[Supplementary materials]

Modular Optimization of Hemicellulose-utilizing Pathway in *Corynebacterium glutamicum* for Consolidated Bioprocessing of Hemicellulosic Biomass

Sung Sun Yim[1], Jae Woong Choi[1], Se Hwa Lee[1], Ki Jun Jeong[1,2,*]

[1]Department of Chemical and Biomolecular Engineering, BK21 Plus program, KAIST, 335 Gwahagno, Yuseong-gu, Daejeon 305-701, Republic of Korea

[2]Institute for the BioCentury, KAIST, 335 Gwahagno, Yuseong-gu, Daejeon 305-701, Republic of Korea

Plasmid manipulation

For construction of modules for xylose utilization, xylose isomerase gene (*xylA*) and xylulokinase gene (*xylB*) from *E. coli* K-12 MG1655 were introduced as operon system under control of three different synthetic promoters (L26, I16 and H30) showing different strengths (Yim et al., 2013). The *xylA* gene with T7 ribosome binding site (RBS) was amplified from *E. coli* MG1655 chromosome with primers, T7RBS-xylA-F and xylA-R. The *xylB* gene with synthetic promoters were amplified from *E. coli* MG1655 with primers, L26-F1, L26-xylB-F2 and xylB-R, I16-F1, I16-xylB-F2 and xylB-R, and H30-F1, H30-xylB-F2 and xylB-R. The *xylA* PCR fragment with T7 RBS and the *xylB* fragments with synthetic promoters were digested with *Xba*I, and linearly ligated together to yield *xylBA* operon fragments with three different synthetic promoters. Each *xylBA* operon with different synthetic promoters was then digested with *Kpn*I and *Not*I, and cloned into pCES208 vector to yield, pXU1 (L26 synthetic promoter), pXU2 (I16 synthetic promoter), and pXU3 (H30 synthetic promoter). For construction of modules for xylose transport, *araE* from *C. glutamicum* ATCC 31831, *araE* from *E. coli* MG1655, and *xylE* from *E. coli* MG1655 were introduced under control of three synthetic promoters (L10, I12, and H72) with different strengths (Yim et al., 2013). Each synthetic promoter was extended in front of each transporter gene sequence by PCR with primers, L10-F1, L10-araE(C)-F2 and araE(C)-R, I12-F1, I12-araE(C)-F2 and araE(C)-R, H72-F1, H72-araE(C)-F2 and araE(C)-R, L10-F1, L10-araE(E)-F2 and araE(E)-R, I12-F1, I12-araE(E)-F2 and araE(E)-R, H72-F1, H72-araE(E)-F2 and araE(E)-R, L10-F1, L10-xylE(E)-F2 and xylE(E)-R, I12-F1, I12-xylE(E)-F2 and xylE(E)-R, H72-F1, H72-xylE(E)-F2 and xylE(E)-R. The PCR fragments of three transporters with three different synthetic promoters were digested with *Nco*I, and cloned into pXU2 to yield nine pXU2T series plasmids (pXU2T1 to pXU2T9). For construction of modules for xylan degradation, *xlnA* from *Streptomyces coelicolor* A3(2) and *xynB* from *Bacillus pumilus* were introduced with native

promoter and signal sequences of *porB*, *cg1514* or *cgR0949* genes isolated from *C. glutamicum* ATCC13032 or *C. glutamicum* R (An et al., 2013; Watanabe et al., 2009; Yim et al., 2015). The *xlnA* gene with promoter and signal sequence of *porB* was amplified from pASJ104 (An et al., 2013) with primers, PporB-F and xlnA-R. The *xynB* gene with T7 RBS was amplified from *Bacillus pumilus* chromosome with primers, T7RBS-xynB-F and xynB-R. The PCR fragments were then fused by PCR with primers, PporB-F and xynB-R, and cloned into *Nco*I restriction enzyme site of pCES208 to yield pXD2. For construction of pXD3, the *porB* signal sequence was extended in front of the *xynB* gene with T7 RBS by PCR with primers, T7RBSporB-xynB-F and xynB-R. The *xynB* fragment with *porB* signal sequence was then fused with the previously mentioned PCR fragment of *xlnA* gene with promoter and signal sequence of *porB* by PCR with primers, PporB-F and xynB-R, and cloned into *Nco*I restriction enzyme site of pCES208 to yield pXD3. In pXD4, signal sequence of *cgR0949* from *C. glutamicum* R was introduced for *xynB* secretion. The *cgR0949* signal sequence was extended in front of the *xynB* gene by PCR with primers, T7RBScgR0949-xynB-F and xynB-R. The *xynB* fragment with *cgR0949* signal sequence was then fused with the previously mentioned PCR fragment of *xlnA* gene with promoter and signal sequence of *porB* by PCR with primer, PporB-F and xynB-R, and cloned into *Nco*I restriction enzyme site of pCES208 to yield pXD4. For pXD5, *cg1514* secretion system was introduced for XlnA secretion. The *xlnA* gene with promoter and signal sequence of *cg1514* was amplified from pCg1514-2-XlnA (Yim et al., 2015) with primers, Pcg1514-F and xlnA-R. The *xlnA* fragment with promoter and signal sequence of *cg1514* was then fused with the previously mentioned PCR fragment of *xynB* gene with *cgR0949* signal sequence by PCR with primer, PporB-F and xynB-R, and cloned into *Nco*I restriction enzyme site of pCES208 to yield pXD5. After finding the optimized systems for each module, the optimized systems were assembled together to construct two plasmids for hemicellulose utilization. To insert xylose utilization module with I16 synthetic promoter (pXU2), *lpp*

terminator sequence was extended at the end of the module by PCR with primers I16-F-KpnI, xylA-tlpp-R1, tlpp-R2-KpnI. The PCR fragment was then digested with *Kpn*I, and cloned into *Kpn*I restriction enzyme site of pCES208 to yield pHCBP200. To insert xylan degradation module with *cg1514* secretion system for *xlnA* and *cgR0949* signal sequence for *xynB*, *lpp* terminator sequence was extended at the end of the module by PCR with primers Pcg1514-F-SpeI, xynB-tlpp-R1, tlpp-R2-SpeI. The PCR fragment was then digested with *Spe*I, and cloned into *Spe*I restriction enzyme site of pCES208 to yield pHCBP205. To insert xylose transport module of *xylE* with L10 synthetic promoter, *lpp* terminator sequence was extended at the end of the module by PCR with primers L10-F-NcoI, xylE-tlpp-R1, tlpp-R2-NcoI. The PCR fragment was then digested with *Nco*I, and cloned into *Nco*I restriction enzyme site of pCES208 to yield pHCBP275.

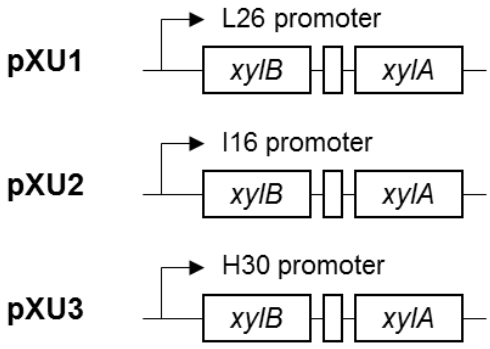
Table S1. List of primers used in PCR experiments.

Primer name	Primer sequence (5' to 3')[a]
T7RBS-xylA-F	ATTAAT**TCTAGA**TAACTTTAAGAAGGAGATATACATATGCAAGCCTATTTTGACCAGCTCG
xylA-R	ATTAAT**GCGGCCGC**GGATCCTTATTTGTCATCGTCATCTTTATAAT
L26-F1	ATTAAT**GGTACC**GTGAGTTTAGAGCAGGGGGGGGGGTTCTTTATGTATGTTCGACGTCGCTTTAGTATGCGTTA
L26-xylB-F2	GACGTCGCTTTAGTATGCGTTAGGATTACTATCGGATCCATGTATATCGGGATAGATCTTGGCA
xylB-R	ATTAAT**TCTAGA**TTATTTGTCATCGTCATCTTTATAATCCGCCATTAATGGCAGAAGTTGCTG
I16-F1	ATTAAT**GGTACC**AGACACCGCGTGCCCGTTATTCTGTGGGGTGGGTATAGTTCTCTAGCGATGTGGTGG
I16-xylB-F2	TAGTTCTCTAGCGATGTGGTGGGCTACAGGATATTATTGGGATCCATGTATATCGGGATAGATCTTGGCA
H30-F1	ATTAAT**GGTACC**AAAGTAACTTTTCGGTTAAGGTAGCGCATTCGTGGTGTTG
H30-xylB-F2	AGCGCATTCGTGGTGTTGCCCGTGGCCCGGTTGGTTGGGCAGGAGTATATTGGGATCCATGTATATCGGGATAGATCTTGGCA
L10-F1	ATTAAT**CCATGG**GGTACCGCAGACGGTTATG
L10-araE(C)-F2	TATTTATTGTTGAAGGAGATAGATTGGATCCATGATGGCAGGGCACATCATC
araE(C)-R	TTAAT**CCATGG**CTATTAATGATGGTGATGGTGATGGACCCTGGCCTTGGTGC
I12-F1	ATTAAT**CCATGG**GGTACCAGTAGTACAGAGATATAGTTC
I12-araE(C)-F2	GCCCAATAGGAGTACGATTGGATCCATGATGGCAGGGCACATCATC
H72-F1	ATTAAT**CCATGG**GGTACCGGAGACAATTTGTGC
H72-araE(C)-F2	CCTCCTAGGAGTATTCTTGGATCCATGATGGCAGGGCACATCATC
L10-araE(E)-F2	AGTTATTTATTGTTGAAGGAGATAGATTGGATCCATGATGGTTACTATCAATACGGAATCTGCTT
araE(E)-R	ATTAAT**CCATGG**CTATTAATGATGGTGATGGTGATGGACGCCGATATTTCTCAACTTCTC
I12-araE(E)-F2	GCCCAATAGGAGTACGATTGGATCCATGATGGTTACTATCAATACGGAATCTGCTT
H72-araE(E)-F2	CCTCCTAGGAGTATTCTTGGATCCATGATGGTTACTATCAATACGGAATCTGCTT
L10-xylE(E)-F2	AGTTATTTATTGTTGAAGGAGATAGATTGGATCCATGAATACCCAGTATAATTCCAGTTATATATTTTCGA
xylE (E)-R	ATTAAT**CCATGG**CTATTAATGATGGTGATGGTGATGCAGCGTAGCAGTTTGTTGTGTTTTC

I12- xylE (E)-F2	GCCCAATAGGAGTACGATTGGATCCATGAATACCCAGTATAATTCCAGTTAT ATATTTTCGA
H72-xylE(E)-F2	CCTCCTAGGAGTATTCTTGGATCCATGAATACCCAGTATAATTCCAGTTATAT ATTTTCGA
PporB-F	ATTAAT**CCATGG**CAAAAAGACACGGTAAATCAGCCTCC
xlnA-R	ATTAATCCATGGTATATCTCCTTCTTAAAGTTAGCTAGCCTATTAATGATGGT GATGGTGATGGGTGCGGGTCCAGCGTTGG
T7RBS-xynB-F	GCTAGCTAACTTTAAGAAGGAGATATACATATGAAGATTATCAATCCAGTGC TCAAAGGGTTTA
xynB-R	ATTAAT**CCATGG**TCATTATTTGTCATCGTCATCTTTATAATCTTCGTCTGTTTC CTCATAACGGAAAT
T7RBSporB-xynB-F	GCTAGCTAACTTTAAGAAGGAGATATACATATGAAGCTTTCACACCGCATCG CAGCAATGGCAGCAACCGCAGGCATCACAGTGGCAGCATTCGCAGCACCTG CTTCCGCATCTAGAAAGATTATCAATCCAGTGC
T7RBScgR0949-xynB-F	GCTAGCTAACTTTAAGAAGGAGATATACATATGCAAATAAACCGCCGAGGCT TCTTAAAAGCCACCGCAGGACTTGCCACTATCGGCGCTGCCAGCATGTTTAT GCCAAAGGCCAACGCCCTTGGAGCATCTAGAGGCCCAGCCGGCCAAAAGAT TATCAATCCAGTGC
Pcg1514-F	ATTAAT**CCATGG**AGCCTGACTAGCGGTGTTTAAG
I16-F-KpnI	ATTAAT**GGTACC**AGACACCGCGTGCCCGTTATTCTGTGGGGTG
xylA-tllp-R1	CAATGTGCGCCATTTTTCACTTCACAGGTCTATTATTTGTCATCGTCATCTTTA TAATCTTTGTC
tlpp-R2-KpnI	ATTAAT**GGTACC**GTAGCGGTAAACGGCAGACAAAAAAAAATGTCGCACAATG TGCGCCATTTTTCAC
Pcg1514-F-SpeI	ATTAAT**ACTAGT**AGCCTGACTAGCGGTGTTTAAGGCACTATTATGTTGCTTT
xynB-tlpp-R1	AAAAAATGTCGCACAATGTGCGCCATTTTTCACTTCACAGGTTCATTATTTGT CATCGTCATCTTTATAA
tlpp-R2-SpeI	ATTAAT**ACTAGT**GTAGCGGTAAACGGCAGACAAAAAAAAATGTCGCACAATG TGCGCC
L10-F-NcoI	ATTAAT**CCATGG**GGTACCGCAGACGGTTATGGTCGCCGCTAGGTCTTGGG
xylE-tlpp-R1	GCGCCATTTTTCACTTCACAGGTCTATTAATGATGGTGATGGTGATGCAGCGT AGCAGTTTGTT
tlpp-R2-NcoI	ATTAAT**CCATGG**GTAGCGGTAAACGGCAGACAAAAAAAATGTCGCACAATG TGCGCCATTTTTCACTTCA

[a] Restriction enzyme sites are shown in bold.

1. Plasmids for xylose utilization module (pXU series)



2. Plasmids for xylose transport module (pXUT series)

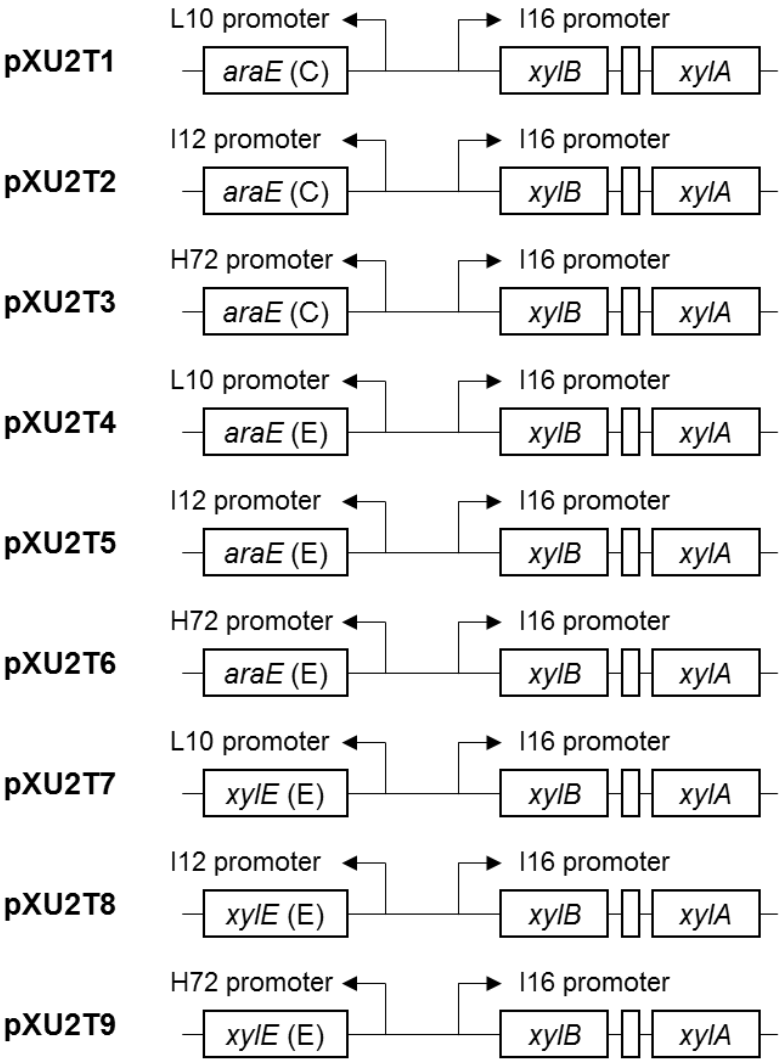
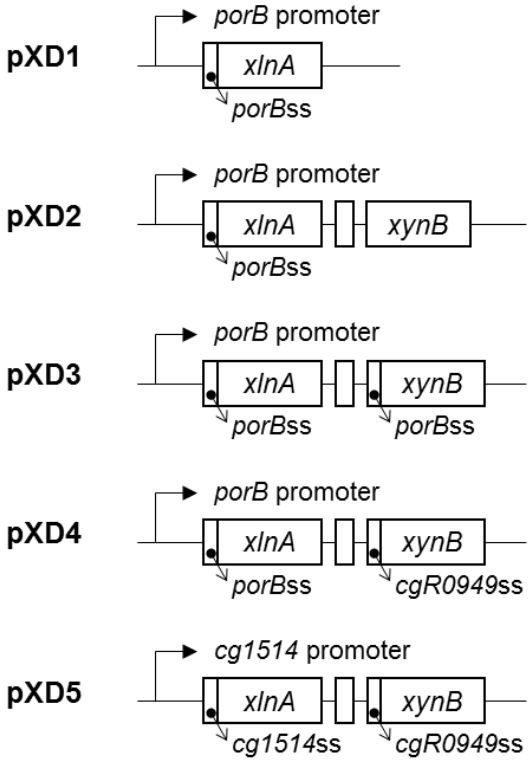


Figure S1. Schematic diagrams of plasmids constructed in this study. (Continued)

3. Plasmids for xylan degradation module (pXD series)



4. Assembled hemicellulose utilization system (pHCBP series)

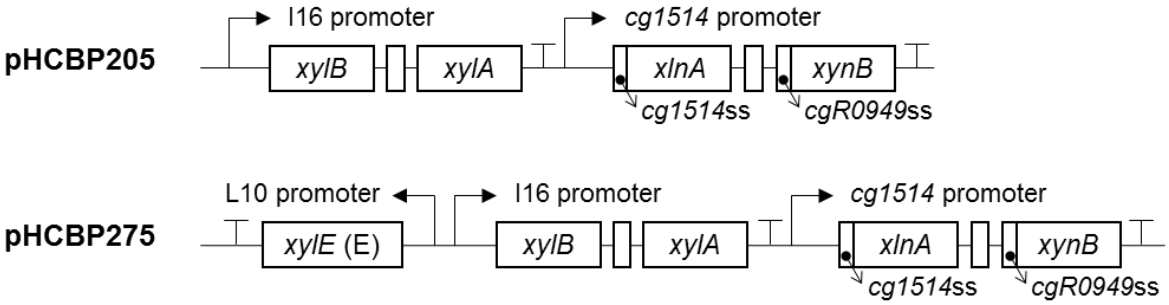


Figure S1. Schematic diagrams of plasmids constructed in this study.

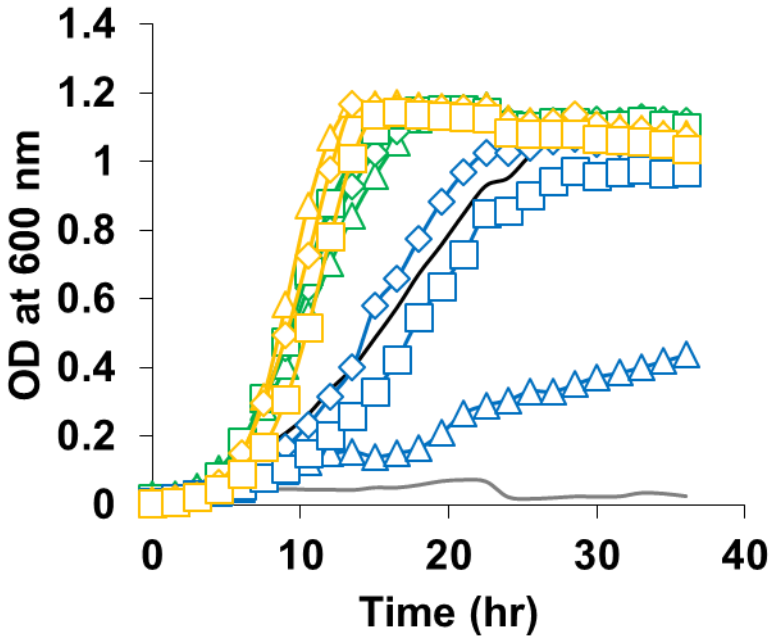


Figure S2. Optimization of xylose transport module. Bioscreen C experiment result for cell growth in minimal media containing 3.6 g/L of xylose as a sole carbon source. Symbols: Gray, *C. glutamicum* harboring pCES208; Black, pXU2; Green, AraE-C added; Blue, AraE-E added; Orange, XylE-E added; △, L10 synthetic promoter; ◇, I12 synthetic promoter; □, H72 synthetic promoter. Dots are average value of triplicate experiments.

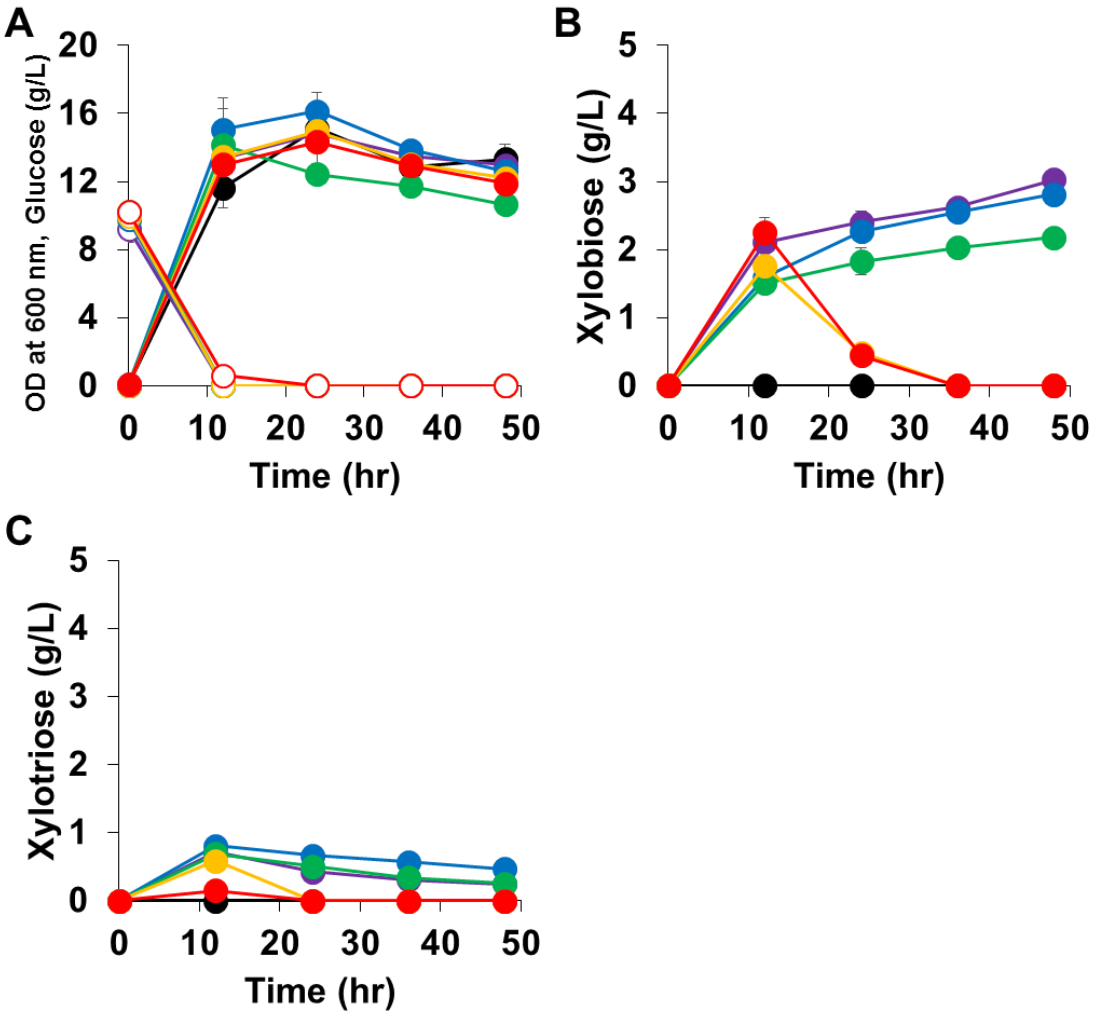


Figure S3. Optimization of xylan degradation module. A) Cell growth and glucose concentration profiles in minimal media containing 10 g/L of glucose and 10 g/L of xylan. B) Xylobiose concentration profiles, C) Xylotriose concentration profiles. Black, *C. glutamicum* harboring pCES208; Purple, pXD1; Blue, pXD2; Green, pXD3; Orange, pXD4; Red, pXD5. All error bars represent standard deviations of triplicate experiments.