Table S1 Sequence of NAD^+-dependent xylose dehydrogenase gene *xylB*

Name	Sequence
Native *xylB*	ATGTCCTCAGCCATCTATCCCAGCCTGAAGGGCAAGCGCGTCGTCATCACCGGC GGCGGCTCGGGCATCGGGGCCGGCCTCACCGCCGGCTTCGCCCGTCAGGGCGC GGAGGTGATCTTCCTCGACATCGCCGACGAGGACTCCAGGGCTCTTGAGGCCG AGCTGGCCGGCTCGCCGATCCCGCCGGTCTACAAGCGCTGCGACCTGATGAAC CTCGAGGCGATCAAGGCGGTCTTCGCCGAGATCGGCGACGTCGACGTGCTGGT CAACAACGCCGGCAATGACGACCGCCACAAGCTGGCCGACGTGACCGGCGCC TATTGGGACGAGCGGATCAACGTCAACCTGCGCCACATGCTGTTCTGCACCCA GGCCGTCGCGCCGGGCATGAAGAAGCGTGGCGGCGGGGCGGTGATCAACTTC GGTTCGATCAGCTGGCACCTGGGGCTTGAGGACCTCGTCCTCTACGAAACCGC CAAGGCCGGCATCGAAGGCATGACCCGCGCGCTGGCCCGGGAGCTGGGTCCC GACGACATCCGCGTCACCTGCGTGGTGCCGGGCAACGTCAAGACCAAGCGCC AGGAGAAGTGGTACACGCCCGAAGGCGAGGCCCAGATCGTGGCGGCCCAATG CCTGAAGGGCCGCATCGTCCCGGAGAACGTCGCCGCGCTGGTGCTGTTCCTGG CCTCGGATGACGCGTCGCTCTGCACCGGCCACGAATACTGGATCGACGCCGGC TGGCGTTGA
Codon optimized *xylB*	ATGTCCTCCGCAATCTACCCATCCTTGAAGGGTAAGCGTGTCGTTATCACTGGA GGTGGTTCTGGTATCGGTGCTGGTTTGACAGCTGGTTTCGCTCGTCAAGGTGCT GAGGTCATCTTCTTGGACATTGCTGACGAGGATTCCCGTGCTTTGGAGGCTGAA TTAGCTGGTTCCCCTATCCCACCAGTCTACAAGCGTTGCGACTTGATGAACTTG GAGGCAATCAAGGCAGTCTTCGCAGAGATCGGAGATGTCGACGTCTTGGTCAA CAACGCTGGTAACGACGACAGACACAAGTTGGCTGACGTCACTGGTGCTTACT GGGACGAGCGTATTAACGTTAACTTGAGACACATGTTGTTCTGCACTCAAGCA GTTGCACCCGGTATGAAGAAGAGAGGTGGTGGTGCTGTCATCAACTTCGGTTC CATCTCTTGGCACTTGGGATTGGAGGACTTGGTCTTGTACGAGACTGCAAAGG CTGGTATCGAGGGTATGACTAGAGCATTGGCTCGTGAGTTGGGTCCAGACGAC ATCCGTGTCACTTGCGTTGTCCCCGGTAACGTTAAGACTAAGCGTCAAGAAAA GTGGTACACACCAGAAGGAGAGGCACAGATTGTCGCTGCTCAGTGCTTGAAG GGTCGTATTGTCCCAGAGAACGTCGCAGCTTTGGTCTTGTTCTTGGCATCCGAC GACGCTTCCTTGTGTACTGGTCACGAGTATTGGATCGATGCTGGTTGGCGTTAA

Table S2 Primers used in this study.

Name	Primer sequences	Products
XYL1&p-F	CACCATTCCCACTATTATCACC	*XYL1* promoter and *XYL1* ORF
XYL1&p-R	CGACTCTAAGGTTGAAAACACATT	
TDHpro-F	tattttaaagcttaaacacaGGGCTAGATTTCGATATGGATATGG	*TDH* promoter
TDHpro-R	ggtagattgcggaggacatTTTTTGTAATTGTGTTTGTTTGTGTGT	
xylB-F	aATGTCCTCCGCAATCTACCCA	*xyl*B
xylB-R	caccatTTAACGCCAACCAGCATCG	
XYL1ter-F	ctggttggcgttaaATGGTGTTAGTCTGATCTAATGCAACA	*XYL1* terminator
XYL1ter-R	atgtcaaccttccctgtTGCCGTTGCCAGAGCTATTG	
URA3-F	gcaACAGGGAAGGTTGACATTGTCTAGC	*URA3* expression cassettes
URA3-R	ggatggtccaattcaaggaaAACACTTAGAATACGCGGAACAATC	
TXYL-F	TTCCTTGAATTGGACCATCCA	*XYL1* 3'flank&T-vector&*XYL1* promoter
TXYL-R	TGTGTTTAAGCTTTAAAATAGTTTAGTTTGG	

Table S3 Ethanol production of *P. kudriavzevii* N-X in YP medium containing different concentrations of D-glucose by shaking flasks at 100 rpm and 37 ℃.

D-glucose concentrations	Fermentation time (h)	Ethanol (g/L)	% of theoretical yield	Productivity(g/L/h)
100	16	46.8±0.51	91.8±1.0	2.93±0.03
150	24	70.5±1.95	92.1±2.6	2.94±0.08
200	36	78.6±1.50	77.1±1.5	2.46±0.05

Table S4 Ethanol production of *P. kudriavzevii* N-X in YP medium containing 150 g/L D-glucose glucose by shaking flasks at 100 rpm and different temperatures.

Temperature (℃)	Fermentation time (h)	Ethanol (g/L)	% of theoretical yield	Productivity(g/L/h)
30	28	60.1±1.21	78.6±1.6	2.15±0.04
35	24	66.4±1.43	86.8±1.9	2.77±0.06
40	24	72.2±1.35	94.3±1.7	3.01±0.06
42	28	63.2±2.40	82.6±3.1	2.26±0.08
45	36	19.2±3.42	25.1±4.5	0.53±0.08

Figure S1 Schematic diagram of expression *xyl* B at *XYL1* locus using the *URA3* pop-out system.

