+)_ManA1 can degrade mannan from *S. cerevisiae*.
4. Proved that XynB is active at a wide range of temperature up to 94.7℃

Procedure:

1. Prepare DNS reagent

DNS reagent recipe is from a published paper(Effects of Different DNS Reagents in Determination of Xylose Content, Wang et al.), and is shown as below.

Weight (g/L)	Component
10.00	3,5-Dinitrosalicylic acid
16.00	NaOH
5.00	Phenol
5.00	Sodium sulfite
300.00	Potassium sodium tartrate

Store at room temperature in brown bottle for a week before use.

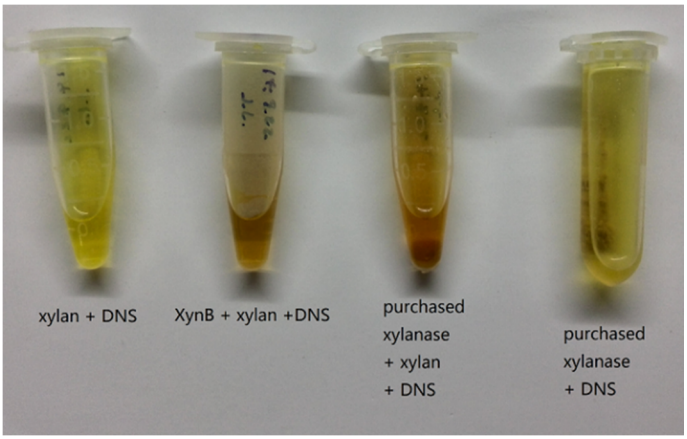
2. To testify enzyme activity, we employed DNS method to measure reductive saccharide released from substrate, and indicate enzyme activity. We found that spectra of DNS reagent is somehow miscellaneous, but optic density improves at 540nm when heated with reductive saccharide.

3. We testified that our substrate, xylan from beech wood (product of MERYER), does not contain reductive saccharide (Figure 1A), and the osmotic shock supernatants do not contain enough reductive saccharide for DNS reagent to detect, compared to untreated samples, osmotic shock supernatant treated samples do not show prominent increase at OD 540 (Figure 1B, C).

Culture BL21(DE3) transformed with empty pET22b+ vector and apply osmotic shock procedure, test enzyme activity for both supernatant and precipitate(Figure 1B, C)

+10 μl	Osmotic shock supernatant(S)	Osmotic shock precipitate(P)
+10 μl	Xylan(0.5 g/mL)	
For each tube, extract 5 μl , add 5 μl DNS, incubate at 99℃ for 10min as sample before enzyme treatment(S0 and P0), the rest 15 μl Incubate at 65℃ for 6h		
+15 μl	DNS reagent	
incubate 99℃ for 10min, sample named as S6 and P6		
assay by Nanodrop		

A



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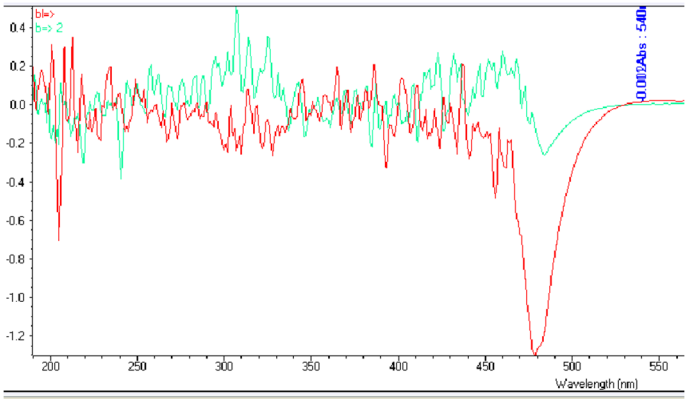

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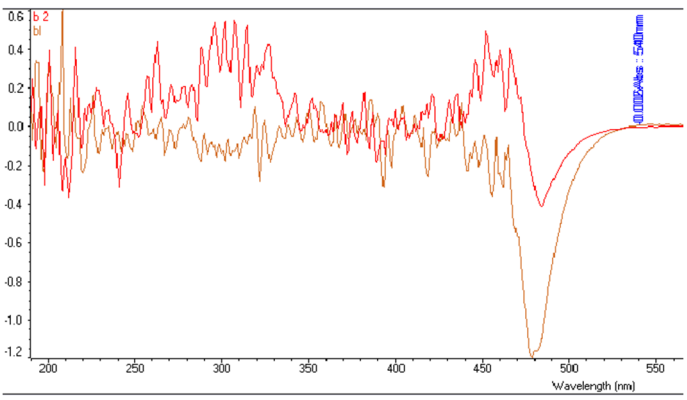


Figure 1, (A) During examination of purchased xylanase, we testified that our substrate (0.05 g/mL) does not contain enough reductive saccharide for DNS method to detect. (B) Green line is the spectra of P6, red line is the spectra of P0. (C) Brown line is the spectra of P0, red line is the spectra of P6.

Test XynB working temperature, find that XynB shows stable performance under a wide range of temperature conditions, even up to 94.7℃

Recipe(μL):

Temperature(℃)		74.5	74.9	76.2	78.1	80.4	83.0	85.8	90.8	94.2	94.7
XynB from C.st (Group A)	0.1mg/mL xylan	10	10	10	10	10	10	10	10	10	10
	XynB from C.st	10	10	10	10	10	10	10	10	10	10
Xylase purchased (Group B)	0.1mg/mL xylan	10	10	10	10	10	10	10	10	10	10
	Xylase purchased(over dose)	10	10	10	10	10	10	10	10	10	10

Incubate in metal bath for 4.5h

Add 10μl DNS reagent to each tube, incubate at 99℃ for 10min

Use 99℃ incubated reaction system containing following components as blank, measure OD 540 of each sample

10μL 0.1mg/mL xylan, 10μL H_2O, 10μL DNS reagent

Nanodrop data, wavelength = 540 nm

Temperature(℃)	OD 540	
	Group A	Group B
74.5	0.119	0.007
74.9	0.135	-0.002
76.2	0.128	-0.065
78.1	0.127	-0.106
80.4	0.156	-0.006
83.0	0.119	-0.002
85.8	0.112	-0.096
90.8	0.145	0.004
94.2	0.151	-0.079
94.7	0.172	0.01

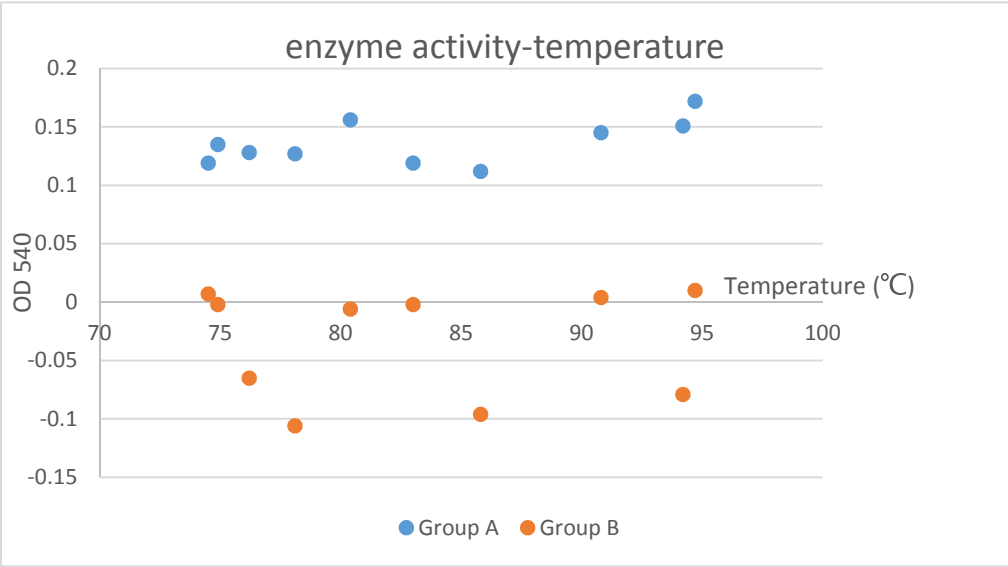


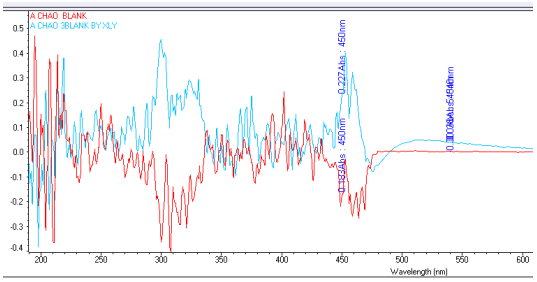
Figure 2, Enzyme shows stable performance under a wide range of temperature conditions

Verify enzyme activity of ArfB and ManA1, compared to untreated samples, OD 540 show increase after protein extraction product treatment.(Figure 3A, B)

ArfB and ManA1 are easy to form inclusion body during expression, so we tested different protein extraction methods, and used ultrasonic sound cell lysis (200W, 1h) for ArfB (product named as A CHAO)and mechanic pressure cell lysis for ManA1 (product named as M LS)to obtain best yield(Data not shown).

Recipe:

+10 μl	A CHAO	H2O	M LS	H2O
+10 μl	Xylan		Mannan	
For each tube, extract 5 μl , add 5 μl DNS, incubate at 99℃ for 10min as sample before enzyme treatment(A CHAO BLANK and M LS BLANK), the rest 15 μl Incubate at 65℃ for 6h				
+15 μL	DNS			
incubate 99'C for 10min				
assay by Nanodrop, use ① as blank for A CHAO and ② as blank for M LS				

A 
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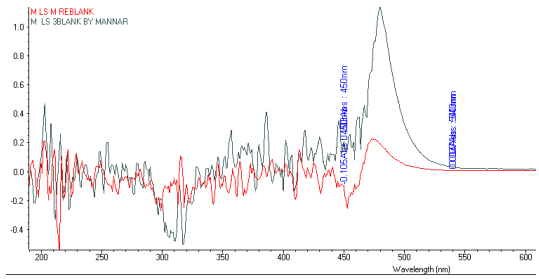

Figure 3, (A) Cyan line is the spectra of A CHAO, red line is the spectra of A CHAO BLANK.(B) Grey line is the spectra of M LS, red line is the spectra of M LS BLANK.