Du et al. 2010

Supplemental Information

Supplemental Table S1. Putative pentose transporters from BLAST search.

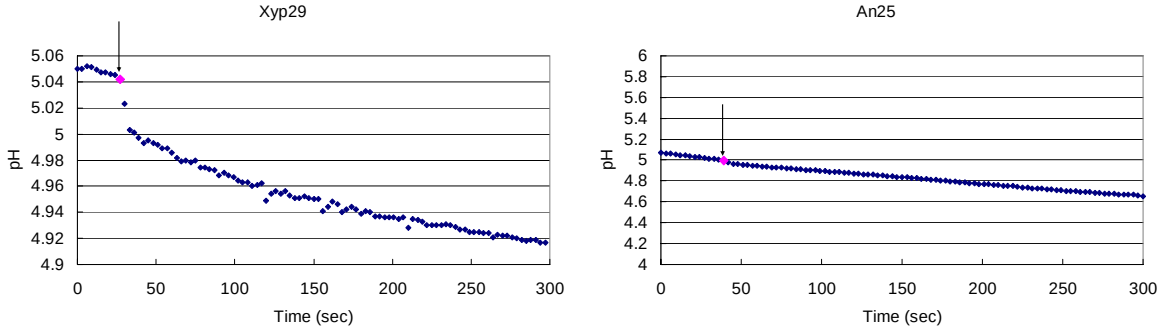
a. BLAST search results using *AUT1* (Locus tag PICST_87108) from *Pichia stipitis* as a probe

Gene Name	Origin	% identity with *AUT1*	Annotation from NCBI	Length (cDNA, nt)	Locus Tag
Ap31 (SUT2)	*P. stipitis*	31	sugar uptake (tentative)	1653	ABN66266
Ap26	*P. stipitis*	26	sugar transporter	1404	XP_001387242
An49	*N. crassa*	49	hypothetical protein NCU01494, similar to MFS sugar transporter	2025	EAA26691
An41	*N. crassa*	41	hypothetical protein NCU09287, similar to galactose-proton symporter	1968	EAA28903
An29-2	*N. crassa*	29	hypothetical protein NCU04963, similar to MFS monosaccharide transporter	1584	EAA30175
An28-3	*N. crassa*	28	hypothetical protein NCU02188, conserved hypothetical protein	1458	EAA30346
An25	*N. crassa*	25	sugar transporter	1653	EAA35128

b. BLAST search results using *GSX1* from *Candida intermedia* as a probe

Gene Name	Origin	% identity with *GXS1*	Annotation from NCBI	Length (cDNA, nt)	Locus Tag
Xy50	*N. crassa*	50	hypothetical protein NCU04537 similar to monosaccharide transporter	1620	EAA26741
Xy31	*N. crassa*	31	hypothetical protein NCU06138, similar to MFS monosaccharide transporter	1752	EAA30764
Xy33	*N. crassa*	33	hypothetical protein NCU00988, similar to MFS quinate transporter	1614	EAA34662
Xyp37 (SUT3)	*P. stipitis*	37	sugar uptake (tentative)	1653	ABN67990
Xyp33 (XUT3)	*P. stipitis*	33	sugar transporter, putative xylose uptake (tentative); predicted transporter (major facilitator superfamily)	1656	EAZ63115
Xyp32 (XUT1)	*P. stipitis*	32	sugar transporter, high affinity, putative; xylose uptake (tentative)	1701	ABN67554
Xyp30 (STL1)	*P. stipitis*	30	sugar transporter, strongly conserved	1587	ABN65745
Xyp31 (XUT2)	*P. stipitis*	31	sugar transporter, xylose transporter (tentative) similarly to GXS1 (STL1)	1404	AAVQ01000002
Xyp29 (STL12) (XUT6)	*P. stipitis*	29	sugar transporter, putative (STL12); xylose uptake (tentative)	1635	ABN68560
Xyp30-1 (HGT3)	*P. stipitis*	30	high affinity xylose transporter (putative), xylose uptake (tentative)	1587	ABN68686
Xyp28 (XUT7)	*P. stipitis*	28	xylose transporter, high affinity, putative similarity to STL13, high affinity sugar transporters	1251	EAZ63044

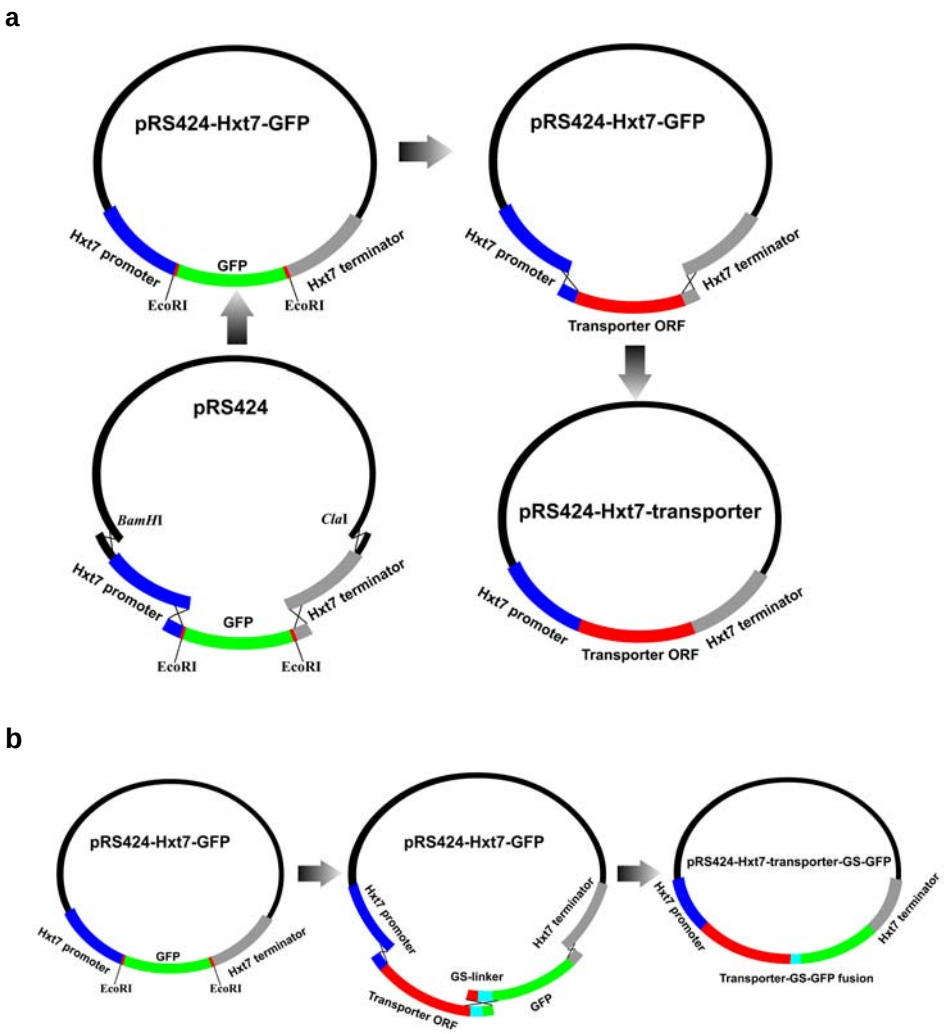
Supplemental Figure S2. Symporter assay of An25 and Xyp29. pH change in un-buffered cell suspension of *S. cerevisiae* EBY.VW4000 strains harboring D-xylose-specific transporter overexpressing plasmids. The pink point indicates when the sugar solution is added into the system.



Supplemental Figure S3. Plasmid construction using the DNA assembler method.

a. Cloning of putative pentose transporters. The pRS424-HXT7-GFP plasmid was used for cloning of putative pentose transporters. In this plasmid, a HXT7 promoter, a GFP gene flanked with the *Eco*RI site at both ends, and a HXT7 terminator, were assembled into the pRS424 shuttle vector linearized by *Cla*I and *Bam*HI. PCR products of the putative pentose transporters flanked with DNA fragments sharing sequence identity to the HXT7 promoter and terminator were co-transferred into CEN.PK2-1C with *Eco*RI digested pRS424-HXT7-GFP using the standard lithium acetate method.

b. Construction of plasmids containing transporter-GFP fusion proteins. The GS-linker was added to the N-terminus of the GFP open reading frame by a PCR primer, resulting in a PCR product of GS-linker-GFP flanked with nucleotide sequences identical to the transporters at the 5'-end and the HXT7 terminator at the 3'-end. Transporter genes were amplified from the original pRS424-HXT7-transporter constructs to generate DNA fragments of the transporters flanked with nucleotide sequences identical to the HXT7 promoter at the 5'-end and the GS-linker-GFP at the 3'-end. These two fragments were then co-transferred into CEN.PK2-1C with pRS424-HXT7-GFP digested with *Eco*RI. The resulting transformation mixture was plated on SC-Trp supplemented with 2% D-glucose.



Supplemental Table S4. Alignment of amino acid sequence of cloned An25 and Xyp29 with their corresponding NCBI entries.

a. Align An25 amino acid sequences (97.3% identity)

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                    10         20         30         40         50         60
An25_NCBI     MAPPKFLGLSGRPLSLAVSTVATTGFLLFGYDQGVMSGIITAPAFNNFFTPTKDNSTMQG
              ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
An25_cloned   MAPPKFLGLSGRPLSLAVSTVATTGFLLFGYDQGVMSGIITAPAFNNFFTPTKDNSTMQG
                    10         20         30         40         50         60

                    70         80         90        100        110        120
An25_NCBI     LITAIYEIGCLIGAMFVLWTGDLLGRRRNIMVGAFIMALGVIIQVTCQAGSNPFAQLFVG
              ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
An25_cloned   LITAIYEIGCLIGAMFVLWTGDLLGRRRNIMVGAFIMALGVIIQVTCQAGSNPFAQLFVG
                    70         80         90        100        110        120

                              130        140        150        160
An25_NCBI     RVVM--------DSV----AECSKTSNRGLLICIEGGVIAFGTLIAYWIDYGASYGPDDL
              ::::        ...     ::::::::::::::::::::::::::::::::::::::
An25_cloned   RVVMGIGNGMNTSTIPTYQAECSKTSNRGLLICIEGGVIAFGTLIAYWIDYGASYGPDDL
                    130        140        150        160        170        180

                  170        180        190        200        210        220
An25_NCBI     VWRFPIAFQLLFAIFICVPMFYLPESPRWLLSHGRTQEADKVIAALRGYEIDGPETIQER
              ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
An25_cloned   VWRFPIAFQLLFAIFICVPMFYLPESPRWLLSHGRTQEADKVIAALRGYEIDGPETIQER
                    190        200        210        220        230        240

                  230        240        250        260        270        280
An25_NCBI     NLIVDSLRASGGFGQKSTPFKALFTGGKTQHFRRLLLGSSSQFMQQVGGCNAVIYYFPIL
              ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
An25_cloned   NLIVDSLRASGGFGQKSTPFKALFTGGKTQHFRRLLLGSSSQFMQQVGGCNAVIYYFPIL
                    250        260        270        280        290        300

                  290        300        310        320        330        340
An25_NCBI     FQDSIGESHNMSMLLGGINMIVYSIFATVSWFAIERVGRRRLFLIGTVGQMLSMVIVFAC
              ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
An25_cloned   FQDSIGESHNMSMLLGGINMIVYSIFATVSWFAIERVGRRRLFLIGTVGQMLSMVIVFAC
                    310        320        330        340        350        360

                  350        360        370        380        390        400
An25_NCBI     LIPDDPMKARGAAVGLFTYIAFFGATWLPLPWLYPAEVNPIRTRGKANAVSTCSNWMFNF
              ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
An25_cloned   LIPDDPMKARGAAVGLFTYIAFFGATWLPLPWLYPAEVNPIRTRGKANAVSTCSNWMFNF
                    370        380        390        400        410        420

                  410        420        430        440        450        460
An25_NCBI     LIVMVTPIMVDKIGWGTYLFFAVMNGCFLPIIYFFYPETANRSLEEIDIIFAKGFVENMS
              ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
An25_cloned   LIVMVTPIMVDKIGWGTYLFFAVMNGCFLPIIYFFYPETANRSLEEIDIIFAKGFVENMS
                    430        440        450        460        470        480

                  470        480        490        500        510        520
An25_NCBI     YVTAAKELPHLTAEEIESYANKYGLVDRDSNGEGGNRHDEEKTRDRPDQSDSDSPAHVEI
              ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
An25_cloned   YVTAAKELPHLTAEEIESYANKYGLVDRDSNGEGGNRHDEEKTRDRPDQSDSDSPAHVEI
                    490        500        510        520        530        540

                  530        540        550
An25_NCBI     DVVDEHGVESGFGDGINTKETR
              ::::::::::::::::::::::
An25_cloned   DVVDEHGVESGFGDGINTKETR
                    550        560
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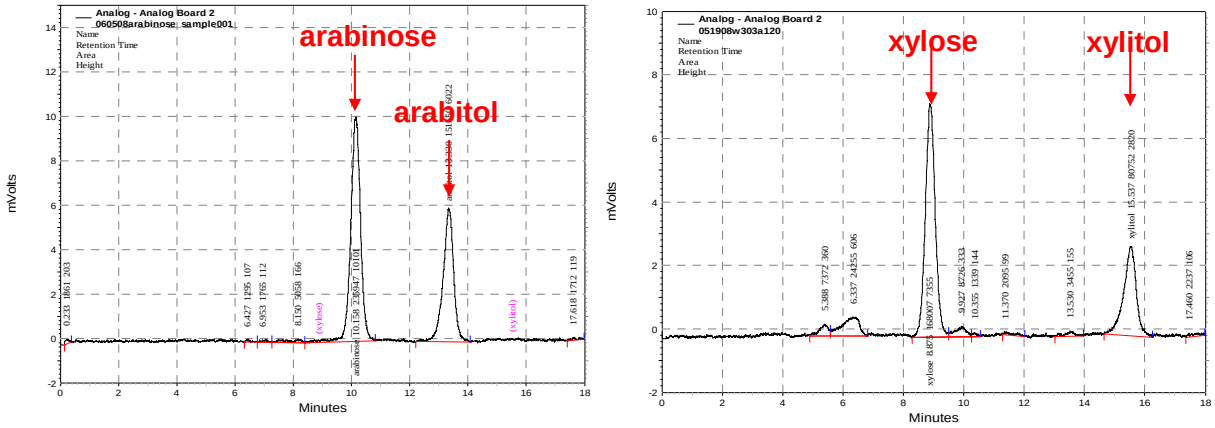
a. Align Xyp29 amino acid sequences (99.6% identity)

```
                  10         20         30         40         50         60
Xyp29_NCBI     MSSVEKSAETASYTSQVSASGSAKTNSYLGLRGHKLNFAVSCFAGVGFLLFGYDQGVMGS
               ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Xyp29_cloned   MSSVEKSAETASYTSQVSASGSAKTNSYLGLRGHKLNFAVSCFAGVGFLLFGYDQGVMGS
                  10         20         30         40         50         60

                  70         80         90        100        110        120
Xyp29_NCBI     LLTLPSFENTFPAMKASNNATLQGAVIALYEIGCMSSSLATIYLGDRLGRLKIMFIGCVI
               ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Xyp29_cloned   LLTLPSFENTFPAMKASNNATLQGAVIALYEIGCMSSSLATIYLGDRLGRLKIMFIGCVI
                  70         80         90        100        110        120

                 130        140        150        160        170        180
Xyp29_NCBI     VCIGAALQASAFTIAHLTVARIITGLGTGFITSTVPVYQSECSPAKKRGQLIMMEGSLIA
               ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Xyp29_cloned   VCIGAALQASAFTIAHLTVARIITGLGTGFITSTVPVYQSECSPAKKRGQLIMMEGSLIA
                 130        140        150        160        170        180

                 190        200        210        220        230        240
Xyp29_NCBI     LGIAISYWIDFGFYFLRNDGLHSSASWRAPIALQCVFAVLLISTVFFFPESPRWLLNKGR
               ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Xyp29_cloned   LGIAISYWIDFGFYFLRNDGLHSSASWRAPIALQCVFAVLLISTVFFFPESPRWLLNKGR
                 190        200        210        220        230        240

                 250        260        270        280        290        300
Xyp29_NCBI     TEEAREVFSALYDLPADSEKISIQIEEIQAAIDLERQAGEGFVLKELFTQGPARNLQRVA
               ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Xyp29_cloned   TEEAREVFSALYDLPADSEKISIQIEEIQAAIDLERQAGEGFVLKELFTQGPARNLQRVA
                 250        260        270        280        290        300

                 310        320        330        340        350        360
Xyp29_NCBI     LSCWSQIMQQITGINIITYYAGTIFESYIGMSPFMSRILAALNGTEYFLVSLIAFYTVER
               ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Xyp29_cloned   LSCWSQIMQQITGINIITYYAGTIFESYIGMSPFMSRILAALNGTEYFLVSLIAFYTVER
                 310        320        330        340        350        360

                 370        380        390        400        410        420
Xyp29_NCBI     LGRRFLLFWGAIAMALVMAGLTVTVKLAGEGNTHAGVGAAVLLFAFNSFFGVSWLGGSWL
               ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Xyp29_cloned   LGRRFLLFWGAIAMALVMAGLTVTVKLAGEGNTHAGVGAAVLLFAFNSFFGVSWLGGSWL
                 370        380        390        400        410        420

                 430        440        450        460        470        480
Xyp29_NCBI     LPPELLSLKLRAPGAALSTASNWAFNFMVVMITPVGFQSIGSYTYLIFAAINLLMAPVIY
               ::::::::::::::::::::::::::::::::::::::::::::::::::::::::::::
Xyp29_cloned   LPPELLSLKLRAPGAALSTASNWAFNFMVVMITPVGFQSIGSYTYLIFAAINLLMAPVIY
                 430        440        450        460        470        480

                 490        500        510        520        530        540
Xyp29_NCBI     FLYPETKGRSLEEMDIIFNQCPVWEPWKVVQIARDLPIMHSEVLDHEKDVIIEKSRIEHV
               :::::::::::::::::::::::::::::::::::: ::: ::::::::::::::::::::
Xyp29_cloned   FLYPETKGRSLEEMDIIFNQCPVWEPWKVVQIARDLPIMHSEVLDHEKNVIIKKSRIEHV
                 490        500        510        520        530        540


Xyp29_NCBI      ENIS
               ::::
Xyp29_cloned   ENIS
```

Supplemental Figure S5. Representative chromatogram of HPLC analysis of intracellular sugar concentrations.



Sugar and corresponding sugar alcohol concentrations were determined using Shimadzu HPLC equipped with a Bio-Rad HPX-87C column (Bio-Rad Laboratories, Hercules, CA) and Shimadzu ELSD-LTII low temperature-evaporative light scattering detector following the manufacturer's protocol. The HPX-87C column was kept at 85 °C using a Shimadzu CTO-20AC column oven. Deionized water was used as mobile phase at a constant flow rate of 0.2 mL/min. During the HPLC process, nitrogen was provided to the ELSD detector at a constant pressure of 350 kPa. 20 uL of filtered sample was injected into the HPLC system with a Shimadzu SIL-20AC HT auto sampler, and each run was stopped at 20 minutes after the injection. The concentration of the sugars and corresponding sugar alcohols were determined using a standard curve generated using a series of external standards. The sugar uptake activity was calculated as mg sugar extracted through osmosis per mL of cell culture at OD~10.