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Supplementary Information for

Multi-layered horizontal operon transfers from bacteria reconstruct a thiamine salvage pathway in yeasts

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Other supplementary materials for this manuscript include the following:

Datasets S1 to S3
Phylogenetic analyses related files can be accessed in Figshare (DOI:
[10.6084/m9.figshare.9800636](https://doi.org/10.6084/m9.figshare.9800636))

Methods for the construction of preliminary phylogenies presented in Figure S2 and S5.

For the preliminary phylogenies presented in Figure S2, the top 5.000 BLASTp hits on NCBI (Refseq database) were selected using *St. bombicola* proteins as queries and an *e-value* cut off of $1e^{-10}$. *tenA*, *thiD*, *thiE* and *thiM* (recovered as *thiEM* because these genes are fused in all W/S-clade species) sequences from other W/S-clade species were retrieved by tBLASTx in local genome databases and the proteins were subsequently predicted in *AUGUSTUS* (web interface) using *Saccharomyces cerevisiae* as reference. Sequences with more than 95% similarity were removed with *CD-HIT* v4.6.7 (1) and the remaining sequences were aligned with *MAFFT* v7.222 (2) using an iterative refinement method (L-INS-i). For *TenA*, *ThiD*, *ThiE* and *ThiM*, poorly aligned sequences were removed with *trimAl* v1.2 (3) using its “gappyout” option. For the alignment comprising the fused *ThiEM* protein which encodes the *ThiE* and *ThiM* domains, columns with gaps in more than 10% of the sequences were removed (-gt 0.9). If this strategy removed more than 40% of the columns in the original alignment, at least 60% were conserved by adding the necessary number of columns in decreasing order of scores (-cons 60). In this way it was ensured that both *ThiE* and *ThiM* portions were conserved in the alignment. Phylogenies were constructed in *IQ-TREE* v1.6.10 (4) with 1.000 ultrafast bootstrap (5) replicates and with selection of the best fitting model. In all cases, W/S-clade sequences grouped with bacteria in the preliminary phylogenies and *W. galacta* groups with different bacteria than other W/S-clade species, suggesting an independent origin.

For the preliminary phylogeny of *YlmB* presented in Figure S5A, the top 5.000 BLASTp hits on NCBI (Refseq database) were selected using *St. bombicola* putative *YlmB* as query and an *e-value* cutoff of $1e^{-10}$. Sequences with more than 95% similarity were removed with *CD-HIT* v4.6.7 (1) and the remaining sequences were aligned with *MAFFT* v7.222 (2) using an iterative refinement method (L-INS-i). For the final phylogeny presented in Figure S5B, only the top 750 hits in UniprotKB (reference_proteomes) were selected with the addition of the closest related hits in *Bacillus subtilis* which include the characterized *YlmB* protein (6). Sequences with more than 98% similarity were removed with *CD-HIT* v4.6.7 (1) and the remaining sequences were aligned with *MAFFT* v7.222 (2) using an iterative refinement method (L-INS-i). Phylogenies were constructed in *IQ-TREE* v1.6.10 (4) with 1.000 ultrafast bootstrap (5) replicates and with the selection of best fitting model.

Supplementary figures

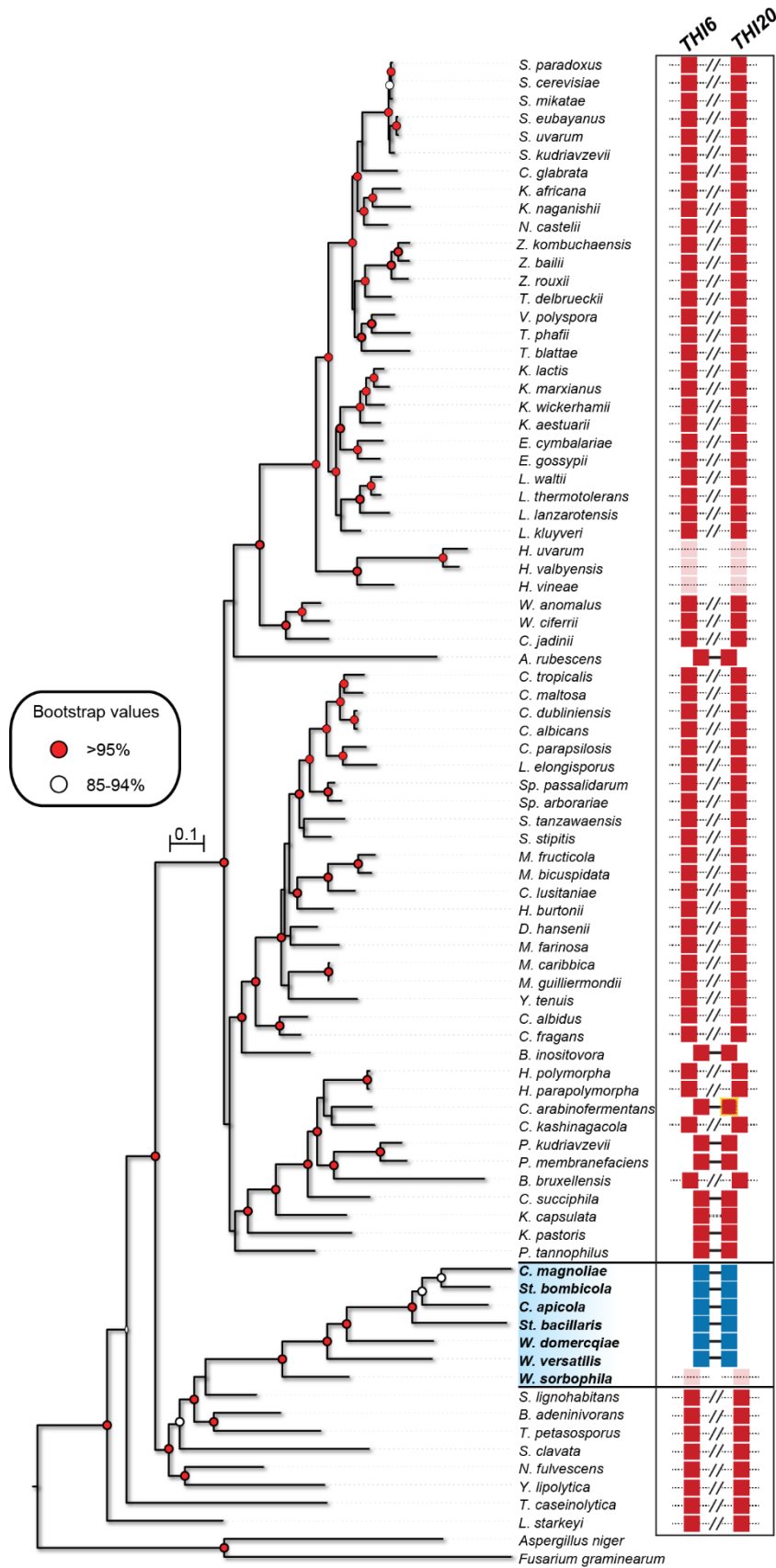


Fig. S1. Distribution of TH16 and TH20 homologues in the Saccharomycotina. The maximum likelihood (ML) tree is the one depicted in Gonçalves C. et al., 2018 (7). A tBLASTx in local genome databases (see Table S4, which includes the respective accession numbers) was performed (E -value < e^{-3}). A BLASTp in

NCBI was performed with the top hits and whenever the top BLASTp hits corresponded to the identity of the query gene, the gene was considered as being present. Physical linkage was asserted by inspecting if the top hits for *THI6* and *THI20* were contiguously located. The red and blue squares represent homologues of fungal origin (top BLASTp hits corresponded to fungal proteins) and bacterial origin (top BLASTp hits corresponded to bacterial proteins), respectively. Faded squares denote absent genes ($e\text{-value} > e^{-3}$). Physical linkage between *THI6* and *THI20* is represented by a black line linking the two squares while “//” means that the genes are distantly located in the respective genome. For *Candida arabinofementans* several paralogues of *THI20* were detected and one of the paralogues is in cluster with *THI6* while the others are located in other genomic regions. In *Kuraishia capsulata* *THI6* and *THI20* are separated by three genes.

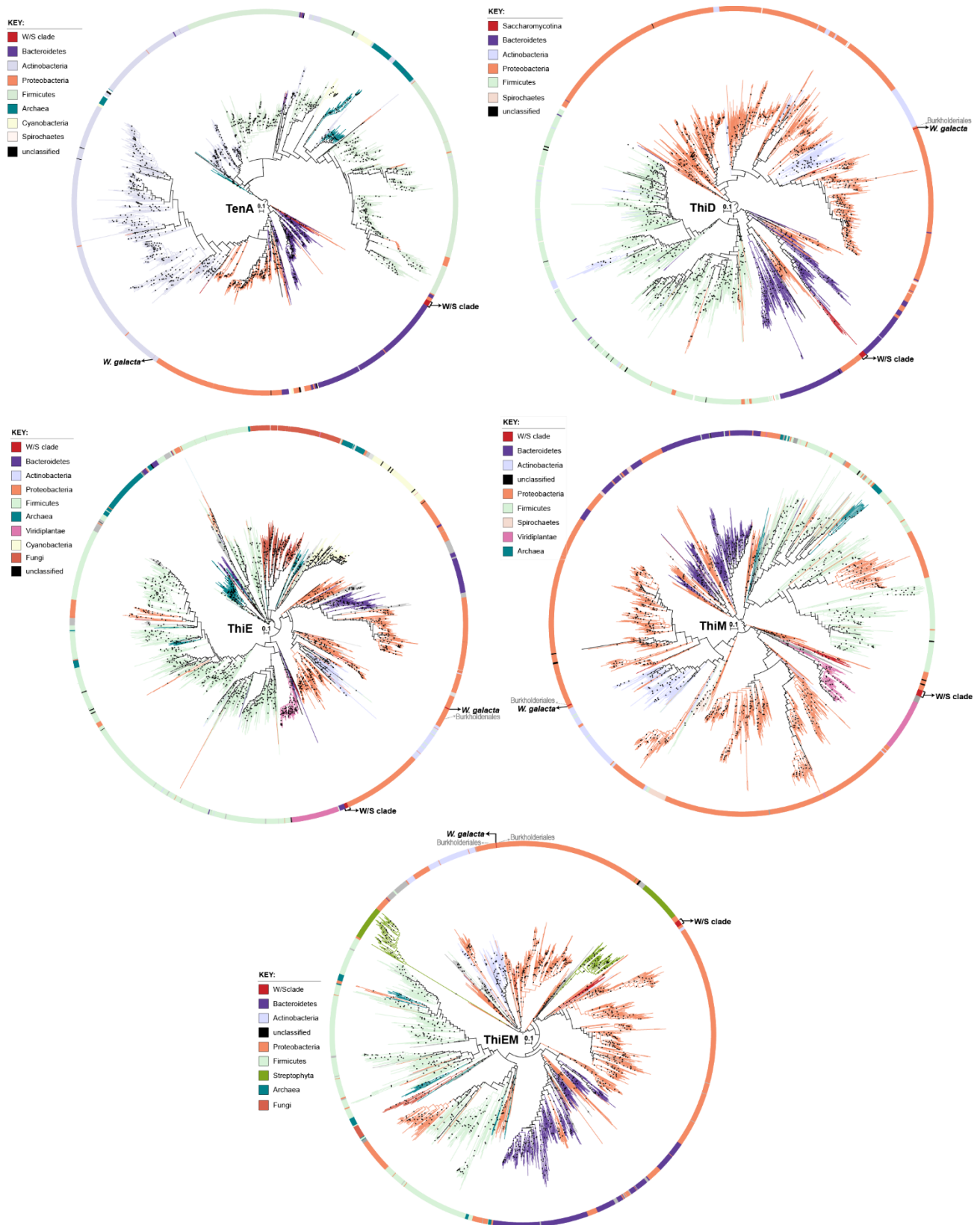


Fig. S2. Preliminary phylogenies of Thi proteins. The top 5,000 hits on NCBI (Refseq database) were selected using *St. bombycolina* proteins as queries. Taxonomic classification (Phylum for Bacteria and Kingdom/Domain for other organisms) was automatically assigned using a customized *E*-utilities script provided by Entrez Direct and is presented in the key on the left next to the respective phylogenetic tree.

Lineages with less than 20 representatives are colored in grey. All phylogenies were midpoint rooted and visualized in iTOL v4 (8).

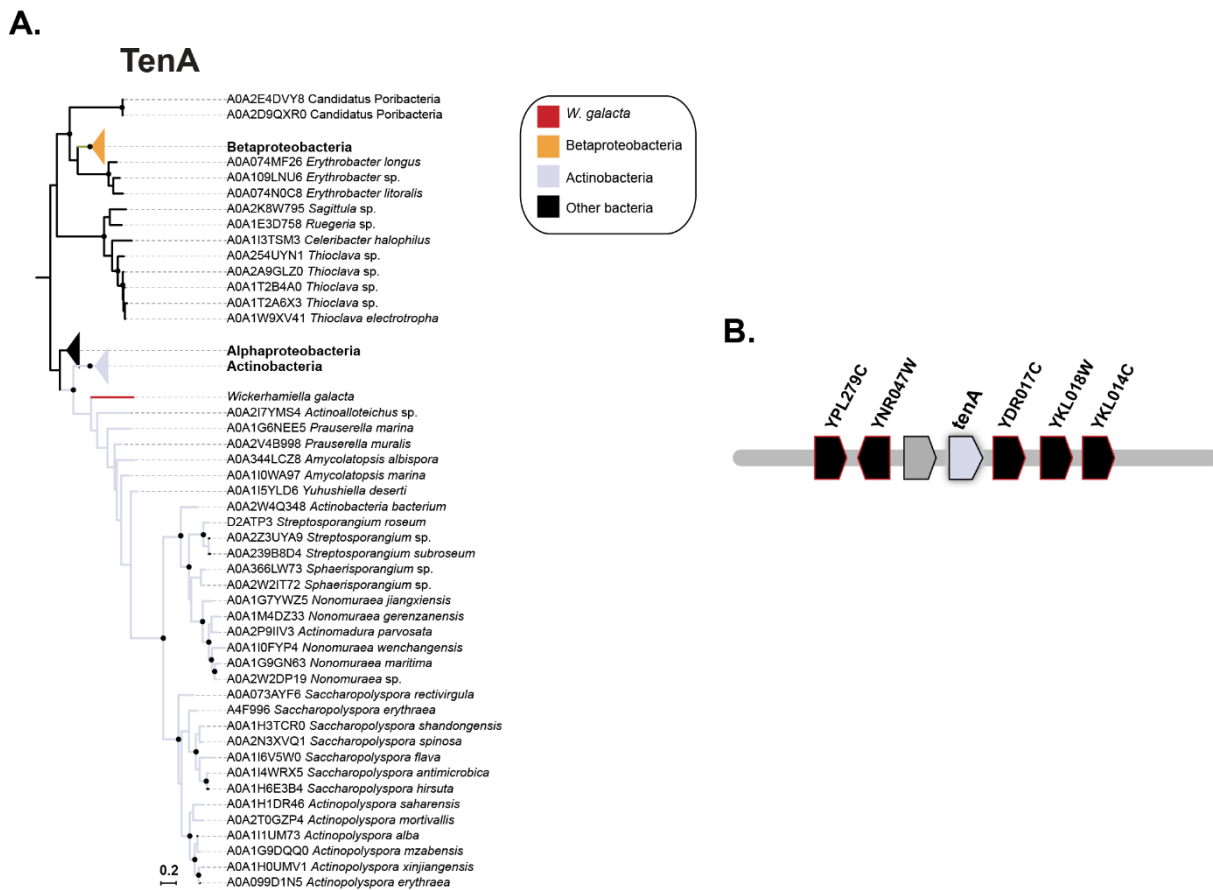


Fig. S3. Maximum likelihood phylogeny of proteins closely related to TenA from *W. galacta*. (A) Phylogeny constructed with the top 750 BLASTp hits in UniprotKB. Branches with support higher than 95% (ultrafast bootstrap) are indicated by black circles. (B) Gene organization in the vicinity of *tenA* from *W. galacta*. Genes are represented by arrows denoting relative transcriptional direction. Genes from fungal origin are outlined in red while genes with dubious annotations are represented in grey. The closest related genes in *S. cerevisiae* are shown on top of the arrows.

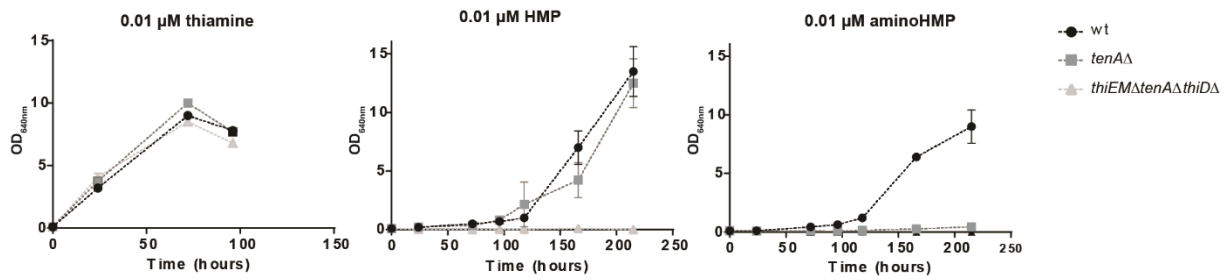


Fig. S4. Growth curves for wt and *THI* deletion mutants (*tenAΔ* and *thiEMΔtenAΔthiDΔ*) grown in YNB medium without aminoacids and without thiamine supplemented with 0.01 μM of thiamine, 0.01 μM of HMP or 0.01 μM of aminoHMP, as indicated. Cultures were pre-grown in the respective media for one to four days (depending on the lag-phase) until late exponential phase was reached and were subsequently inoculated in the same medium to an OD_{640nm} of 0.2. Growth was monitored for 220 hours at 30°C with shaking (180 r.p.m.).

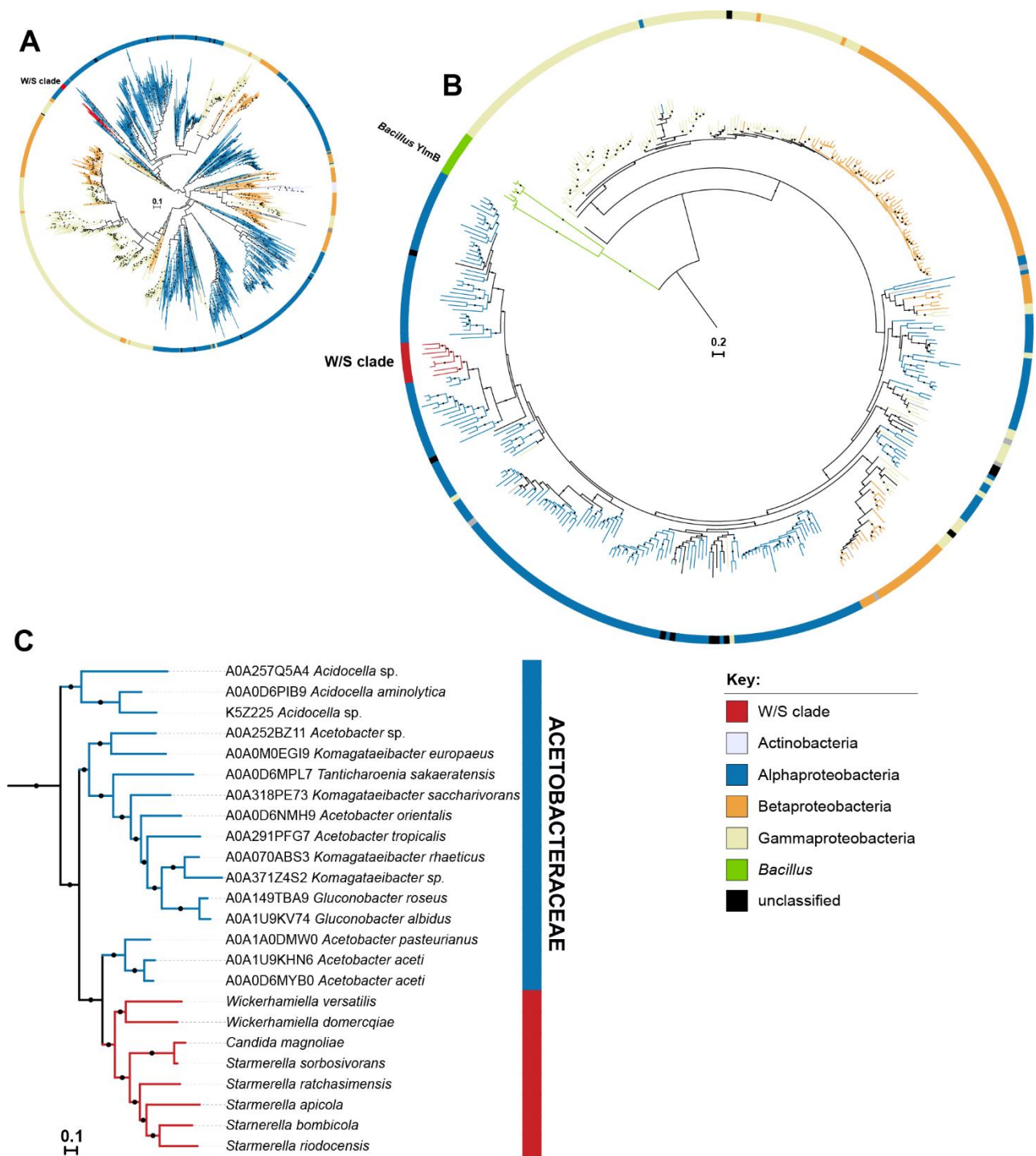


Fig. S5. Maximum likelihood phylogeny of proteins closely related to putative YlmB from W/S-clade species. (A) Preliminary phylogeny constructed with the top 5,000 hits to putative YlmB from *St. bombicola*. Complete **(B)** and pruned **(C)** ML phylogeny constructed with the top 750 hits to putative YlmB from *St. bombicola* with the addition of functionally characterized YlmB proteins from *Bacillus subtilis* (indicated in green). Branches with support higher than 95% (ultrafast bootstrap) are indicated by black dots. Phylogenies were midpoint rooted and visualized in iTOL v4 (8) and branches are colored according to the taxonomic classification as indicated in the key.

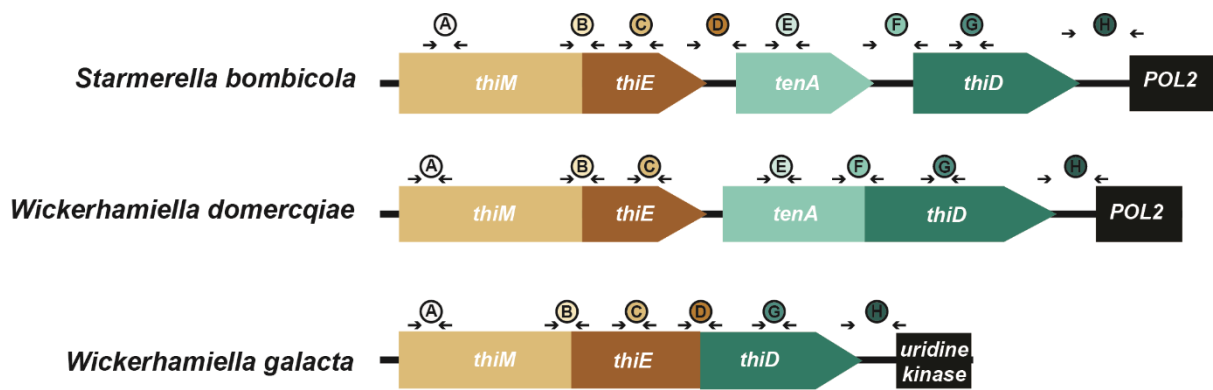


Fig. S6. Primer pairs used for RT-qPCR. Primer sequences and efficiencies are shown in Table S3.

Supplementary Tables

Table S1. Complete list of fungal taxa, abbreviated species names and genome databases and accession numbers of RNA polymerase proteins used to construct the species tree (Figure 1A and Figure S1). Abbreviated species names are given for each species as used in the phylogeny. W/S-clade species are highlighted in blue. Species used as outgroups are highlighted in orange.

Species	Abbreviated names	Strain(s)	Genome database
<i>Aspergillus niger</i>	<i>A. niger</i>	CBS 513.88	BROAD/JGI
<i>Fusarium graminearum</i> (<i>Gibberella zeae</i>)	<i>F. graminearum</i>	PH-1	SGD/BROAD
<i>Blastobotrys adenivorans</i>	<i>B. adenivorans</i>		NCBI
<i>Ascoidea rubescens</i>	<i>A. rubescens</i>	NRRL Y17699	JGI
<i>Babjeviella inositovora</i>	<i>A. inositovora</i>	NRRL Y-12698	JGI
<i>Candida tenuis</i>	<i>C. tenuis</i>	NRRL Y-1498	JGI
<i>Candida albicans</i>	<i>C. albicans</i>	WO-1	SGD/BROAD
[<i>Candida</i>] <i>apicola</i>	<i>C. apicola</i>	NRLL Y-50540	NCBI
<i>Candida arabinof fermentans</i>	<i>C. arabinof fermentans</i>	NRRL YB-2248	JGI
<i>Candida caseinolytica</i>	<i>C. caseinolytica</i>	NRRL Y-17796	JGI
<i>Candida dubliniensis</i>	<i>C. dubliniensis</i>	CD36	SGD/YGOB
<i>Candida glabrata</i>	<i>C. glabrata</i>	CBS 138	SGD/YGOB
[<i>Candida</i>] <i>magnoliae</i>	<i>C. magnoliae</i>	PYCC 2903	NCBI
<i>Candida parapsilosis</i>	<i>C. parapsilosis</i>	CDC317	BROAD/NCBI
<i>Candida tanzawaensis</i>	<i>C. tanzawaensis</i>	NRRL Y-17324	JGI
<i>Candida tropicalis</i>	<i>C. tropicalis</i>	MYA-3404	SGD/BROAD
<i>Clavispora lusitaniae</i>	<i>C. lusitaniae</i>	ATCC 42720	BROAD/NCBI
<i>Cyberlindnera jadinii</i>	<i>C. jadinii</i>	NBRC 0988	NCBI
<i>Debaryomyces hansenii</i>	<i>D. hansenii</i>	CBS767	SGD/NCBI
<i>Dekkera bruxellensis</i>	<i>D. bruxellensis</i>	AWRI1499	NCBI
<i>Eremothecium cymbalariae</i>	<i>E. cymbalariae</i>	DBVPG#7215	NCBI/YGOB
<i>Eremothecium gossypii</i>	<i>E. gossypii</i>	ATCC 10895	SGD/NCBI
<i>Hyphopichia burtonii</i>	<i>H. burtonii</i>	NRRL Y-1933	JGI
<i>Kazachstania africana</i>	<i>K. africana</i>	CBS 2517	YGOB
<i>Kazachstania naganishii</i>	<i>K. naganishii</i>	CBS 8797	YGOB
<i>Kluyveromyces aestuarii</i>	<i>K. aestuarii</i>	ATCC 18862	NCBI
<i>Kluyveromyces lactis</i>	<i>K. lactis</i>	NRRL Y-1140	SGD/YGOB
<i>Kluyveromyces wickerhamii</i>	<i>K. wickerhamii</i>	UCD 54-210	NCBI
<i>Komagataella pastoris</i>	<i>K. pastoris</i>	GS115 + CBS 7435	SGD/NCBI
<i>Lachancea kluyveri</i>	<i>L. kluyveri</i>	NRRL Y-12651	SGD/YGOB
<i>Lachancea thermotolerans</i>	<i>L. thermotolerans</i>	CBS 6340	SGD/YGOB
<i>Lachancea waltii</i>	<i>L. waltii</i>	NCYC 2644	SGD/YGOB
<i>Lipomyces starkeyi</i>	<i>L. starkeyi</i>	NRRL Y-11557	JGI
<i>Lodderomyces elongisporus</i>	<i>L. elongisporus</i>	NRRL YB-4239	BROAD/NCBI
<i>Metschnikowia bicuspidata</i>	<i>M. bicuspidata</i>	NRRL YB-4993	JGI
<i>Meyerozyma guilliermondii</i>	<i>M. guilliermondii</i>	ATCC 6260	BROAD/NCBI
<i>Millerozyma farinosa</i>	<i>M. farinosa</i>	CBS 7064	SGD/NCBI

Species	Abbreviated names	Strain(s)	Genome database
<i>Nadsonia fulvescens</i> var. <i>elongata</i>	<i>N. fulvescens</i>	DSM 6958	JGI
<i>Naumovozyma castellii</i>	<i>N. castellii</i>	CBS 4309	YGOB
<i>Ogataea polymorpha</i>	<i>O. polymorpha</i>	NCYC 495 leu1.1	JGI
<i>Ogataea parapolyomorpha</i>	<i>O. parapolyomorpha</i>	DL-1	NCBI
<i>Pachysolen tannophilus</i>	<i>P. tannophilus</i>	NRRL Y-2460	JGI/NCBI
<i>Pichia kudriavzevii</i>	<i>P. kudriavzevii</i>	M12	NCBI
<i>Pichia membranifaciens</i>	<i>P. membranifaciens</i>	NRRL Y-2026	JGI
<i>Saccharomyces cerevisiae</i>	<i>S. cerevisiae</i>	S288c	SGD/BROAD
<i>Saccharomyces eubayanus</i>	<i>S. eubayanus</i>	FM1318	NCBI
<i>Saccharomyces kudriavzevii</i>	<i>S. kudriavzevii</i>	IFO 1802	SSS Website
<i>Saccharomyces mikatae</i>	<i>S. mikatae</i>	IFO 1815	SSS Website SGD/BROAD
<i>Saccharomyces paradoxus</i>	<i>S. paradoxus</i>	NRRL Y-17217	SSS Website NCBI
<i>Saccharomyces uvarum</i>	<i>S. uvarum</i>	CBS 7001	SSS Website SGD/BROAD
<i>Saprochaete clavata</i>	<i>Sa. clavata</i>	CNRMA 12.647	NCBI
<i>Scheffersomyces stipitis</i>	<i>S. stipitis</i>	CBS 6054	JGI/NCBI
<i>Spathaspora passalidarum</i>	<i>S. passalidarum</i>	NRRL Y-27907	JGI/NCBI
<i>Starmerella bacillaris</i>	<i>St. bacillaris</i>	PYCC 3044	NCBI
<i>Starmerella riodocensis</i>	<i>St. riodocensis</i>	NRRL Y-27859	NCBI (Y1000+ project)
<i>Starmerella ratchamatensis</i>	<i>St. ratchamatensis</i>	CBS 10611	NCBI (Y1000+ project)
<i>Starmerella sorbosivorans</i>	<i>St. sorbosivorans</i>	CBS 8768	NCBI (Y1000+ project)
<i>Starmerella bombicola</i>	<i>St. bombicola</i>	PYCC 5882	NCBI
<i>Sugiyamaella lignohabitans</i>	<i>Su. lignohabitans</i>	NRRL YB-1473	NCBI
<i>Tetrapispora blatae</i>	<i>T. blatae</i>	CBS 6284	YGOB
<i>Tetrapispora phaffii</i>	<i>T. phaffii</i>	CBS 4417	YGOB
<i>Torulaspora delbrueckii</i>	<i>T. delbrueckii</i>	CBS 1146	YGOB
<i>Trichomonascus petasosporus</i>	<i>T. petasosporus</i>	NRRL YB-2093	JGI
<i>Vanderwaltozyma polyspora</i>	<i>V. polyspora</i>	DSM 70294	YGOB/NCBI
<i>Wickerhamiella domercqiae</i>	<i>W. domercqiae</i>	PYCC 3067	NCBI
<i>Wickerhamiella cacticola</i>	<i>W. cacticola</i>	NRRL Y-27362	NCBI (Y1000+ project)
<i>Wickerhamiella hasegawae</i>	<i>W. hasegawae</i>	JCM 12559	NCBI (Y1000+ project)
<i>Wickerhamiella galacta</i>	<i>W. galacta</i>	NRRL Y-17645	NCBI (Y1000+ project)
<i>Wickerhamiella occidentalis</i>	<i>W. occidentalis</i>	NRRL Y-27364	NCBI (Y1000+ project)
<i>Wickerhamiella pararugosa</i>	<i>W. pararugosa</i>	NRRL Y-17089	NCBI (Y1000+ project)
<i>Wickerhamiella versatilis</i>	<i>W. versatilis</i>	JCM 5958	RIKEN
<i>Wickerhamomyces anomalus</i>	<i>W. anomalus</i>	NRRL Y-366	JGI
<i>Yarrowia lipolytica</i>	<i>Y. lipolytica</i>	CLIB 122	SGD/NCBI
<i>Zygosaccharomyces bailii</i>	<i>Z. bailii</i>	CLIB 213	NCBI
<i>Zygosaccharomyces kombuchaensis</i>	<i>Z. kombuchaensis</i>	CBS 8849	Local database
<i>Zygosaccharomyces rouxii</i>	<i>Z. rouxii</i>	CBS 732	SGD/JGI

Table S2. Information concerning strains and genome assemblies used in this work.

Species	Genome assembly reference	Information was included in:
<i>Sugiyamaella lignohabitans</i> CBS10342	ASM164002v2	Figure 1B
<i>Wickerhamiella galacta</i> NRRL Y-17645	ASM304524v1	Figure 1B, Figure 2, Figure 4
<i>Wickerhamiella hasegawae</i> JCM 12559	ASM412510v1	Figure 1B
<i>Wickerhamiella pararugosa</i> NRRL Y-17089	ASM412523v1	Figure 1B
<i>Wickerhamiella occidentalis</i> NRRL Y-27364	ASM412509v1	Figure 1B
<i>Wickerhamiella sorbophila</i> DS02	ASM225199v2	Figure 1B
<i>Wickerhamiella versatilis</i> JCM 5958	JCM_5958_assembly_v001	Figure 1B, Figure 2, Figure 3
<i>Wickerhamiella domercqiae</i> PYCC 3067	ASM303370v1	Figure 1B, Figure 2, Figure 3
<i>Starmerella sorbosivorans</i> CBS 8768	ASM412500v1	Figure 1B, Figure 2, Figure 3
<i>Candida magnoliae</i> PYCC 2903	ASM303343v1	Figure 1B, Figure 2, Figure 3
<i>Starmerella bacillaris</i> PYCC 3044	ASM303376v1	Figure 1B, Figure 2, Figure 3
<i>Starmerella apicola</i> NRRL Y-50540	ASM100541v1	Figure 1B, Figure 2, Figure 3
<i>Starmerella ratchamatensis</i> CBS 10611	ASM412497v1	Figure 1B, Figure 2, Figure 3
<i>Starmerella riodocensis</i> NRRL Y-27859	ASM412495v1	Figure 1B, Figure 2, Figure 3
<i>Starmerella bombicola</i> PYCC 5882	ASM303378v1	Figure 1B, Figure 2, Figure 3
<i>Dysgonomonas mossii</i> DSM22836	ASM37640v1	Figure 2
<i>Mangrovibacterium marinum</i> DSM 28823	ASM304662v1	Figure 2
<i>Caballeronia udeis</i> ES_PA-B7	ASM361007v1	Figure 2
<i>Burkholderia novacaledonica</i> LMG 28615T	LMG28615	Figure 2
<i>Peptostreptococcus russellii</i> RT-10B	ASM301205v1	Figure 2
<i>Veillonella dispar</i> DORA_11	GCA_000508585.1	Figure 2

Table S3. Primers used for Real Time PCR. Efficiencies of each primer pair were calculated using five ten-fold DNA dilutions.

Name	Sequence 5'-3'	Purpose	Primer Efficiency (%)
qPCR_thiD_Fw	AGATACTGATGGCACTCTCATCG	primers for qRT-PCR of <i>thiD</i> from <i>St. bombicola</i>	101
qPCR_thiD_Rv	GTGAAGTGGCCATGACCTC	primers for qRT-PCR of <i>thiD</i> from <i>St. bombicola</i>	
qPCR_thiE_Fw	TTATGGCGTCGGAGCCTACC	primers for qRT-PCR of <i>thiM</i> portion from <i>St. bombicola</i>	99,6
qPCR_thiE_Rv	CGAGTAGTGGCTTCAGATGTGTC	primers for qRT-PCR of <i>thiM</i> portion from <i>St. bombicola</i>	
qPCR_thiEfim_Fw	GAAGCGTCAAAGCAATTAGTGG	primers for qRT-PCR of <i>thiE</i> portion from <i>St. bombicola</i>	92,5
qPCR_thiEfim_Rv	CAGCTTTTGGGCTTCTGCCAG	primers for qRT-PCR of <i>thiE</i> portion from <i>St. bombicola</i>	
qPCR_tenA_Fw	GCTGAGCAGGAATTGCACGAC	primers for qRT-PCR of <i>tenA</i> from <i>St. bombicola</i>	94,5
qPCR_tenA_Rv	GCGCATAGGGATGTTGACG	primers for qRT-PCR of <i>tenA</i> from <i>St. bombicola</i>	
qPCR_thiM718_Fw	CAGTTGCTGGAGAGCTCGCTG	primers for qRT-PCR of the fragment in between <i>thiM</i> and <i>thiE</i> from <i>St. bombicola</i>	95,7
qPCR_thiE1024_Rv	GGAACATGGAACATCGCATACC	primers for qRT-PCR of the fragment in between <i>thiM</i> and <i>thiE</i> from <i>St. bombicola</i>	
qPCR_Pol2_Fw	TGACGGCAGAGGCTATCTTGTCT	primers for qRT-PCR of <i>POL2</i> from <i>St. bombicola</i>	94,1
qPCR_Pol2_Rv	CGTAAACGCTAGCGATGACAGTGG	primers for qRT-PCR of <i>POL2</i> from <i>St. bombicola</i>	
qPCR_thiD_779Fw	GTAGAGGTCATGCCCAGTTCA	primers for qRT-PCR of the fragment in between <i>thiD</i> and <i>POL2</i> from <i>St. bombicola</i>	94,5
qPCR_Pol2_82Rv	TGGCATCAAACATGTCGGTCCAG	primers for qRT-PCR of the fragment in between <i>thiD</i> and <i>POL2</i> from <i>St. bombicola</i>	
qPCR_Widcom_tenA334_Fw	CTAAGGTGTCTGCAGTTGAGC	primers for qRT-PCR of <i>tenA</i> from <i>W. domercqiae</i>	94,8
qPCR_Widcom_tenA545Rv	GTGGCTGCTGCAAGCTCATCG	primers for qRT-PCR of <i>tenA</i> from <i>W. domercqiae</i>	
qPCR_Widcom_thiD850Fw	CTGGTGCCTTTGTCCAAGCTC	primers for qRT-PCR of <i>thiD</i> from <i>W. domercqiae</i>	93,1
qPCR_Widcom_thiD1089Rv	TGCAAGCGAAATTAAGCGATCAGC	primers for qRT-PCR of <i>thiD</i> from <i>W. domercqiae</i>	
Widcom_thiM67Fw	GCGGTAGCATCTAACTACGCTG	primers for qRT-PCR of <i>thiM</i> portion from <i>W. domercqiae</i>	94,3
Widcom_thiM308Rv	GCCACTAGAGCTCTGCATGTATC	primers for qRT-PCR of <i>thiM</i> portion from <i>W. domercqiae</i>	
Widcom_thiE1116Fw	TCTTGACTCAGCGTGGGATC	primers for qRT-PCR of <i>thiE</i> portion from <i>W. domercqiae</i>	97,4
Widcom_thiE1364Rv	GCTACTCCAGCAGCACCTGTG	primers for qRT-PCR of <i>thiE</i> portion from <i>W. domercqiae</i>	
Widcom_thiM684Fw	TGGTGTGCCGGTGAGCTTGC	primers for qRT-PCR of the fragment in between <i>thiM</i> and <i>thiE</i> from <i>W. domercqiae</i>	108,3
Widcom_thiE953Rv	CTTGACCTCAACCGCGCAG	primers for qRT-PCR of the fragment in between <i>thiM</i> and <i>thiE</i> from <i>W. domercqiae</i>	
Widcom_tenA518_Fw	CATGCGATGAGCTTGACGACGC	primers for qRT-PCR of the fragment in between <i>tenA</i> and <i>thiD</i> from <i>W. domercqiae</i>	86,7
Widcom_thiD790_Rv	CGTCTGAGAATACACTCCAAGCG	primers for qRT-PCR of the fragment in between <i>tenA</i> and <i>thiD</i> from <i>W. domercqiae</i>	
Widcom_POL2_661Fw	CGTCTCGCAATTGATCTTGATGTGC	primers for qRT-PCR of <i>POL2</i> from <i>W. domercqiae</i>	90,0
Widcom_POL2_915Rv	CAATGCTCTGACTTAGCTCAGC	primers for qRT-PCR of <i>POL2</i> from <i>W. domercqiae</i>	
Widcom_tenAthiD_1451Fw	GCTGCGTGGATCAACAAGTGG	primers for qRT-PCR of the fragment in between <i>thiD</i> and <i>POL2</i> from <i>W. domercqiae</i>	94,4
Widcom_POL2_36Rv	CGCGATGAAAGACGCGACGC	primers for qRT-PCR of the fragment in between <i>thiD</i> and <i>POL2</i> from <i>W. domercqiae</i>	
Wgalacta_thiM266Fw	GTCTCCATTCGTGCGGCCATTG	primers for qRT-PCR of <i>thiM</i> portion from <i>W. galacta</i>	94,9
Wgalacta_ThiM485Rv	CGTCTCAGAGCTAACAGCGC	primers for qRT-PCR of <i>thiM</i> portion from <i>W. galacta</i>	
Wgalacta_thiE312Fw	GCTCGATGGTCTGGCAGAGAT	primers for qRT-PCR of <i>thiE</i> portion from <i>W. galacta</i>	100,5
Wgalacta_thiE503Rv	GCTTCAAGTTGACTACTGCCG	primers for qRT-PCR of <i>thiE</i> portion from <i>W. galacta</i>	
Wgalacta_thiD527Fw	GGCATCTACCACAGACCTTACTAG	primers for qRT-PCR of <i>thiD</i> from <i>W. galacta</i>	101,6
Wgalacta_thiD700Rv	CCTTGACGGATTGTGCAAGGC	primers for qRT-PCR of <i>thiD</i> from <i>W. galacta</i>	
Wgalacta_thiM798Fw	GGAGCAGCTGACTTTGAGCG	primers for qRT-PCR of the fragment in between <i>thiM</i> and <i>thiE</i> from <i>W. galacta</i>	105,6
Wgalacta_thiE81Rv	CGAGTGCCAGCTCTACGTCG	primers for qRT-PCR of the fragment in between <i>thiM</i> and <i>thiE</i> from <i>W. galacta</i>	
Wgalacta_thiE312Fw	GCTCGATGGTCTGGCAGAGAT	primers for qRT-PCR of the fragment in between <i>thiD</i> and <i>thiE</i> from <i>W. galacta</i>	92,5
Wgalacta_thiD105Rv	CACGCTGAGACCATAAGCTCC	primers for qRT-PCR of the fragment in between <i>thiD</i> and <i>thiE</i> from <i>W. galacta</i>	
Wgalacta_thiD750Fw	TGTAGGTCATGGAAGTGGTCCG	primers for qRT-PCR the fragment between <i>thiD</i> and uridine kinase from <i>W. galacta</i>	116,9
Wgalacta_uridineRv	GCCACTCCCTGAACATCCAGC	primers for qRT-PCR the fragment between <i>thiD</i> and uridine kinase from <i>W. galacta</i>	

Table S4. Primers used for the disruption of *THI* genes in *St. bombycola* PYCC 5882.

Name	Sequence 5'-3'	Purpose
thiE_upNotI_Fw	TATCATGCGGCCGCGTGATACCTGGCATCAG	Amplification of the upstream and downstream regions of thiEM
thiE_downNcoI_Rv	ATGATACCATGGGGATTCCAGCACCTCC	
thiE_transf_Fw	TGAGTGGATTCTCAGGATC	Amplification of final construction for thiEM deletion
thiE_transf_Rv	GGCAATGGAAAGTGCTG	
tenAthiD_upstreamSphI_Fw	CGGGCACGATGCATTC	Amplification of the upstream and downstream regions of thiD
tenAthiD_downstream_KpnI_Rv	ATGATAGGTACCCACAGGTTGTGCACGC	
thiD_transf_Fw	TAGCTCTCATTGCTGCC	Amplification of final construction for thiD deletion
thiD_transf_Rv	AATTAGATCGCGACGCGT	
tenAKO_KpnI_Rv	GACTTAGGTACCGAGTGCCATCAGTATCTTC	Amplification of the upstream and downstream regions of tenA
tenA_603_SmaI_Fw	GACTTACCCGGGGATGACCGAGGCATTTGT	
tenA_transf_Fw	GGTTACAGATCCAGAGC	Amplification of final construction for tenA deletion
tenA_transf_Rv	GAGTGCCATCAGTATCTTC	
thiE_upNotI_Fw	TATCATGCGGCCGCGTGATACCTGGCATCAG	Amplification of the upstream and downstream regions of the entire operon
tenAthiD_downNotI_Rv	ATGATAGCGGCCGCTCACGAGCATATGGAGTC	
thiE_transf_Fw	TGAGTGGATTCTCAGGATC	Amplification of final construction for the deletion of the entire operon
THlope_transf_Rv	CTTCGCTACCGATATCG	

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