

Supplementary Information: Flux balance analysis predicts NADP phosphatase and NADH kinase are critical to balancing redox during xylose fermentation in *Scheffersomyces stipitis*

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Contents

1	Integrated omics analysis	1
2	Review of other redox balancing mechanisms	3
2.1	Succinate bypass	3
2.2	Complex I bypass	4
2.3	Alternative oxidase	4
3	Cloning, enzyme expression, optimization, and characterization	4
4	Phylogenetic analysis of <i>PHO3.2</i> and <i>XYL1</i> homologs	8
5	Phenotypes of xylose-fermenting yeasts based on phylogeny and genome annotations	11
6	<i>UTR1</i> amino acid alignment	12

1 Integrated omics analysis

The xylose fermenting transcriptome from several studies were compared to find common expression patterns (Jeffries et al., 2007; Jeffries and Van Vleet, 2009; Yuan et al., 2011; Wohlbach et al., 2011). The expression of the xylose reductase (XR)-xylitol dehydrogenase (XDH) pathway, sugar transporters, lignocellulose-related enzymes, pentose phosphate pathway and redox metabolism in *S. stipitis* is consistent with other native xylose fermenters in the CTG clade. Far more genes were upregulated than downregulated with xylose as a carbon source than glucose (Yuan et al., 2011).

Redox metabolism. Glucose 6-phosphate dehydrogenase (encoded by *ZWF1*) had increased expression in multiple transcriptome studies (Jeffries et al., 2007; Jeffries and Van Vleet, 2009; Yuan et al., 2011). NAD(H) kinase (encoded by *UTR1*) was upregulated during xylose fermentation (Yuan et al., 2011). This may lead to an increase in the NADP concentration, an increase in the NADPH concentration, or regeneration of NADPH flux depending on its enzyme kinetics. We created a *S. stipitis* Utr1p structure by modelling it

with human NAD kinase (3PFN) using Phyre2 (Kelley et al., 2015). We were unable to analyze how the CTG clade-specific conserved motifs may impact the binding of NAD or NADH since they lie outside of the conserved domain. The upregulation of *ZWF1* and *UTR1* support an NADPH preferring XR, regardless of whether *UTR1* encodes an NAD kinase or NADH kinase. *PHO3.2* had a marginal change in transcript levels during xylose fermentation; its expression was confirmed via proteomics during xylose fermentation (Huang and Lefsrud, 2012).

Suboptimal growth. *S. stipitis* has been observed to have suboptimal growth during xylose fermentation when compared to glucose fermentation (Ligthelm et al., 1988). There are several possibilities given our flux simulations and a review of *S. stipitis* omics data: a drop in the ATP yield caused by regenerating NADPH from NAD(H) kinase than the oxidative pentose phosphate pathway, a futile cycle between phosphofructokinase and fructose-1 6-bisphosphatase-1, which are both expressed in transcriptomics and proteomics studies (Yuan et al., 2011; Huang and Lefsrud, 2012), a bypassed Complex I or interruption of the TCA cycle (Shi et al., 2002), and the accumulation of other polyols (Ligthelm et al., 1988; Su et al., 2015).

There was no downregulation of Complex I, despite some evidence it is not active during xylose fermentation (Shi et al., 2002; Yuan et al., 2011). Huang and Lefsrud (2012) has confirmed its expression by shotgun proteomics. Mitochondrial superoxide dismutase’s expression was lower with xylose fermentation than glucose fermentation, which provides some indirect evidence that Complex or the electron transport chain is less relevant during xylose fermentation. Complex I and other electron transport chain complexes generate free radicals that must be scavenged by superoxide dismutase and other compounds. In contrast, the cytoplasmic superoxide dismutase, *SOD2.1*, had increased expression during xylose fermentation. Glyceraldehyde 3-phosphate dehydrogenase, encoded by *TDH1* and *TDH2*, also have higher transcript levels under xylose fermentation than glucose, which indicates glycolysis is more relevant to xylose fermentation than the ETC.

Expression of trans-aconitate methyltransferase (*TMT1*) increased 9 fold with xylose fermentation, although its transcript levels were low (7.17 RPKM) (Yuan et al., 2011). Trans-aconitate is an inhibitor of aconitase (Saffran and Prado, 1949), so it is possible that Tmt1p is acting to reduce its inhibition in response to an interrupted TCA cycle or blocked Complex I (Cai et al., 2001).

Ribitol has a higher yield than other polyols during glucose and xylose fermentation with *S. stipitis* (Ligthelm et al., 1988; Su et al., 2015). A possible pathway for ribitol accumulation may involve ribulose 5-phosphate reductase (PICST_45705) and ribitol kinase (PICST_86603), which were both upregulated during xylose fermentation (Yuan et al., 2011). This pathway would reoxidize NADH and regenerate ATP, rather than dephosphorylate ribitol 5-phosphate via a phosphatase. These genes could be deletion targets to reduce

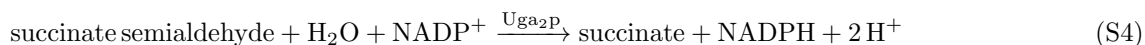
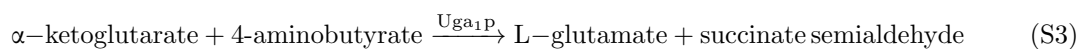
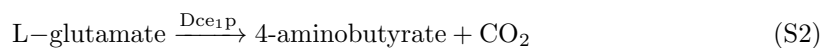
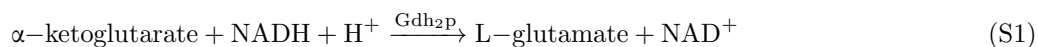
ribitol yield in native xylose fermenters. Alternatively, our Pho3.2p assay has shown its substrate promiscuity may lead to polyol accumulation.

Other expression patterns. Interestingly, isocitrate lyase (*ICL1*) had increased expression with xylose, but reduced malate synthase (*MLS1.2*) expression with xylose. Recent characterization has shown the glyoxylate cycle to be active in batch and chemostat aerobic growth for *S. stipitis* (Papini et al., 2012). Huang and Lefsrud (2012) found evidence of an active glyoxylate cycle in xylose fermentation, with the expression of isocitrate lyase (*ICL1*), malate synthase (*MLS1.1*), and NADP-dependent isocitrate dehydrogenase (*IDP2*) throughout the 64-hour fermentation. The glyoxylate cycle is suppressed during glucose growth in *S. cerevisiae* (Zampar et al., 2013). It is possible that it is less active in xylose fermentation than glucose fermentation. Further experiments may yield more insight into the role of the glyoxylate cycle in Crabtree-negative yeasts, and xylose growth.

2 Review of other redox balancing mechanisms

2.1 Succinate bypass

The cytoplasmic succinate bypass in *S. stipitis* has been proposed to supply NADPH for XR (Jeffries et al., 2007; Jeffries and Van Vleet, 2009); this mechanism was based on the upregulation of *GDH2* and *UGA1* in microarrays. Jeffries et al. (2007) assumed Gdh2p assimilates NH_4 with NADH, but characterization of *GDH2* knockouts in *S. stipitis* found its role to be related to glutamate catabolism (Freese et al., 2011). Glutamate or other amino acids must accumulate for the succinate bypass to supply NADPH for XR, or another nitrogenous compound must be degraded to release NH_4 . Furthermore, there would be excess NAD(P)H regenerated from xylose to α -ketoglutarate. These shortcomings stress the need to evaluate transcriptomics with flux balance analysis.



2.2 Complex I bypass

Complex I is bypassed during xylose fermentation in *S. stipitis* (Shi et al., 2002; Jeffries et al., 2007), yet no transcriptomics studies support downregulation of Complex I subunits. Complex I subunits have been detected via shotgun proteomics during xylose fermentation (Huang and Lefsrud, 2012), which indicates it is expressed and could be bypassed. An expressed but bypassed Complex I has also been observed in *Cyberlindnera jadinii* (Ohnishi, 1972). There is no indication that bypassing Complex I has a direct role in balancing redox cofactors during xylose fermentation.

External NADH dehydrogenase, encoded by *NDE1*, is expressed during xylose fermentation study (Huang and Lefsrud, 2012). *NDE1* provides an alternative mechanism to reoxidize NADH to NAD in the cytoplasm, and it is not linked to proton translocation. These results could account for suboptimal growth seen in xylose fermentation if both proteins are expressed during xylose growth.

2.3 Alternative oxidase

Alternative oxidase has been postulated to balance redox cofactors by scavenging for oxygen when it is present in low oxygen concentration or at high cell densities (Jeppsson et al., 1995). *In silico* simulations show that alternative oxidase has no impact on xylitol yield regardless of the cofactor selectivity for XR; however, there is a reduction in the growth rate when Aox1p oxidizes ubiquinone *in silico*. Disruption of *AOX1* led to an increase in ethanol in *S. stipitis* (Shi et al., 2002).

3 Cloning, enzyme expression, optimization, and characterization

Primer sequences used to clone *PHO3* and *PHO3.2* into pPICZ α ,B for expression in *Komagataella phaffii* are listed in Table S1. We used *K. phaffii* as a host because we were unable to collect any soluble protein by expressing Pho3 and Pho3.2p in *Escherichia coli*; presumably glycosylation was required or the signal peptide impacted its expression. The N-terminal sequences of *PHO3* and *PHO3.2* were truncated to remove their native signal peptides, and tagged with the α -factor secretion signal peptide from *S. cerevisiae* at their N-terminus.

His-tag antibodies (results not shown) and the agar acid phosphatase assay were used to find *K. phaffii* clones with the highest expression of Pho3p and Pho3.2p (Figure S1) (Dorn, 1965). No phosphatase activity was initially detected in the supernatant of mutants expressing Pho3p and Pho3.2p after 24 hours of growth on methanol (Figure S2). Sonication treatment to the cells increased phosphatase activity in the supernatant of Pho3p and Pho3.2p (Figure S2). Pho3p and Pho3.2p were predicted to be 36 kDa without glycosylation,

Table S1: Primer sequences for *PHO3* and *PHO3.2* inserts into pPICZ α ,B without their native signal peptides.

Primer	Sequence
PstI-PHO3 (mature) FR	GTT GTT CTG CAG TTA AAA CAA TTC TCT TGT CTA ACG AC
PHO3 (mature)-NotI RC	GTT GTT GCG GCC GCG CTG GAA AAC AAA GGT TGC A
EcoRI-PHO3.2 (mature) FR	GTT GTT GAA TTC TGA AGA CCA TCC TCT TGA CCA A
PHO3.2 (mature)-NotI RC	GTT GTT GCG GCC GCA GAG AAC AAT GGT TCC AAC A

but were actually 55 kDa with glycosylation (Figure S3). The malachite green assay shows higher promiscuity with Pho3.2p than Pho3p (Figure S4).

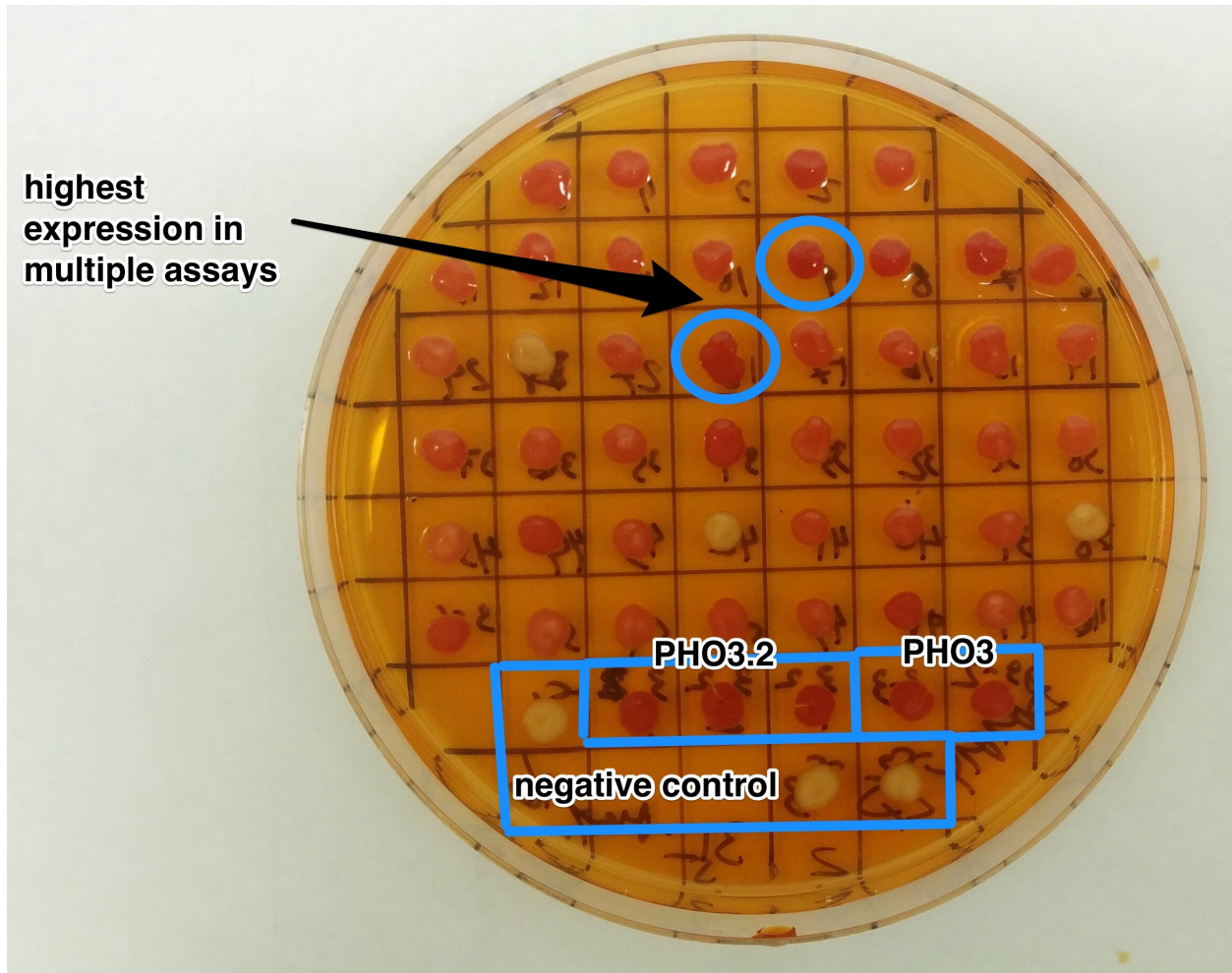


Figure S1: Acid phosphatase screen as described by Dorn (1965). Three out of 45 colonies did not have any detectable acid phosphatase activity.

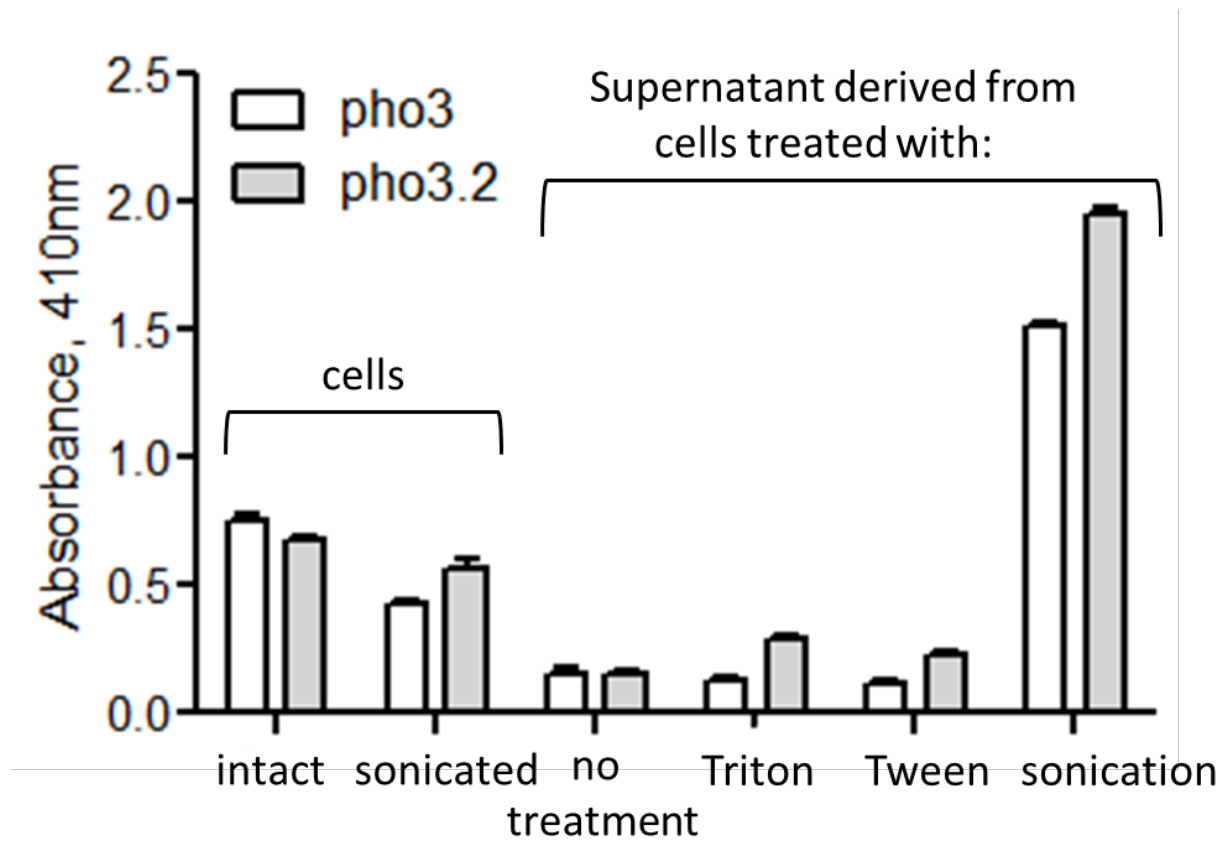


Figure S2: Impact of purification method on phosphatase activity after 24 hours of growth on methanol. Sonication led to the highest activity of phosphatase in the supernatant.

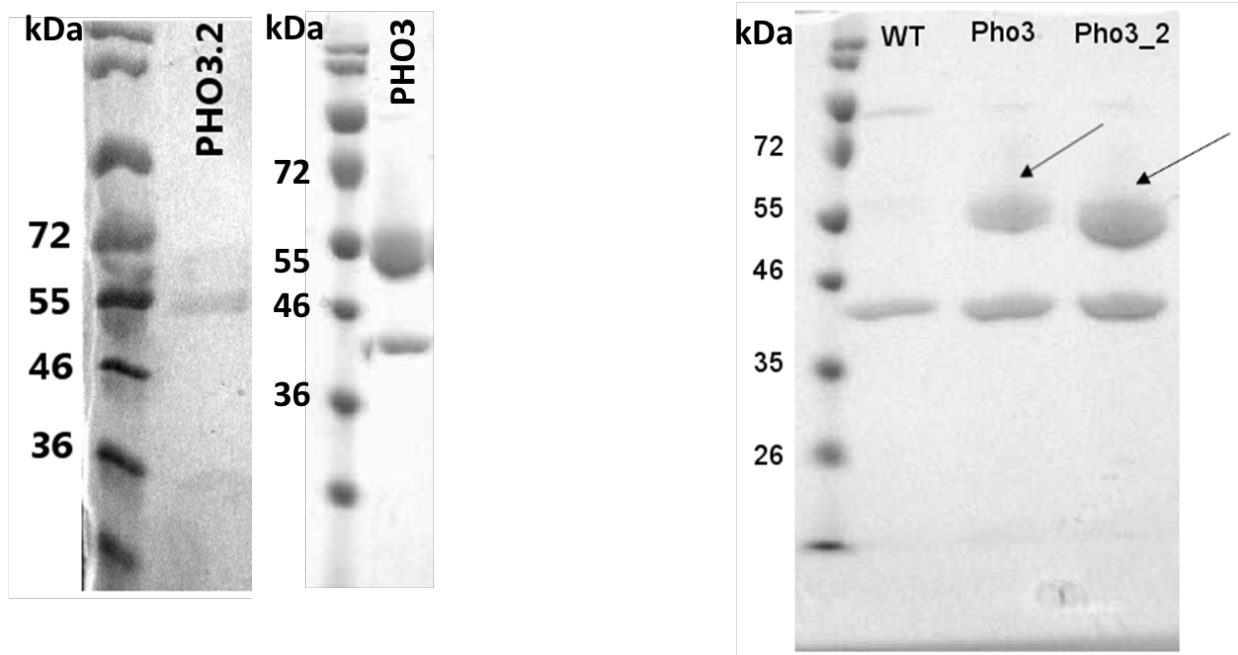


Figure S3: PAAG showing purified proteins from wild-type, *PHO3*, and *PHO3.2*-expressing mutants in *Komagataella phaffii*. The *Komagataella phaffii* Adh2p contaminant is also shown in the gel.



Figure S4: Malachite green assay results for Pho3p and Pho3.2p (no replicates). Scale is % change in absorbance after five minutes. Pho3.2p has broader activity than Pho3p.

4 Phylogenetic analysis of *PHO3.2* and *XYL1* homologs

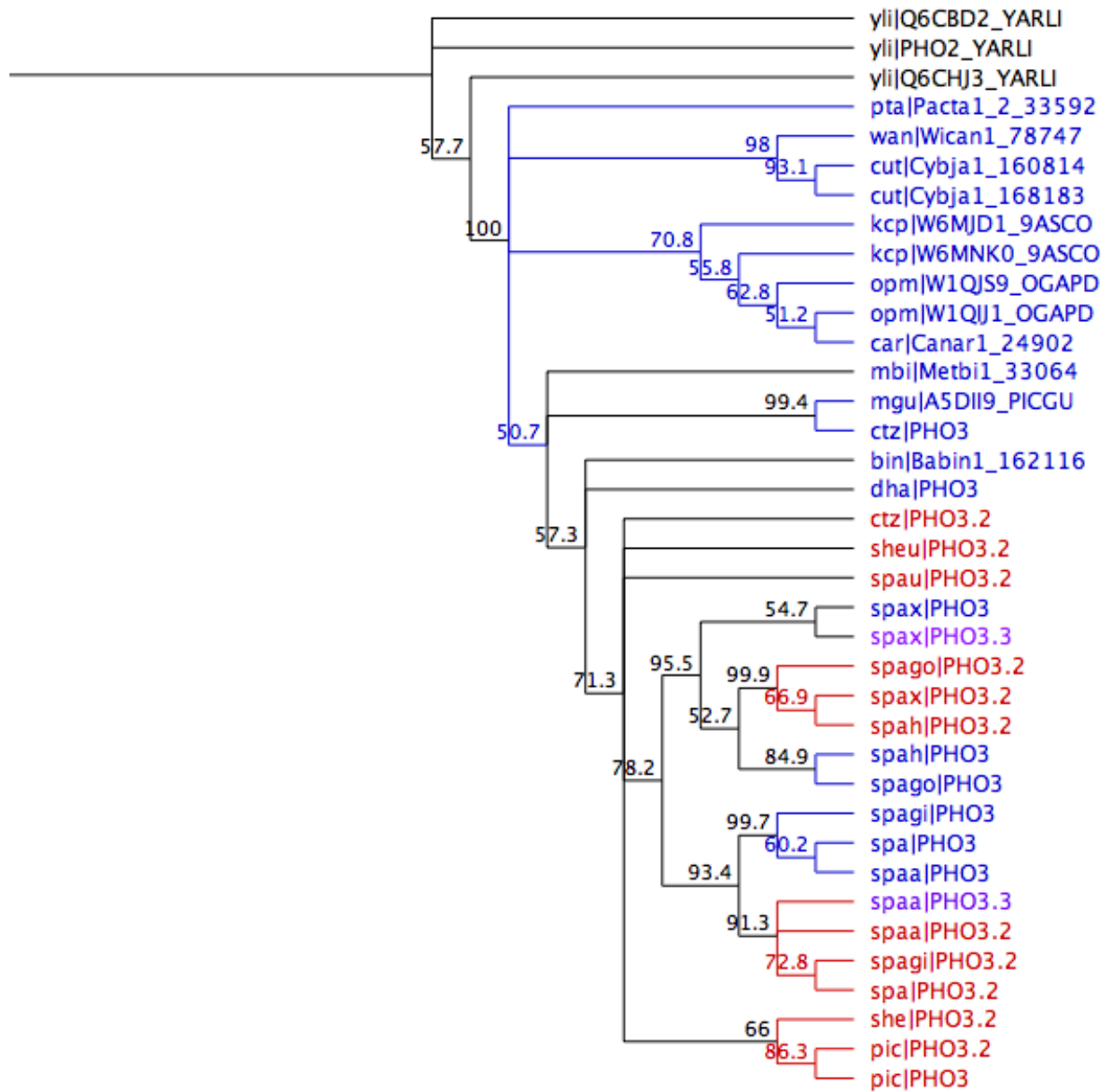


Figure S5: Phylogenetic reconstruction of *PHO3.2* (acid phosphatase) homologs in budding yeasts. *PHO3.2* derived from a tandem duplication in a common ancestor of *Suhomyces tanzawaensis*, *Scheffersomyces* and *Spathaspora* species. The red leaves highlight the *PHO3.2* paralogs. The purple leaves highlight an additional uncharacterized *PHO3* or *PHO3.2* paralog.

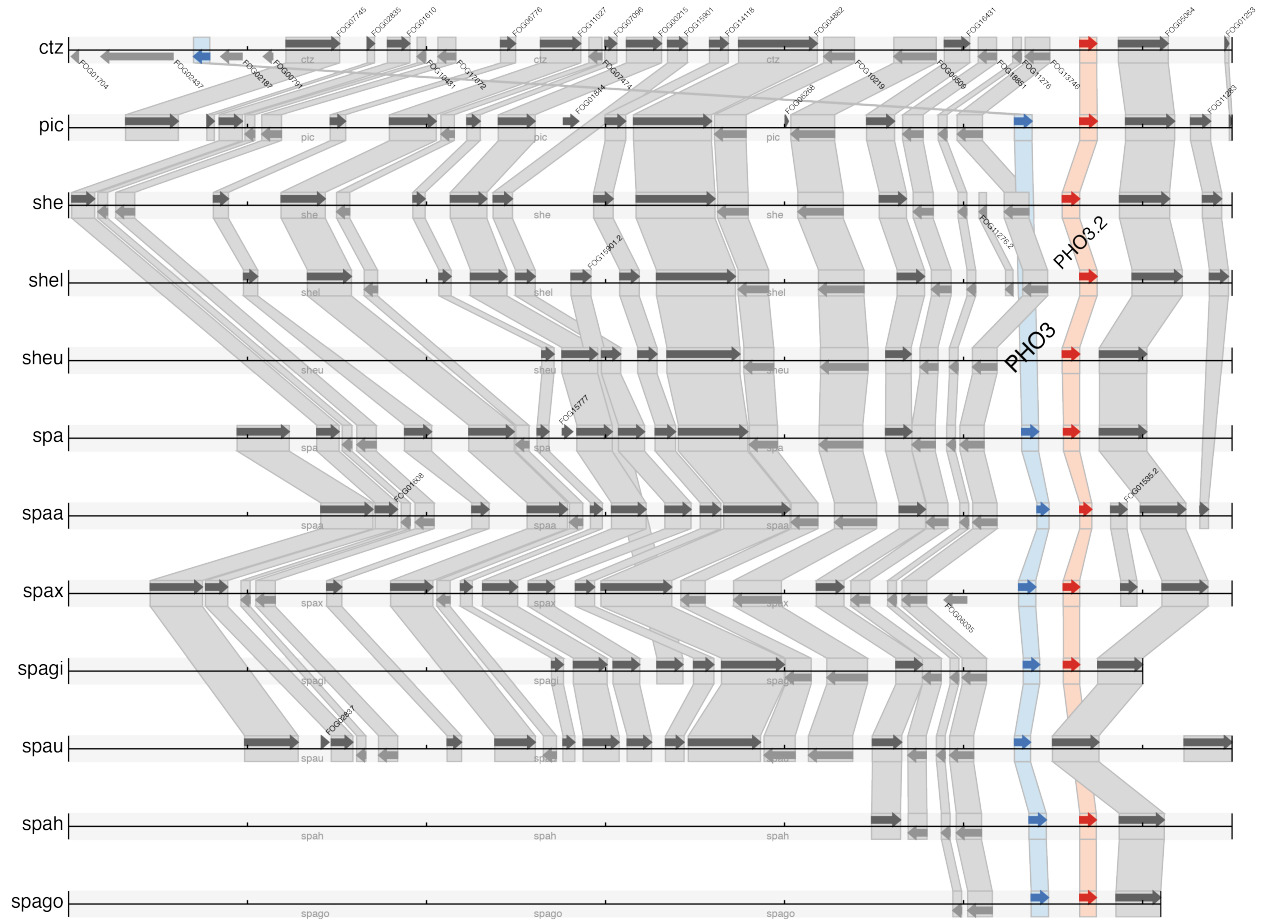


Figure S6: Synteny of the *PHO3* (blue) and *PHO3.2* (red) loci in *Suhomyces tanzawaensis*, *Scheffersomyces* and *Spathaspora* species. A genomic inversion of *PHO3* occurred in an ancestor of *Suhomyces tanzawaensis*. *PHO3* is less conserved in *Scheffersomyces* species than *PHO3.2*.

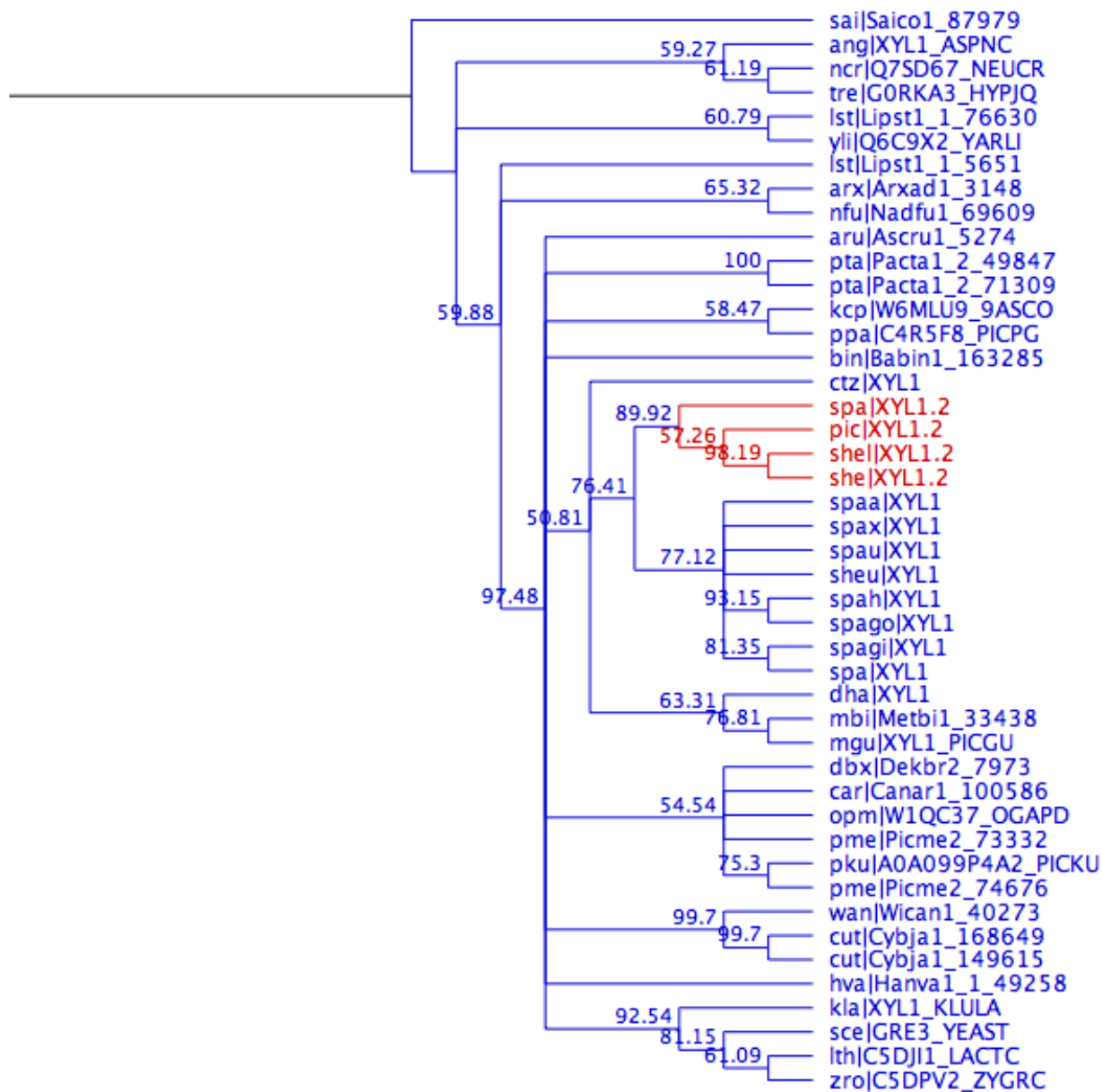


Figure S7: Phylogenetic reconstruction of *XYL1* (xylose reductase) homologs in budding yeasts, Pezizomycotina fungi, and *Saitoella complicata*; the *XYL1* ortholog appears to be absent in Basidiomycota (Correia et al., 2017; Mi et al., 2012). Red leaves highlights the *XYL1.2* paralog, which has NAD(P)H-dependent xylose reductase activity. *Pachysolen tannophilus* has an independent duplication of *XYL1* (Correia et al., 2017), which also led to NAD(P)H-dependent XR activity (Ditzelmüller et al., 1985).

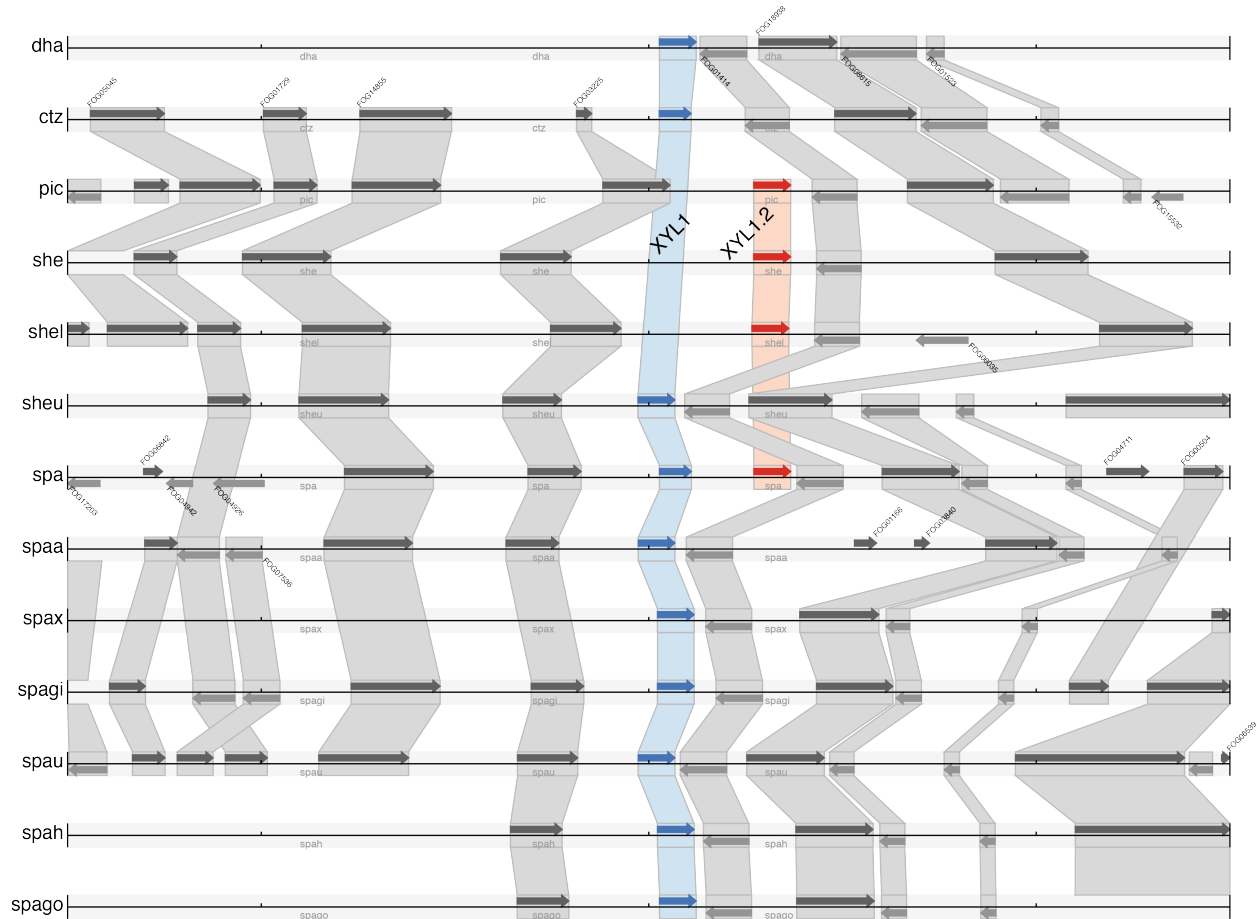


Figure S8: Synteny of *XYL1* (blue) and *XYL1.2* (red) loci in *Scheffersomyces* and *Spathaspora* species. *XYL1.2* originated from a tandem duplication of *XYL1* upstream of trimethyllysine dioxygenase (FOG01414). *XYL1* was subsequently lost in some *Scheffersomyces* species.

5 Phenotypes of xylose-fermenting yeasts based on phylogeny and genome annotations

The phenotypes of xylose-fermenting yeasts can be categorized by the products they accumulate under oxygen-limiting or anaerobic conditions, although there is no strict criteria defining each group:

- xylitol
- xylitol and ethanol
- ethanol
- xylitol, acetate, ethanol

Xylitol. This phenotype is observed during oxygen limitation with yeasts that possess NADPH-dependent XR, such as *Debaromyces hansenii* (Converti and Domínguez, 2001), *Candida parapsilosis* and *Meyerozyma guilliermondii* (Nolleau et al., 1995). Interestingly, one strain of *Candida parapsilosis* has an XR that uses NADH (Lee et al., 2003) but it has not been explored why this yeast does not have a cofactor imbalance during xylose fermentation (Nolleau et al., 1995). Minor amounts of ethanol can accumulate with these yeasts under some conditions.

Xylitol and Ethanol. *Candida tropicalis*, *Candida tenuis*, *Scheffersomyces shehatae*, and some novel species of *Spathaspora* all ferment xylose to xylitol and ethanol (Ligthelm et al., 1988; Wohlbach et al., 2011; Lopes et al., 2016). These yeasts have XR with varying selectivities of NADH and NADPH (Cadete et al., 2013). It is unclear why *S. shehatae* accumulates large amounts of xylitol (greater than 10%), despite having the *XYL1.2* and *PHO3.2* orthologs. *Spathaspora* species with strictly NADPH-dependent XR have been observed to ferment xylose to ethanol but only during aerobic conditions (Cadete et al., 2013).

Ethanol. *S. stipitis* and *Spathaspora passalidarum* are the only known yeasts that accumulate minor amounts of polyols during xylose fermentation. They have NAD(P)H-dependent XR (Cadete et al., 2013; Veras et al., 2017) with the highest selectivities to NADH, and also possess *PHO3.2* orthologs.

Xylitol, Ethanol, and Acetate. To our knowledge, this phenotype has only been observed in *P. tannophilus*; it may also be present in a recently sequenced relative *Candida peltata*. *P. tannophilus* exhibits significant polyol yield compared to *S. stipitis* and *S. shehatae*. The polyol yield results from an inability to reoxidize NADH. This has been attributed to an XR that is solely catalyzed by NADPH, a second XR that has a significantly higher activity with NADPH than NADH (Ditzelmüller et al., 1984a,b, 1985; Verduyn et al., 1985), and a XDH that has less favourable enzyme kinetics in converting xylitol to xylulose (Yang and Jeffries, 1990). NADPH regeneration is likely to be driven by NADP-dependent acetaldehyde dehydrogenase (*ALD6.1*) (Correia et al., 2017) in *P. tannophilus*. In contrast, CTG yeasts do not have *ALD6.1* and likely use NADH kinase during oxygen limitation. Curiously, Jeffries (1983) found that *P. tannophilus* accumulated ethanol aerobically when grown with glucose and nitrate.

6 *UTR1* amino acid alignment

Flux balance analysis predicts NADPase and NADH kinase are required to balance redox cofactors during xylose fermentation. We were unable to confirm *S. stipitis* Utr1p activity using *Escherichia coli* or *K. phaffii* as expression hosts. The protein alignment of the CTG clade and Saccharomycetaceae Utr1p sequences show the CTG clade has unique motifs at the N and C-termii.

UTR1 NAD(H) kinase protein alignment

■ conserved submotif in CTG clade
■ conserved submotif in Saccharomycetaceae clade

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S_stipitis      .....
D_hanseni      .....
M_guilliermondii .....
C_albicans     .....MSHKKTQSQLSSQMKNLNTPPIDFNSTSSNNTMPSEPNSSQPQQQSSQPEAKTEP
M_farinosa     .....
C_tenuis       .....
C_parapsilosis .....MSDSPQSQTLEQLKNLSTKVKDKDALHPLTKLVSPSEPVDIQQ
C_orthopsilosis .....MSDYTQSQLTEQLKHLSTKSERDVLHPLTKIVSPSEPMDL
C_dubliniensis MSHNTQSQLSSQMKNLDTSPISINPTSTNITMPEPEPKPQSSQSSQPEAKTEP
C_tropicalis   .....
C_maltosa      .....
T_delbrueckii .....
E_gosypii     .....
K_lactis      .....
K_marxianus    .....
L_thermotolerans .....
Z_bailii      .....
Z_rouxii      .....
C_glabrata     .....
N_castelli    .....
S_cerevisiae   .....
V_polyspora    .....

                                     1      10      20      30      40
S_stipitis      .....MFTDKKSQLTARLHGEMTNTDRPTNPTKRASSRGYGTVVINGHS
D_hanseni      .....MSKYGLESH
M_guilliermondii .....MNPQSIPPRRVSGSIMANSHA
C_albicans     QTIRPATFTTSGNSSSSSISTLSADIIQPLHQLSINNNNSTVTQPAPQSSSFQRRNNP
M_farinosa     .....MSKNDGIERSI
C_tenuis       .....MAIAVSPKSTAIEPTKEAPLVPSSPVAVAPGSVSR
C_parapsilosis IREGRQQRERTSESSSSSISLSPTEILEPPSNQPQVNSTPALASQPQHSSFRKHSI
C_orthopsilosis QQIRAAARQQRTESSSSSSVSSLPEVLDPSPNQAPNSTPTLASPQPQHTSFRKHSI
C_dubliniensis QTIRAAATFTTSGNSSSSSISTLSDIIQPLHQLSLNNNNNSTVTQPQTSQSSSFQRRSNP
C_tropicalis   .....MYATEEKKIEISDLRFLQQAIEYSTAMNNNNNNNNSSSSSNY
C_maltosa      .....
T_delbrueckii .....MSLPNSAEECVTR
E_gosypii     .....MVKRRQRAPVTRASTTEKRP LNHPDWV
K_lactis      .....MVEGHPLEKVLASALTSSSNSSSRSSIPLTFEVTHQHKTIKRFQNV
K_marxianus    .....MVNHPLEEKALPTGMECSSNSSSR
L_thermotolerans .....MARHGDAPFVPLSRELSPLSRTLTDGGEKSHGT
Z_bailii      .....MVIPIPMDEILEQDSE
Z_rouxii      .....MGSLSNSSNMDSIDDT
C_glabrata     .....MIETKDDKHYMVFPPIEDTSEITMYN
N_castelli    .....MKENDMNGVDKVVNE
S_cerevisiae   .....
V_polyspora    .....MKRS

                                     50      60      70      80      90
S_stipitis      I I A N T S H A N G F R S Q S Q S Q I Q S Q N S S G T T S P V G N H I S R E A P M I H N K L Y C E Q V N K K I . . .
D_hanseni      Q S Q L T Q G L R R N R S H R Q S L G Q N K L S K S P W E E N D A L N R E A P M I H N K L Y C D S V K K N V K L R
M_guilliermondii A S P S P I V S P T A A R P I D T E A F S P H G T I S P L T S S S P V S A P P M I H N K L Y C E Q A R . . . . .
C_albicans     Q R F N R N Q L N V Y T . D F N S T T S S A S S I S S S P K D F F T R E P . . P R I H S K L I C E E I A S A N N . .
M_farinosa     E S D K T T Q I V E D E V S N R D S G D S E S R S P T Q G D S T K R Y V P G L P I T H N K L Y H G S A K K N S R L K
C_tenuis       K P S G G A H K N G V R K H R K S L R E G G S S R S T S P D G S S I R R V P P K I H N K L Y C E Q V N K I K . . .
C_parapsilosis H K F N R S Q Y S L S S M S S T P Y G S T V P S I S S S P K D N F A R E P . . P R I H A K L Y C E D V A K S N . .
C_orthopsilosis H K F N R S Q Y S L S S I S S T P Y G S T V P S M S S S P K D N F A R E P . . P R I H A K L Y C E D V A K N N . .
C_dubliniensis Q R F S R N Q L N V Y T . D F G S T T S S V S S M S S S P K D F F T R E P . . P R I H N K L I C E E I A S A N N . .
C_tropicalis   H R A Q F T T G S S T T T N S S T S S L S E L T T S S S F Q R K N N F V P H S P K I H S K L I C D D V K A A S . . G
C_maltosa      .....MSTSNST
T_delbrueckii I Y K D C D K K S K G N S K T V E Q K N T S Y T K Q R R K S A T H R S V P S L G F C K E L N Y I D D D K K R E R I N
E_gosypii     L V A S E D T M G H I S D D D S K A S S V N L A L I D D S E Q D I V S V T D E P K L V E M A A E V G A A A A D T I A
K_lactis      L T S D S A T Q D D G N D D P S R N Q G N E V S E Q F H L L Q Y P E Q H Q H Q N K H Q H Q H Q Q H E K G D L D
K_marxianus    T S V P L S F E V K R Q N R H N G C E R F Q N V M C S D G M E P N D P D H L L H K E L Q Q V D D F Q G E E N D N V D
L_thermotolerans N S K G K G E I T N K S K R S K S V S H L A D R N K I L G S R M T P I K P E E Q K V P E E P L E D F L E E P H R V D
Z_bailii      Q S S S A E D E G T L A G P I A Q V R S L Q D I K N D R D S K I T R S D P A L D G F R A V K T G D D G N . C E R I R
Z_rouxii     L E S E R S S S N S V S I P M S Q C R S L Q D I R R D N R E N T V K S D S S I R F F P D Q H V L D E G N E R I R N I
C_glabrata     E E M K R T D S C T K E K A L Q K W L A D A E E S S S S A S E Y K I A N Q D T S R H D S D V A L D I S N A K D M L R
N_castelli    S S T A L S E K G E S C T S T S S F P K L Q K A S S V P P K L T K I S Q D S E S Y H D D L A L E D G S P L D T C A
S_cerevisiae   E D G R N D H H N N N N N L M K K A M M N N E Q I D R T Q D I D N A K E M L R K I S S E S S S R R S S L L N K D S S
V_polyspora    L E L I K K D G D V V S P D L K L A R T K P D T S D I K L K W K L Y D E P K S T D I E S K T K K I T D G Y D G C I
    
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	100	110	120	130	140	150			
<i>S_stipitis</i>	..TSSTN	MLQK	...SVD.E	IRSVKSH	TELAETAN	GVRMLAKNLSKTTIQ	LDVRAIMI		
<i>D_hanseni</i>	..QVSSV	LSRL	...GTD.D	LRSVKSH	TELAETAN	GVRMLAKNLSKTTIQ	LDVRAIMI		
<i>M_guilliermondii</i>	..SKPKK	ASATL	KLSD.P.E	LKTVRSH	AEALAO	TAN	GVRMLAKNLSKTTIQ	LDVRAIMI	
<i>C_albicans</i>	..RAAKE	VL SRL	...STD.E	LRSVKSH	TELAETAN	GVRMLAKNLSKTTIQ	LDVRAIMI		
<i>M_farinosa</i>	..RLPSN	VLRLK	...SNE.D	LNRVRS	TELAETAN	GVRMLAKNLSKTTIQ	LDVRAIMI		
<i>C_tenuis</i>	..RAPST	VLNKL	...NHD.E	LKSVRS	TELAETAN	GVRMLAKNLSKTTIQ	LDVRAIMI		
<i>C_parapsilosis</i>	..KTAKD	VL SRL	...SSD.E	LRSVKSH	TELAETAN	GVRMLAKNLSKTTIQ	LDVRAIMI		
<i>C_orthopsilosis</i>	..KTAKD	VL SRL	...SSD.E	LRSVKSH	TELAETAN	GVRMLAKNLSKTTIQ	LDVRAIMI		
<i>C_dubliniensis</i>	..RAAKE	VL SRL	...STD.E	LRSVKSH	TELAETAN	GVRMLAKNLSKTTIQ	LDVRAIMI		
<i>C_tropicalis</i>GN.T	PRS	IKSH	TELAETAN	GVRMLAKNLSKTTIQ	LDVRAIMI	
<i>C_maltosa</i>	LTTSSTT	TSASS	TSISTT	PRS	AGSSF	THLAE	TAN	GVRMLAKNLSKTTIQ	LDVRAIMI
<i>T_delbrueckii</i>	DLNEAKA	MIRRL	SGDSHPK	VTA	TAKSH	FQLSS	TAN	GVRMLAKNLSKTTIQ	LDVRAIMI
<i>E_gossypii</i>	AAKDSQRS	EDSL	KPLPHQC	MRK	VKSYA	QLSS	TAN	GVRMLAKNLSKTTIQ	LDVRAIMI
<i>K_lactis</i>	EVLCTQR	MFRKL	STGSDD	..VKK	VYSHA	QLSS	TAN	GVRMLAKNLSKTTIQ	LDVRAIMI
<i>K_marxianus</i>	EMLTTER	MFRKL	STGSDD	..MKA	YSHA	QLSS	TAN	GVRMLAKNLSKTTIQ	LDVRAIMI
<i>L_thermotolerans</i>	...TAEK	VFRRL	SVGSND	..MR	RATSH	QLSS	TAN	GVRMLAKNLSKTTIQ	LDVRAIMI
<i>Z_bailii</i>	NINDAKEM	IKQLS	..VGD	KLNS	AKSQLK	LSSTAN	GVRMLAKNLSKTTIQ	LDVRAIMI	
<i>Z_rouxii</i>	..NDAKE	MIKQL	LNIGGK	..RL	TS	AKSQLK	LSSTAN	GVRMLAKNLSKTTIQ	LDVRAIMI
<i>C_glabrata</i>	RISSERS	SPMSM	...SAHNT	SKS	SNTH	FQYAS	TAN	GVRMLAKNLSKTTIQ	LDVRAIMI
<i>N_castelli</i>	ELKTIVKE	MVRR	ISSESP	P.GAK	HKKH	VEYAS	TAN	GVRMLAKNLSKTTIQ	LDVRAIMI
<i>S_cerevisiae</i>	LVNGNANS	GGGT	SINGTRGS	SKS	SNTH	FQYAS	TAN	GVRMLAKNLSKTTIQ	LDVRAIMI
<i>V_polyspora</i>	EVSRRDS	KSTSK	SMPNVSEM	KAS	FKPH	FKYAS	HAN	GVRMLAKNLSKTTIQ	LDVRAIMI

	160	170	180	190	200									
<i>S_stipitis</i>	VTKARDNS	LIYLTR	EVVEW	LLTQ	ERDIT	VYVDAK	LENSK	RENTDD	IRTQIPKANG	LLR				
<i>D_hanseni</i>	VTKARDNS	LIYLTR	EIVDY	LLAKN	KDIT	VYVDRN	LQSKR	RENAVN	LYETVPKAKK	YVK				
<i>M_guilliermondii</i>	VTKARDNS	LVVLT	RELV	EWLL	GO	SRDIA	VYVD	KGLEK	SKRE	NARE	I	FESSEKA	QNR	LLR
<i>C_albicans</i>	ITKARDNG	LIYLTK	EVVEW	LLDQ	HPHIT	IYAD	EKLAK	SKRE	NPES	II	ANYPNGC	KLK		
<i>M_farinosa</i>	VTKARDNS	LIYLTR	EVVEW	LLTR	DKETI	VYVD	KNLQ	DSKRE	DLAG	LH	ETVPKAKT	HVK		
<i>C_tenuis</i>	VTKARDNS	LIYLTR	EVVEW	LLLN	KRNKI	IYVD	SKLEA	SKRE	NYSG	L	VESVPTAR	QYVK		
<i>C_parapsilosis</i>	VTKARDNA	LVLVLT	RELV	EWLL	SNHTE	ITVF	VDSK	LEH	SKRE	DS	KRMV	KQYPSAS	KHLR	
<i>C_orthopsilosis</i>	VTKARDNA	LVLVLT	RELV	EWLL	LNHTE	ITVF	VDSK	LEH	SKRE	DS	KRMV	KQYPSAS	KHLR	
<i>C_dubliniensis</i>	ITKARDNG	LIYLTK	EVVEW	LLGQ	HPQIT	IYVD	EKLEK	SKRE	NPQD	II	TNYPNGC	KLK		
<i>C_tropicalis</i>	ITKARDNS	LIYLTK	EVVEW	LLGQ	HPHIT	IYVD	EKLEK	SKRE	NPQD	II	TNYPNGC	KLK		
<i>C_maltosa</i>	ITKARDNS	LIYLTK	EVVEW	LLGQ	HPHIT	IYVD	EKLEK	SKRE	NPQD	II	TNYPNGC	KLK		
<i>T_delbrueckii</i>	VTKARDNS	LIYLTR	EVVEW	LLLN	KRNKI	IYVD	SKLEA	SKRE	NYSG	L	VESVPTAR	QYVK		
<i>E_gossypii</i>	VTKARDNS	LIYLTR	EVVEW	LLLN	KRNKI	IYVD	SKLEA	SKRE	NYSG	L	VESVPTAR	QYVK		
<i>K_lactis</i>	VTKARDNS	LIYLTR	EVVEW	LLLN	KRNKI	IYVD	SKLEA	SKRE	NYSG	L	VESVPTAR	QYVK		
<i>K_marxianus</i>	VTKARDNS	LIYLTR	EVVEW	LLLN	KRNKI	IYVD	SKLEA	SKRE	NYSG	L	VESVPTAR	QYVK		
<i>L_thermotolerans</i>	VTKARDNS	LIYLTR	EVVEW	LLLN	KRNKI	IYVD	SKLEA	SKRE	NYSG	L	VESVPTAR	QYVK		
<i>Z_bailii</i>	VTKARDNS	LIYLTR	EVVEW	LLLN	KRNKI	IYVD	SKLEA	SKRE	NYSG	L	VESVPTAR	QYVK		
<i>Z_rouxii</i>	VTKARDNS	LIYLTR	EVVEW	LLLN	KRNKI	IYVD	SKLEA	SKRE	NYSG	L	VESVPTAR	QYVK		
<i>C_glabrata</i>	VTKARDNS	LIYLTR	EVVEW	LLLN	KRNKI	IYVD	SKLEA	SKRE	NYSG	L	VESVPTAR	QYVK		
<i>N_castelli</i>	ITKARDNS	LIYLTR	EVVEW	LLLN	KRNKI	IYVD	SKLEA	SKRE	NYSG	L	VESVPTAR	QYVK		
<i>S_cerevisiae</i>	VTKARDNS	LIYLTR	EVVEW	LLLN	KRNKI	IYVD	SKLEA	SKRE	NYSG	L	VESVPTAR	QYVK		
<i>V_polyspora</i>	VTKARDNS	LIYLTR	EVVEW	LLLN	KRNKI	IYVD	SKLEA	SKRE	NYSG	L	VESVPTAR	QYVK		

	210	220	230	240	250	260																								
<i>S_stipitis</i>	FWDK	KKFAL	KNPEK	FDLV	VT	LG	GGD	TVLY	ASNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H				
<i>D_hanseni</i>	YWDK	KKFAL	QNPEK	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H				
<i>M_guilliermondii</i>	FWDK	QFAM	RNPEI	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H				
<i>C_albicans</i>	YWNK	KLTT	KNPEI	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H				
<i>M_farinosa</i>	FWT	RKLS	MRNPEA	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H				
<i>C_tenuis</i>	FWT	KEFT	INNPEI	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H				
<i>C_parapsilosis</i>	YWNK	KLTT	KNPEI	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H				
<i>C_orthopsilosis</i>	YWNK	KLTT	KNPEI	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H				
<i>C_dubliniensis</i>	YWNK	KLTT	KNPEI	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H				
<i>C_tropicalis</i>	FWT	RKLS	MRNPEA	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H				
<i>C_maltosa</i>	YWT	KGLA	MKHP	EL	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H			
<i>T_delbrueckii</i>	YWNK	KLTT	KNPEI	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H				
<i>E_gossypii</i>	YWT	PELV	SERGD	LD	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H			
<i>K_lactis</i>	YWT	PELV	SERGD	LD	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H			
<i>K_marxianus</i>	YWT	PELV	SERGD	LD	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H			
<i>L_thermotolerans</i>	TWS	PELV	AKKDD	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H				
<i>Z_bailii</i>	YWD	DDF	I	AKH	DF	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H		
<i>Z_rouxii</i>	YWD	KEF	V	AQ	H	DF	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H	
<i>C_glabrata</i>	YWT	KEF	I	D	N	DF	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H	
<i>N_castelli</i>	YWN	QEF	I	A	K	H	DF	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H
<i>S_cerevisiae</i>	YWT	KDF	I	R	H	DF	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H	
<i>V_polyspora</i>	YWN	QEF	L	D	N	N	DF	FDLV	VT	LG	GGD	TVLY	VSNLF	FOR	V	PPV	ISF	AL	GL	S	G	F	L	T	N	F	K	F	E	H

	270	280	290	300	310
<i>S_stipitis</i>	F R E R M N T V I A S G V K A Y L R M R F T C R V H T A D G K L I C E Q Q V L N E L V T				
<i>D_hanseni</i>	F R E R M S N V L D A G V R A Y L R M R F T C R V H R A D G K L I C E Q Q V L N E L V V				
<i>M_guilliermondii</i>	F P K H M V K V L E R G V R A N L R M R F T C R V H H A D G R L V S E Q Q V L N E L V V				
<i>C_albicans</i>	F R T V L S K C F D S G V K A N L R M R F T C R V H T D E G K L I C E Q Q V L N E L V V				
<i>M_farinosa</i>	F R E K M T Q V L E S G V R A Y L R M R L T C R V H T A D G K L V C E Q H V L N E L V V				
<i>C_tenuis</i>	F R S K M L S V L E S G V R A N L R M R F T A R V H R S D G Q L V C E Q Q V L N E L V V				
<i>C_parapsilosis</i>	Y K S K L N H C L D S G V K A N L R M R F T C R V H T A E G K L I C E Q Q V L N E L V V				
<i>C_orthopsilosis</i>	Y K S K L N H C L D S G V K A N L R M R F T C R V H T A E G K L I C E Q Q V L N E L V V				
<i>C_dubliniensis</i>	F R T V L N K C F D S G V K A N L R M R F T C R V H T D E G K L I C E Q Q V L N E L V V				
<i>C_tropicalis</i>	F K R I L N R C I E S G V K A N L R M R F T C R V H S S D G K L I G Q Y Q T L N E L V V				
<i>C_maltosa</i>	F K N V L N T C I N S G V N A N L R M R F T C R V H N N E G K L I A Q Q Q V L N E L V V				
<i>T_delbrueckii</i>	F R E D L I A T V L N N R I K I T N L R M R L D C K A Y R R R P P I I D E N T G K K I C V T E L V G G H Q V L N E L T I				
<i>E_gossypii</i>	F R E D L S K A L Q S K I R T N M R M R L C C K V Y R R L P C S S S K G N K K K Y E Y V E T H H V L N E L T I				
<i>K_lactis</i>	F K H A L S K I L N K I K T K M R M R L C C Q L F R K R I K K V D E E A R K T H I K Y T M E G E Y H V L N E L T I				
<i>K_marxianus</i>	F K H D L S K V L N K I K S K M R M R L C C Q L Y K R R I K R K D E E S G K T H I K Y D L K I G E Y H V L N E L T I				
<i>L_thermotolerans</i>	F R Q S L P R V L N S K I R S K M R M L C C R V F R K R K P N K E N N N S R S R K K F T M I G E Y H V L N E L T I				
<i>Z_bailii</i>	F R E D L P K I L N N K I K T N L R M R L E C K V Y R R H P P V L D P R T G E K I A V A E L I S Q R Q V L N E L T V				
<i>Z_rouxii</i>	F R E D L P K I L N N K I K T N L R M R L E C K V Y R C H P P M V D S R T G E K V A V A E L M Q R Q I L N E L T I				
<i>C_glabrata</i>	F R T D L T K I L N S K V K T N L R M R L E C K V Y R R H E P E V D P E T G K K I C V V E H I D T H H I L N E V T I				
<i>N_castelli</i>	F K Q D L R K I L T E K V K I N L R M R L E C K I Y H R N K P E Y D S E T G K K V C I M E Q V S T H H V L N E M T I				
<i>S_cerevisiae</i>	F R E D L P R I M N H K I K T N L R M R L E C T I Y R R R H P E V D P N T G K K I C V V E K L S T H H I L N E V T I				
<i>V_polyspora</i>	F R K D L P L I L N N K I K T N L R M R L E C K V F R R R D P V V N P E T G K K I F V S E L I S E H H V L N E L T V				

	320	330	340	350	360
<i>S_stipitis</i>	D R G P S P Y V T Q L E L Y G D G S L L T I A Q A D G L I I A T P T G S T A Y S L S A G G S L V H P G V S A I S V T				
<i>D_hanseni</i>	D R G P S P Y V T Q L E L Y G D G S L L T V A Q A D G L I I A T P T G S T A Y S L S A G G S L V H P G V S A I S V T				
<i>M_guilliermondii</i>	D R G P S P Y V T Q L E L Y G D G S L L T V A Q A D G L I I A T P T G S T A Y S L S A G G S L V H P G V S A I S V T				
<i>C_albicans</i>	D R G P S P Y V T H L E L Y G D G S L L T V A Q A D G L I I A T P T G S T A Y S L S A G G S L V H P G V S A I S V T				
<i>M_farinosa</i>	D R G S S P Y V T Q L E L Y G D D S L L T I A Q A D G L I I A T P T G S T A Y S L S A G G S L V H P G V S A I S V T				
<i>C_tenuis</i>	D R G P S P Y V T N L E L Y G D G S L L T I A Q A D G L I I A T P T G S T A Y S L S A G G S L V H P G V S A I S V T				
<i>C_parapsilosis</i>	D R G P S P F V T N L E L Y G D G S L L T I A Q A D G L I I A T P T G S T A Y S L S A G G S L V H P G V S A I S V T				
<i>C_orthopsilosis</i>	D R G P S P F V T N L E L Y G D G S L L T I A Q A D G L I I A T P T G S T A Y S L S A G G S L V H P G V S A I S V T				
<i>C_dubliniensis</i>	D R G P S P Y V T H L E L Y G D G S L L T V A Q A D G L I I A T P T G S T A Y S L S A G G S L V H P G V S A I S V T				
<i>C_tropicalis</i>	D R G P S P Y V T Q L E L Y G D G S L L T V A Q A D G L I I A T P T G S T A Y S L S A G G S L V H P G V S A I S V T				
<i>C_maltosa</i>	D R G P S P F V T Q L E L Y G D G S L L T V A Q A D G L I I A T P T G S T A Y S L S A G G S L V H P G V S A I S V T				
<i>T_delbrueckii</i>	D R G P S P F I S M L E L Y G D N S L L T M A Q A D G L I I A T P T G S T A Y S L S A G G S L V Y P S V N A I A V T				
<i>E_gossypii</i>	D R G P S P F L S M L E L Y G D H S L L T V A Q A D G L I I A T P T G S T A Y S L S A G G S L V Y P S V N A I A V T				
<i>K_lactis</i>	D R G P S P F I S M L E L Y G D G S L L T V A Q A D G L I I A S P T G S T A Y S L S A G G S L V Y P S V N A I A V T				
<i>K_marxianus</i>	D R G P S P F I A M L E L Y G D G S L L T V A Q A D G L I I A S P T G S T A Y S L S A G G S L V Y P S V N A I A V T				
<i>L_thermotolerans</i>	D R G P S A F I S M L E V F G D N S L L T V A Q A D G L I I A T P T G S T A Y S L S A G G S L V Y P S V N A I A V T				
<i>Z_bailii</i>	D R G P S P F I S N L D Y G D D S L L T V A Q A D G L I I A T P T G S T A Y S L S A G G P L V Y P S V N A V V V T				
<i>Z_rouxii</i>	D R G P S P F I S N L E V Y G D N S L L T V A Q A D G L I I A T P T G S T A Y S L S A G G P L V Y P S V N A V C V T				
<i>C_glabrata</i>	D R G P S P F I S M L E L Y G D G N L M T V A Q A D G L I I A T P T G S T A Y S L S A G G S L Y P T V N A I A V T				
<i>N_castelli</i>	D R G T C P F I S N L E L Y G D D S L M T V A Q A D G L I I A T P T G S T A Y S L S A G G A L V H P S I N A I S V T				
<i>S_cerevisiae</i>	D R G P S P F L S M L E L Y G D G S L M T V A Q A D G L I A A T P T G S T A Y S L S A G G S L V H P T V N A I A L T				
<i>V_polyspora</i>	D R G S S P F I S M L E L Y G D S L L T V A Q A D G L I V S T P T G S T A Y S L S A G G S L V Y P S V N A I A V T				

	370	380	390	400	410	420
<i>S_stipitis</i>	P I C P H T L S F R P I L L P D G M F L K V K V P D T S R S T A W A S F D G K V R T E L R K G D Y V T I Q A S P F P					
<i>D_hanseni</i>	P I C P H T L S F R P I L L P D G M F L K V K V P S T S R S T A W A S F D G K V R T E L H K G D Y V T I H A S P F P					
<i>M_guilliermondii</i>	P I C P H T L S F R P I L L P D G M V L K V R V P L T S R S T A W A S F D G K E R L E L K R G D Y V T I R A S P Y P					
<i>C_albicans</i>	P I C P H T L S F R P I L L P D G M F L K V K V P S S R A T A W A S F D G K V R T E L K K G Y V T I Q A S P F P					
<i>M_farinosa</i>	P I C P H T L S F R P I L L P D G M F L K L R V P W D S R S T A W A S F D G K V R T E L C R G D Y V T V Q A S P Y P					
<i>C_tenuis</i>	P I C P H T L S F R P I L L P D G M F L K V K V P F A S R S T A W A S F D G K V R T E L L Q G D Y V T I Q A S P F P					
<i>C_parapsilosis</i>	P I C P H T L S F R P I L L P D G M F L K I K V P T S R S T A W A S F D G K V R K E L S K G Y V T I Q A S P F P					
<i>C_orthopsilosis</i>	P I C P H T L S F R P I L L P D G M F L K I K V P L T S R S T A W A S F D G K V R K E L S K G Y V T I Q A S P F P					
<i>C_dubliniensis</i>	P I C P H T L S F R P I L L P D G M F L K V K V P S S R A T A W A S F D G K V R T E L K K G Y V T I Q A S P F P					
<i>C_tropicalis</i>	P I C P H T L S F R P V L L P D G M F L K V K V P D G S R A T A W A S F D G K R T E L K K G D Y V T I Q A S S F P					
<i>C_maltosa</i>	P I C P H T L S F R P I L L P D G M N L K V K T P N S R G T A W A S F D G K V R T E L K K G Y V T I Q A S S F P					
<i>T_delbrueckii</i>	P I C P H T L S F R P I L L P E S M T L K V R V S M K S R A T A W A A F D G K S R L E L K K G D Y T I T I Q A S P Y S					
<i>E_gossypii</i>	P V C P H T L S F R P I L L P D S M R L R I K V P K R S R G T A W A A F D G K S R V E L Q K G D Y I S V T A S P Y S					
<i>K_lactis</i>	P I C P H T L S F R P I L L P D S M T L K V K V P K A S R S T A W A A F D G K N R V E M K R G D Y I V I N A S P Y S					
<i>K_marxianus</i>	P I C P H T L S F R P I L L P D S M T L K V K V P R T S R S T A W A A F D G K N R V E M Q K G D Y I V I T A S P Y S					
<i>L_thermotolerans</i>	P I C P H T L S F R P I L L P D S M K L K V K V P L N S R A T A W A A F D G K N R V E L F K G D Y V C I T A S P H S					
<i>Z_bailii</i>	P V C P H T L S F R P I L L P D S M N L K V K V S M K S R A T A W A S F D G K E R T E L Q K G D Y I T V Q T S P Y A					
<i>Z_rouxii</i>	P I C P H T L S F R P I L L P D S M N I K I R V S Q G S R A T A W A A F D G K R I E L Q K G D Y I T V Q S S P Y A					
<i>C_glabrata</i>	P I C P H T L S F R P I L L P D S M T L K V K V S L K A R G T A W A G F D G K D R C E L K Q G D F I T I S A S P Y V					
<i>N_castelli</i>	P I C P H T L S F R P I L L P E N M N L K V K V S L K A R G N A W A S F D G K G R F E L Q K G D Y I T V S A S P Y A					
<i>S_cerevisiae</i>	P I C P H A L S F R P I L L P E S I N L K V K V S M K S R A P A W A A F D G K D R I E L Q K G D F I T I C A S P Y A					
<i>V_polyspora</i>	P I C P H T L S F R P I L L P D S M N L K V R V S L K S R A T A W A A F D G K N R V E L Q P G D Y I S I A A S P Y A					

	430	440	450	460	470
S_stipitis	FPTV	ISSKTEYIDSVSRNLNWNARE	OKPFSHLISDQSQ	RRMMRKSTSAA
D_hanseni	FPTV	ISSKTEYIDSVSRNLNWNARE	OKPFTHLISENN	KKIYENSPAN
M_guilliermondii	FPTV	ISSKTEYIDSVSRNLHWNVRE	SOKPFTHLISAKN	KQLFEGGHGDAQFD	IDYDE
C_albicans	LPTV	MSSKTEYIDSVSRNLHWNIRE	QOKPFSSYLKPET	RQSI	AESERLDNLHISSEQD
M_farinosa	FPTV	ISSKTEYIDSVSRNLNWNARE	KOKPLSSYLSQES	TDMLNTEAAQA
C_tenuis	FPTV	ISSKTEYIDSVSRNLHWNVRE	QORPFSYKLNDS	NEDVESQI
C_parapsilosis	FPTV	IASKTEYMDSVSRNLNWNVRE	QOKPFSSYLKPET	RQMMV
C_orthopsilosis	FPTV	IASKTEYMDSVSRNLNWNVRE	QOKPFSSYLKPET	RQMMV
C_dublinsiensis	LPTV	MSSKTEYIDSVSRNLHWNIRE	QOKPFSSYLKPET	RQSI	AESE.....RLENLHI
C_tropicalis	FPTV	IASPTEYFDSVSRNLHWNVRE	QOKPLGNQTKDID	GDM	DNLH.....ISSEQ
C_maltosa	FPTV	VSTKTEYFDSVSRNLHWNVRE	QOKPL.....KTEE	.G.....EDLEN	
T_delbrueckii	FPTV	ESHPTDFIDSI	RTLNWNVRE	QORSFTHMLSRKN	QEKYASDAGNTRADDEEVEE
E_gossypii	FPTV	EHSPTEFIDSI	RTLNWNVRE	SOKSYAHMLSQKN	QLRYESDACHKTPTSSDSAD
K_lactis	FPTV	EARSTEFIDSI	RTLNWNVRE	SOKSFTHMLSRKN	QOKYEIHTVTRQDSEEEEE
K_marxianus	FPTV	EARSTEFIDGI	RTLNWNVRE	SOKSFTHMLSRKN	QOKYEIHTKAKLQSEEEEE
L_thermotolerans	FPTV	ESSPTEFIDSI	RTLNWNVRE	QOKSFAMHLSQKN	QKNYVSDTEKQKQDQPEVRA
Z_bailii	FPTV	ESHRTTEFIESIS	RSLNWNVRE	QOKSFTHMLSRKN	REKYVTDKS...GRDEWEE
Z_rouxii	FPTV	ESHSTTEFIESIS	RSLNWNVRE	QOKSFTHMLSRKN	QEKYVTDKE...GEDDLH
C_glabrata	FPTV	ESSPTEFINIS	RTMNVNRE	QOKSFTHMLSRKN	KEKYNTTEKVVRESKAKSEEEEE
N_castelli	FPTV	ESSPTEFFDGI	RTLNWNVRE	QOKSFTHMLSRKN	RKLAIESNYEHDSDDELEE
S_cerevisiae	FPTV	EASPTTEFINIS	RQLNWNVRE	QOKSFTHMLSRKN	QEKYAHEANKVRNQAEPLEV
V_polyspora	FPTV	ESSSSEFIDSI	RTLNWNVRE	QOKSFTHMLSRKN	KEKFAIESYKMDSDSSVVEE

	480	490	500	510	520	530
S_stipitis	TQDLENLHIDNGAAED	DFDINYS	SSDSDSTPSDNETEEDLPY	IPLPGDGINTPPPGNT		
D_hanseni	LDSHIN...NLSLAPDE	DFDIDY	TDEENSSLSDEIDPFVPSPEEGLET	PPPTYGT...F		
M_guilliermondii	NDELGTADTEITGSE	PESEVEY	DDDRPCFAHPNAKITLDHQ		
C_albicans	ESNH...EEPEIT	DFDINYT	DNERDSSSSTPSEESNEECANTTT		
M_farinosa	DGAQHRGSRSSVPLDD	YDINYS	SAYDSSDSELNSPMPSSPNSGFC	TPFSSNFNGL...		
C_tenuis	ESLSLNASITIDNDNAD	YDINFS	DENPEENTSYSSKDIPELPLSLGGGLSTPPSH	TI		
C_parapsilosis	ENDNGEFKPEQNE	DNEDFDINYS	SDQEESEEKTSSTNTTSETNS	SEDMSYLP	LGGGTQTP	
C_orthopsilosis	ENDNGEDKPKEND	DNDFDINYS	SDQEESEEKTSSTNTTSETNS	SEDMSYLP	LGGGTQTP	
C_dublinsiensis	SSEQDEVNHEEHEIT	EDFDINYT	DNERDSSSSTPSEESNEEHV	NATT.....		
C_tropicalis	DEESEPDITEDDEED	DFDINFT	DTERSSYSSTPSSDDIHYLSTNGAET	TPQMSY...		
C_maltosa	LHISTEVSA	PHSGEEF	DFDINFD	GSFSTPNSEENLFLP	SNGGANTPQNTSYL...	
T_delbrueckii	VITDKNKKPKFRLHDNAD	DDND	ENEAELEDDTSLAPVSPKSKPIDQ	IRSPQANFTI		
E_gossypii	DKASDPPEEPAPTEQQP	ADSG	SDSSSNTGRDLSVLR	RAPRRRRKGR	RACRRPD...	
K_lactis	LEDDQSDDYSTDSDSEL	NE			
K_marxianus	LEESTNTAASD	SERDSNSE	SDSDSDVE		
L_thermotolerans	PSDEEEEGESDS	SESPQFER	PKAASSAASP	PKLPNFQL	
Z_bailii	REDDEVLVVQAEDPAQAS	NMMHKASDRNS	IGGKPSFTV		
Z_rouxii	EGDHREVVLQAEDKDKQA	QKMIERTLAEQKAEKDSVC	ANGGAAKTNE	TV.....		
C_glabrata	IEERKLS	SAFDMSSLEAVE	KKEAKEDEGDDE	DETADRCLL	KKTS	SGSK.....
N_castelli	RKLEKQLS	GHELTDSSETE	NENSNDYEEI	RVALPKIETVHV	
S_cerevisiae	IRDKYSLEADATKEN	NNGS	DES	VNCEACKL	KPSSV	PKPSQARFSV.....
V_polyspora	LEVNEKIGDERLDM	DKIES	LLDQAD	IKEKVH	FS

	540	550	560	570
S_stipitis	..YGGFEGT	CFAHHPAKITL	DSSATSTNSTRYSSSTTGSGSED
D_hanseniEDRP	CFAHHPNAKIS	INGSSSSMNFSSPTSAESD	TLTMYPKHRINNNHTPN
M_guilliermondii
C_albicans
M_farinosa	...SYDERO	CFAHHPNAKIS	INPSALNSISSSPLSTN	SEVLTINPESALHHK.....
C_tenuis	SYTNLEDRH	CFAHHPNAHVTF	GSSSDNSGHSNFL
C_parapsilosis	TNNQDDRC	CYAHHPARVHL	NGN
C_orthopsilosis	TNNQDDRC	CYAHHPARVHL	NGS
C_dublinsiensis
C_tropicalis	..LNNVDERC	CFAHHPNARVHL	SGGKS
C_maltosa	..NNIDERC	CFAHHPKARVHL	NGRE
T_delbrueckii
E_gossypii
K_lactis
K_marxianus
L_thermotolerans
Z_bailii
Z_rouxii
C_glabrata
N_castelli
S_cerevisiae
V_polyspora

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