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ORIGINAL ARTICLE

Stb5p is involved in *Kluyveromyces lactis* response to 4-nitroquinoline-N-oxide stress

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Abstract

In yeast, the *STB5* gene encodes a transcriptional factor belonging to binuclear cluster class (Zn_2Cys_6) of transcriptional regulators specific to ascomycetes. In this study, we prepared the *Kluyveromyces lactis stb5Δ* strain and assessed its responses to different stresses. We showed that *KlSTB5* gene is able to complement the deficiencies of *Saccharomyces cerevisiae stb5Δ* mutant. The results of phenotypic analysis suggested that *KlSTB5* gene deletion did not sensitize *K. lactis* cells to oxidative stress inducing compounds but led to *Klstb5Δ* resistance to 4-nitroquinoline-N-oxide and hygromycin B. Expression analysis indicated that the loss of *KlSTB5* gene function induced the transcription of drug efflux pump encoding genes that might contribute to increased 4-nitroquinoline-N-oxide and hygromycin B tolerance. Our results show that *Kl*Stb5p functions as negative regulator of some ABC transporter genes in *K. lactis.*

Keywords Transcriptional regulator · Oxidative stress · Drug resistance · *Kluyveromyces lactis*

Introduction

Kluyveromyces lactis, the yeast species with predominantly respiratory metabolism, represents an alternative model to the commonly used *Saccharomyces cerevisiae* for studies related to carbon and oxygen metabolism. While these two yeast species are phylogenetically close to each other, they display some important differences. *K. lactis* is a Crabtree-negative yeast with a respiratory-fermentative lifestyle, respiration being regulated by oxygen availability. The Crabtree-positive *S. cerevisiae* is adapted to high-sugar supply in its natural environment, performs aerobic fermentation, and compensates the low-energy yield by a high-glycolytic flux. The glucose is metabolized through glycolysis in *S. cerevisiae*; in *K. lactis*, it is mainly channeled into the pentose phosphate pathway (PPP). *K. lactis* is able to utilize xylose, xylitol, cellobiose, arabinose, and lactose. The important advantage over other types of yeast is the ability of *K. lactis* to grow on whey, which has economic and ecological benefits. With its potential to secrete large proteins and rapidly achieve high-culture densities, *K. lactis* has been used to produce proteins on an industrial scale in the food and feed industry. *K. lactis* unlike *S. cerevisiae* has not undergone whole genome duplication (WGD), the redundancy of genes is, therefore, lower (Bussereau et al. 2006; González-Siso et al. 2009; Rodicio and Heinisch 2013). Previous studies have shown that most genes are conserved in these yeast species. The different physiological responses result from modified regulatory interactions (Breunig et al. 2000; Mehlgarten et al. 2015).

The most important class of yeast transcriptional regulators is composed of proteins bearing the $Zn(II)_2Cys_6$ zinc cluster (MacPherson et al. 2006; Clarke et al. 2013). These proteins display variable secondary structures and enormous functional diversity. They are involved in the regulation of numerous processes such as the control of the metabolism of sugars, amino acids, fatty acids, as well as drug resistance. Different combinations of transcription factors (forming homodimers or heterodimers) may differentially regulate the expression of their target genes. The genome of *S. cerevisiae* encodes 56 proteins in this category, and 58 putative $Zn(II)_2Cys_6$ proteins were detected in the genome of *K. lactis* (Bussereau et al.

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2006). However, in *K. lactis*, only a handful of them have been characterized.

In our previous work, we have characterized the master regulator of drug resistance in *K. lactis*, the *Kl*Pdr1p (Balkova et al. 2009). In *S. cerevisiae,* Pdr1p has been shown to form a heterodimer with another transcription factor, Sin3 binding protein 5 (*Sc*Stb5p) (Akache et al. 2004). Studies in *S. cerevisiae* found that *Sc*Stb5p is a $Zn(II)_2Cys_6$ transcription factor (Kasten and Stillman 1997; Akache et al. 2001) which regulates genes involved in the oxidative stress response by increasing the supply of NADPH through the pentose phosphate pathway (Larochelle et al. 2006). The absence of *Sc*Stb5p results in a growth defect, sensitivity to cold (below 20 °C), and susceptibility to various drugs (Akache et al. 2001; Larochelle et al. 2006; Matsufuji et al. 2010).

Here, we investigated the role of the protein encoded by the open reading frame (ORF) KLLA0A09251g and show that it is a *K. lactis* orthologue of *Sc*Stb5p. Deletion or overexpression of *KlSTB5* had substantial effect on *K. lactis* drug susceptibility. We also showed that the *KlSTB5* gene can complement the defects in the *S. cerevisiae stb5Δ* mutant strain and acts as a transcriptional regulator of several genes implicated in *K. lactis* drug resistance.

Materials and methods

Strains and culture conditions

The following yeast haploid strains were used: *K. lactis CBS2359ku80* (*MATα ku80::loxP*; Kooistra et al. 2004) and *S. cerevisiae* BY4741 (*MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0*) as parental strains and their isogenic *stb5Δ* deletion mutants. Cells were grown in liquid glucose-rich (YPD) medium (2% glucose, 1% yeast extract, 2% Bacto peptone), or minimal (YNB) medium containing 0.67% yeast nitrogen base without amino acids and 2% glucose, supplemented with the required amino acids and/or bases at 28 °C to a density of 1.0×10^8 cells/mL. Phenotypic analyses were performed on YP or YNB medium containing 2% glucose (*w*/*v*), 2% glycerol, or 2% lactate as carbon source in serial tenfold dilutions. The media were solidified with 2% Bacto agar. YP and YNB plates were incubated at 28 °C for 2 and 4 days respectively. For drug susceptibility testing, yeast cell suspensions were prepared from mid-exponential cell cultures grown in YNB, diluted to 1.0×10^7 cells/mL. Five-microliter aliquots from tenfold dilutions were spotted onto YPD media containing respective drugs. Drugs were applied at the concentrations indicated in the figures. The *Escherichia coli* DH5α strain was used as a host for transformation, plasmid amplification, and preparation. Bacteria were grown at 37 °C in Luria-Bertani (LB) medium (1% tryptone, 1% NaCl, 0.5% yeast extract, and pH 7.6) supplemented with 100 μg/mL ampicillin for the selection of transformants.

Cloning of the *KlSTB5* gene

Genomic DNA from *K. lactis CBS2359ku80* was extracted and used as a template for the amplification of *KlSTB5* gene. PCR was carried out with a high-fidelity DNA polymerase (ThermoFisher Scientific, Frankfurt am Main, Germany) using forward and reverse primers (Table 1) and generated 4 kb fragment. It was then ligated into the *BamH*I-*Xho*I digested pRS306K (*URA3 ARS1 KARS2 ori* Amp^R) vector, obtained from J.J. Heus (Leiden University, Leiden, The Netherlands). The pRS306K-*KlSTB5* plasmid amplified in *E. coli* DH5α was verified by restriction analysis and sequencing with automated DNA sequences (ABI Prism 3100, Applied Biosystems, Foster City, USA).

Construction of the *K. lactis* deletion strain

The chromosomal *KlSTB5* gene was deleted by one-step gene replacement procedure (Rothstein 1983). Plasmid pUG6 (Güldener et al. 1996) was used as template for amplification of the *kanMX* cassette using primers listed in Table 1. The 2 kb fragment obtained containing 49/43 bp target homologous flanking sequences of the *KlSTB5* gene was used to transform *K. lactis CBS2359ku80* cells. The *KlSTB5* gene deletion was verified by PCR.

Genetic manipulations, transformations, and DNA preparations

Standard protocols for generating recombinant DNAs, the restriction enzyme analyses, gel electrophoresis, and hybridization were used (Sambrook et al. 1989). Plasmid DNA from *E. coli* was prepared by the alkaline lysis method. *K. lactis* strains were transformed by electroporation (Sánchez et al. 1993) using Bio-Rad gene pulser at 1.0 kV, 25 μF, and 400 Ω in 0.2-cm cuvettes. *S. cerevisiae* transformation was carried out using the modified lithium acetate protocol (Thompson et al. 1998).

Quantitative real-time PCR

Yeast cells were grown in minimal medium containing 2% glucose to the mid-logarithmic phase. Total RNA was isolated by the hot acidic phenol extraction method (Ausubel et al. 1989). First-strand cDNA was synthetized from 1 μg of total RNA using 200 U of the Revert AID™H Minus M-MuLV Reverse transcriptase (ThermoFisher Scientific, Frankfurt am Main, Germany). Quantitative real-time PCRs were performed in triplicate using the Applied Biosystems 7900 HT fast real-time PCR system.

Table 1 List of oligonucleotides used in this study

Primers for qRT-PCR analysis	Nucleotide sequence (5′ → 3′)
KlACT1-F	AAT GCA AAC TGC TTC TCA AT
KlACT1-R	AAC AGA TGG ATG GAA CAA AG
KlPDR1 -F	ATT TCA ACC TGC CGT TTC
KlPDR1 -R	CCG TTG CCA TTA CTA TCT CTA
KlPDR5 -F	AGG TAC GAG GGT TGT AAT
KlPDR5 -R	CAT ATT CTT GAT CCA CGC AG
KlPDR12-F	TTG TTC TGC TAC TGG GTT
KlPDR12-R	CGT GTC TGT CCT TTG GTT
KlPDR16 -F	CTG ACT CGG AAG GAA GTT
KlPDR16 -R	ACT TGT CTA TGG GAG GTT G
KlPMA1-F	CCG CTA TTG TCG AAT CTC CAG
KlPMA1-R	CCA AGG CTC TGA AAC CTC TG
KlTPO1-F	AGT GTT CGG TTC TTC ATT G
KlTPO1-R	AGT TGG GTC ATG ACT TTC TT
KlYAP1 -F	ATC AAC CGC TAA ACG ACC TT
KlYAP1 -R	ACT CGA TCC GGA ATT GTT G
KlKNQ1 -F	TTG CTG GAG GTA TCG TTC
KlKNQ1 -R	CTT TGT CGG TAG TTT GGT TAC
KlSNQ2 -F	GCC AGA CAA ACA ACA TTG AGA G
KlSNQ2 -R	CCT GAG ACA CCC TTG AAA TGA G
KlERG6 -F	TCT CTG CGT TGG TTT CC
KlERG6 –R	TCT ACG TTC AGC ATC AGC
Primers for *KlSTB5* amplification	Nucleotide sequence (5′ → 3′)
forward	GAT CAG GAT CCT ACC ATA GTG TGA AAC CAG ATT CC
reverse	GAT CTA CTC GAG TTC CAA AGA ATG ATG CCA CTT CC
Primers for deletion cassette amplification	Nucleotide sequence (5′ → 3′)
forward	GCG TAA TAG CAC TGC AAT TGT GTG AGA AAC TAG TGC CAT ATC AGT CGT CTT AGG TGA CAC TAT AGA ACG C
reverse	TTC ATT AAT CAG ATA CAC ACT TTT TTA TTA TAT CTT TAT ATA CCC CTG ATT CTG TGG ATA ACC
Primers for verification of *KlSTB5* gene deletion	Nucleotide sequence (5′ → 3′)
P5	CTG TAA CAT CAT TGG CAA CGC TA
P6	GAT CAG GAT CCA AGC TTC CGA TTC CTT AGT TCA AGA AC
P7	GAT ATA CTC GAG CCC TCA GAC GAT ACT ATC CAC

Independent PCRs were performed using the same cDNA for both the gene of interest and the *KlACT1* gene, using the HOT FIREPol® EvaGreen® qPCR Mix Plus (ROX) (Solis BioDyne, Tartu, Estonia). The *KlACT1* mRNA level was used as the internal control. The relative value obtained for each target gene in the wild type was set as 1 and the remaining values are relative to that value.

β-glucuronidase reporter assay

K. lactis cells transformed with the P_{KlPDR5}-*gusA* chimeric construct were grown in minimal liquid medium to the mid-logarithmic phase and harvested by centrifugation for 5 min at 3000*g* at 4 °C. Pellet was washed with β-glucuronidase extraction buffer (10 mM sodium phosphate pH 7.0, 10 mM Na_2EDTA, 10 mmol/L dithiothreitol, 0.1% sodium lauryl sarcosine, 0.1% Triton X-100) and resuspended in 500 μL of the same buffer. An equal volume of ice-chilled, acid-washed glass beads (0.45 mm diameter) was added and the cells were homogenized ten times for 1 min at 4 °C. After 10-min centrifugation at 10,000*g* at 4 °C obtained crude extracts were used for the measurement of β-glucuronidase activity. It was determined at 30 °C in the extraction buffer containing 1 mmol/L p-nitrophenyl-β-D-glucopyranoside (PNPG) and the reaction was followed spectrophotometrically. The specific activity was normalized to the amounts of protein determined according to Bradford (1976).

Results

Phenotypic analysis of *S. cerevisiae* and *K. lactis* lacking the *KlSTB5* gene

To analyze whether the *Kl*Stb5p is required for growth on non-fermentable carbon sources, the *K. lactis STB5* gene was disrupted in the wild type and phenotypically analyzed by spotting serial dilutions on different carbon sources and compared to those of the respective *S. cerevisiae* null mutant. The phenotypes of *S. cerevisiae* and *K. lactis* wild type and the *stb5Δ* deletion mutants were compared on minimal and rich media supplemented either with fermentative or respiratory carbon sources. The *S. cerevisiae stb5Δ* strain failed to grow on the minimal medium with 2% glycerol or 2% lactate. However, the growth of *K. lactis* strain lacking *KlSTB5* was not impaired on any of these media. Introducing the heterologous *KlSTB5* gene into *Scstb5Δ* mutant cells recovered *Scstb5Δ* mutant growth to wild-type levels in both glycerol and lactate containing media (Fig. 1a). Previous studies showed that the deletion of *ScSTB5* results in sensitivity to cold (20 °C) (Akache et al. 2001; Noble et al. 2013). Our results (Fig. 1b) showed that the overexpression of *KlSTB5* gene in *Scstb5Δ* mutant strain restored its growth at low temperature. On the other hand, the phenotype of *Klstb5Δ* mutant contrasts with that of the *Scstb5Δ* mutant as no discernible effect regarding carbon source and decreased temperature was detected.

Studies in *S. cerevisiae* characterized Stb5p as a regulator of oxidative stress response and drug resistance (Larochelle et al. 2006). To analyze whether the *KlSTB5* deletion also results in sensitivity to drugs, we compared the growth of *Scstb5Δ* and *Klstb5Δ* deletion mutants in the presence of various drugs. Figure 2 demonstrates that *Scstb5Δ* null mutant is susceptible to 4-nitroquinoline-N-oxide, hygromycin B, caffeine, hydrogen peroxide, and antifungal azoles (itraconazole and miconazole) in comparison to wild-type strain. Overexpression of the *KlSTB5* gene in the *Scstb5Δ* mutant restored the wild-type phenotype. Contrary to *S. cerevisiae*, the *Klstb5Δ* strain was able to grow comparably to the wild-type in the presence of 4-nitroquinoline-N-oxide, hygromycin B, caffeine, hydrogen peroxide, itraconazole, and miconazole. The presence of *KlSTB5* gene in multiple copies impaired the growth of *Klstb5Δ* transformant in the presence of 4-nitroquinoline-N-oxide and hygromycin B (Fig. 2).

KlPDR5, *KlSNQ2*, and *KlERG6* genes transcription is upregulated in response to drugs in *Klstb5*Δ mutant

To identify genes that require the presence of *Kl*Stb5p for their expression, mRNA levels of selected genes were compared in *K. lactis* wild-type and *Klstb5Δ* mutant. The selected genes listed in Table 2 encode transcription factors (*KlPDR1*, *KlYAP1*), genes involved in lipid metabolism (*KlPDR16*, *KlERG6*), major facilitator superfamily transporters (*KNQ1*, *KlTPO1*), ABC transporters (*KlPDR5*, *KlSNQ2*, *KlPDR12*), and plasma membrane H^+-ATPase *(KlPMA1*). As Fig. 3a shows, there was no significant difference in transcript levels in the *Klstb5Δ* in comparison with the wild type. However, after exposition of log-phase wild-type and *Klstb5Δ* cell cultures to 1 mM hydrogen peroxide for 60 min, induced transcription of *KlERG6* and *KlSNQ2* genes was observed in *Klstb5Δ* mutant (Fig. 3b). *KlERG6* gene encoding S-adenosine dependent sterol C-24 methyltransferase involved in the ergosterol biosynthetic pathway was induced about 2.5-fold and *KlSNQ2* encoding ABC transporter involved in multidrug resistance about twofold in the *Klstb5Δ* mutant cells. Transcription of *KNQ1*, *KlTPO1*, and *KlPDR5* genes was stimulated to a lesser extent in response to hydrogen peroxide in the *Klstb5Δ* mutant (Fig. 3b).

To identify genes that are regulated by *Kl*Stb5p under azole stress, we compared the expression of selected genes after exposition of log-phase wild-type and *Klstb5Δ* cell cultures to 0.001 μg/mL itraconazole for 60 min. Upon itraconazole exposure, the genes encoding transcription factors *KlPDR1* and *KlYAP1* were significantly upregulated in the *Klstb5Δ* mutant (Fig. 3c). Transcription of genes encoding MDR transporters *KlPDR5*, *KlSNQ2*, *KlTPO1*, and also the *KlPMA1* was stimulated as a result of *KlSTB5* gene deletion. As the *KlPDR5* gene promoter contains the PDRE elements to which *Kl*Stb5p apparently binds, we analyzed the influence of the *Klstb5Δ* mutation on *KlPDR5* promoter activity. *KlPDR5*

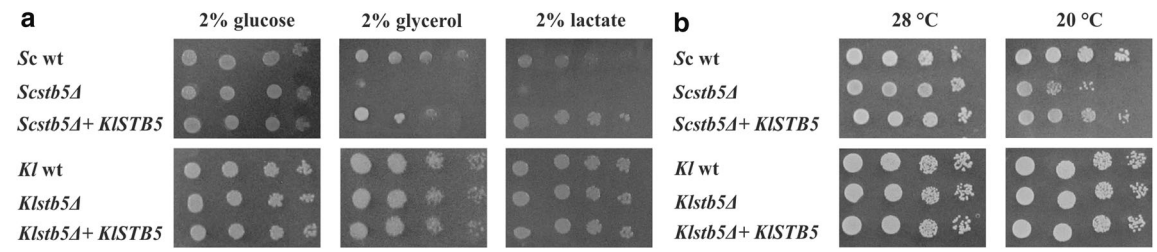

Fig. 1 Complementation assays. *KlSTB5* from *K. lactis* was expressed in *S. cerevisiae Scstb5Δ* mutant strain. **a** Growth of *S. cerevisiae* and *K. lactis* cells on YNB medium with 2% glucose, 2% glycerol, and 2% lactate; **b** growth of *S. cerevisiae* and *K. lactis* cells on YPD agar plates at 20 and 28 °C respectively. Each spot represents 5 μL of the tenfold cell suspension dilution series. The initial cell concentration was 1×10^7cells/mL

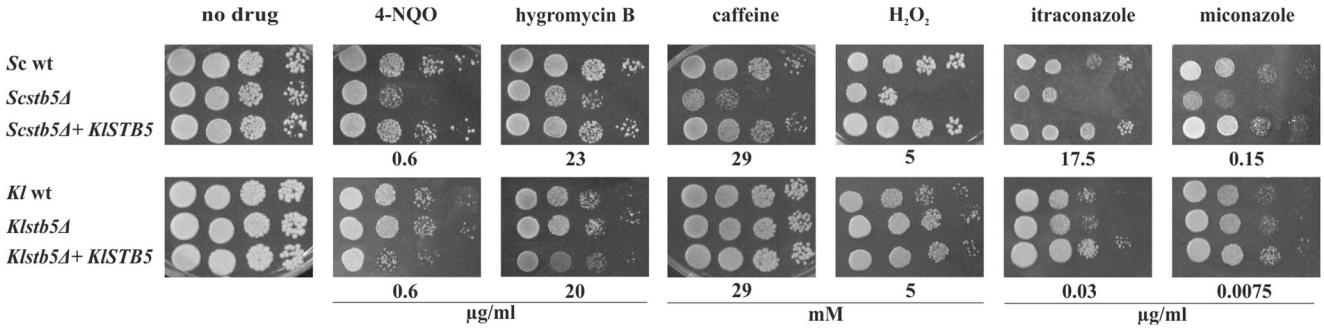

Fig. 2 Phenotype of *S. cerevisiae* and *K. lactis* wild-types, their *stb5Δ* mutants and transformants containing the *KlSTB5* gene on a multicopy plasmid in the presence of various drugs. Cells were spotted as tenfold dilution series on YPD plates and incubated at 28 °C for 2 days

promoter region was fused to the β-glucuronidase *gusA* reporter gene (P_{KlPDR5}—*gusA*) and the construct was introduced into the wild-type and *Klstb5Δ* mutant cells. GUS expression in the wild-type strain and *Klstb5Δ* transformants was determined. Itraconazole induced the activity of β-glucuronidase both in wild-type and in *Klstb5Δ* mutant (Table 3). Increased activity of the reporter construct in the *Klstb5Δ* mutant thus corroborates the results of the qRT-PCR analysis showing increased transcript level of *KlPDR5* gene. Itraconazole strongly induced the transcription of *KlSNQ2* gene (about sixfold) in the *Klstb5Δ* mutant cells that correlates with the observed resistance of *Klstb5Δ* mutant to 4-nitroquinoline-N-oxide in phenotypic assays. The transcription of *KlERG6* gene was induced about threefold in the *Klstb5Δ* mutant compared to the wild-type (Fig. 3c). Levels of mRNA from selected genes (Table 2) were compared also after exposition of log-phase wild-type and *Klstb5Δ* cell cultures to 0.25 μg/mL 4-nitroquinoline-N-oxide for 60 min. The transcription of genes encoding MDR transporters *KlPDR5* and *KlSNQ2* genes was markedly stimulated as a result of *KlSTB5* gene deletion upon 4-nitroquinoline-N-oxide exposure. Enhanced transcription of the *KlERG6* gene was also observed in the *Klstb5Δ* mutant

Table 2 Selected *K. lactis* genes analyzed in response to *KlSTB5* disruption

K. lactis designation	*S. cerevisiae* homolog	Description
KLLA0A09119g	*PDR1*	major transcription factor that regulates the pleotropic drug response; zinc cluster protein that is a master regulator involved in recruiting other zinc cluster proteins to pleotropic drug response elements (PDREs) to fine tune the regulations of multidrug resistance genes.
KLLA0A01760g	*YAP1*	basic leucine zipper (bZip) transcription factor; required for oxidative stress tolerance; activated by H_2O_2 through the multistep formation of disulphide bonds and transit from the cytoplasm to the nuclei; Yap1p is degraded in the nuclei after the oxidative stress had passed.
KLLA0D19679g	*PDR16*	PITP protein controlled by the multiple drug resistance regulator Pdr1p, controls levels of various lipids, may regulate lipid synthesis
KLLA0A01738g	*ERG6*	delta (24)- sterol-C-methyltransferase, converts zymosterol to fecosterol in the ergosterol biosynthetic pathway
KLLA0C18931g	*KNQ1*	plasma membrane transporter of the major facilitator superfamily involved in efflux of 4-NQO
KLLA0F18106g	*TPO1*	polyamine transporter of the MFS; catalyze uptake of polyamine at alkaline pH and excretion at acidic pH; member of the 12- spamer drug:H^+ antiporter DHA1 family
KLLA0F21692g	*PDR5*	plasma membrane ATP- binding cassette (ABC) transporter actively regulated by Pdr1p; also involved in sterol transport, cation resistance and cellular detoxification during exponential growth
KLLA0D03432g	*SNQ2*	multidrug transporter involved in multidrug resistance and resistance to singlet oxygen species
KLLA0B09702g	*PDR12*	weak-acid inducible multidrug transporter required for weak organic acid resistance
KLLA0A09031g	*PMA1*	plasma membrane P2-type H^+ ATP-ase, pumps protons out of the cell; major regulator of cytoplasmatic pH and plasma membrane potential; long- loved protein asymmetrically distributed at plasma membrane between mother cells and buds

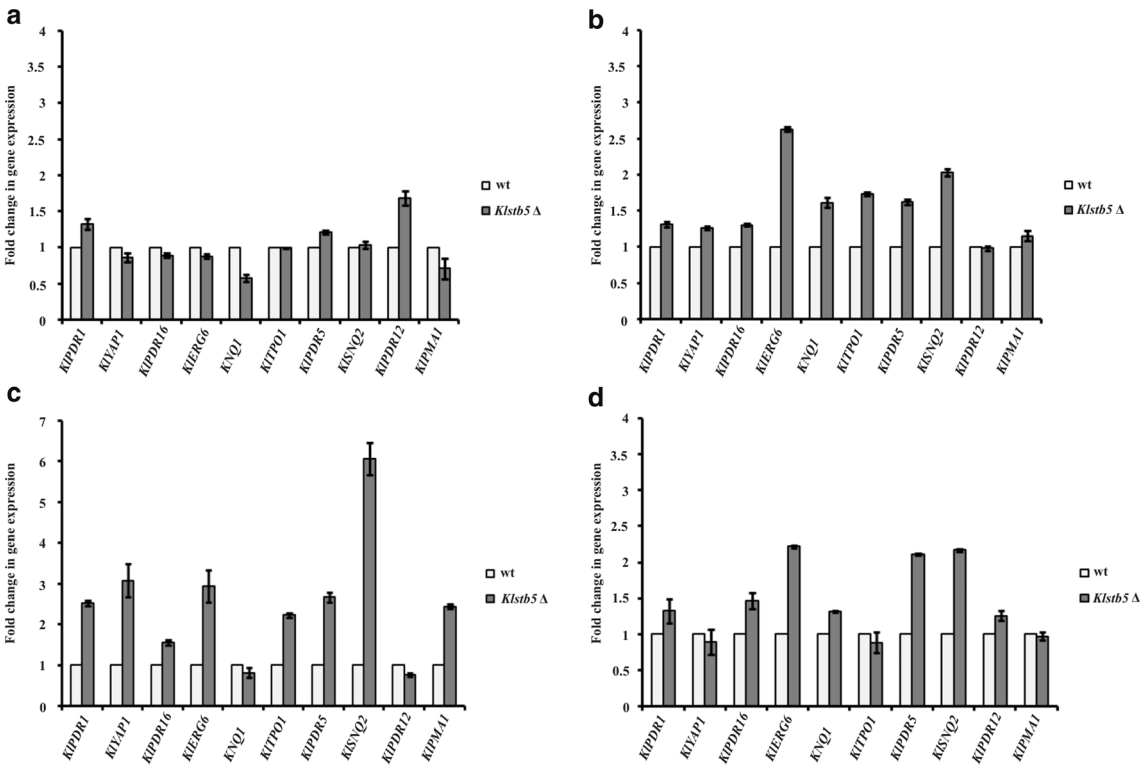

Fig. 3 mRNA levels from the selected *K. lactis* genes by qRT-PCR. **a** Gene transcription levels in *Klstb5Δ* deletion mutant compared with those in the wild-type strain. **b** Relative gene transcription levels in *Klstb5Δ* mutant cells grown in the presence of 1 mM hydrogen peroxide for 60 min. **c** Relative gene transcription levels of *Klstb5Δ* mutant cells grown in the presence of itraconazole (0.001 μg/mL) for 60 min. **d** Relative gene transcription levels in *Klstb5Δ* mutant cells grown in the presence of 4-nitroquinoline-N-oxide (0.25 μg/mL) for 60 min. The gene transcript levels in parental strain were set as 1. Data represent mean values of three measurements performed on two independent biological samples. Error bars indicate the standard deviation (SD). Note: systematic names of the *K. lactis* genes of *S. cerevisiae* othologues: KLLA0A09119g/PDR1; KLLA0A01760g/YAP1; KLLA0D19679g/ PDR16; KLLA0A01738g/ ERG6; KLLA0C18931g/ KNQ1; KLLA0F18106g/ TPO1; KLLA0F21692g/ PDR5; KLLA0D03432g/ SNQ2; KLLA0B09702g/ PDR12; KLLA0A09031g/ PMA1

(Fig. 3d). Collectively, our results suggest a negative role of *Kl*Stb5p in the transcription of *KlPDR5*, *KlSNQ2*, and *KlERG6* genes in the presence of various drugs.

Discussion

KlSTB5 gene (KLLA0A09251g) encodes a binuclear zinc cluster protein conserved among hemiascomycete yeast (Bussereau et al. 2006). The *K. lactis* protein shares 50% identity and 66% similarity with its *S. cerevisiae* counterpart. Previous studies in *S. cerevisiae* showed that *Sc*Stb5p activates the expression of most genes of the pentose phosphate pathway and other genes involved in the production of NADPH, a cofactor in conferring resistance to oxidative stress (Akache et al. 2004; Larochelle et al. 2006; Cadiere et al. 2010). The *Sc*Stb5p forms heterodimers with *Sc*Pdr1p and regulates the expression of different genes involved in *S. cerevisiae* drug resistance (Akache and Turcotte 2002).

In this work, we have initiated a comparative analysis of Stb5p function in the Crabtree-positive *S. cerevisiae* and the distantly related Crabtree-negative *K. lactis*. We demonstrate that the *K. lactis* ortholog of *ScSTB5* gene is able to compensate the loss of *ScSTB5* and enables the *Scstb5Δ* mutant strain to grow on non-fermentable carbon sources, at low temperature, and in the presence of various drugs. In contrast to *S. cerevisiae, K. lactis* does not require Stb5p for growth on non-fermentable carbon sources. As the non-fermentable substrates provide yeast with energy produced via aerobic respiration, the observed differences could result from the

Table 3 β-glucuronidase activity produced by the P_{PDR5}-gusA promoter construct without induction (no stress) and after induction by itraconazole (ITR 0.001 μg/mL, 60 min)

		β- glucuronidase activity [nmol.min^{-1}.mg^{-1}]	
strains	plasmid	no induction	ITR
wt	P_{PDR5}-*gusA*	03.5 ± 0.2	11.5 ± 1.5
Klstb5Δ	P_{PDR5}-*gusA*	4.9 ± 0.4	28.75 ± 3.5

β-glucuronidase activities are the average of three independent experiments including standard deviation

differences in primary carbon metabolism in *S. cerevisiae* and *K. lactis.*

In contrast to *S. cerevisiae*, *K. lactis* is characterized by a higher glucose flow through the pentose phosphate pathway than through glycolysis and as a consequence by a higher production of NADPH in the cytosol (González-Siso et al. 2009; Rodicio and Heinisch 2013). The production of NADPH by the glucose-6-phosphate dehydrogenase reaction is a major factor contributing to the detoxification of ROS in *K. lactis* and its tolerance to oxidative stress. *Kl*Stb5p does not appear, however, to regulate genes of the pentose phosphate pathway which is in agreement with the data obtained for *CgSTB5* in *C. glabrata* (Klimova et al. 2014). To explore the role of *Kl*Stb5p in *K. lactis* we prepared the *Klstb5Δ* deletion mutant and compared its ability to grow in the presence of various oxidants and drugs. Although we could not detect any marked difference in growth between wild-type and that of the *Klstb5Δ* deletion mutant in the presence of 4-nitroquinoline-N-oxide, hygromycin B, hydrogen peroxide, and antifungal azoles, the overexpression of *KlSTB5* conferred susceptibility to 4-nitroquinoline-N-oxide in *K. lactis*. This observation correlates with the upregulated *KlSNQ2* gene transcription in the *Klstb5Δ* mutant, pointing to the fact that *Kl*Stb5p could be a negative regulator of *KlSNQ2* expression. Besides this, the *KlSTB5* gene deletion also positively affects the transcription of genes encoding transcription factors *KlPDR1* (master regulator of multidrug resistance), *KlYAP1* (involved in oxidative stress response), *KlPDR5* (main drug efflux pump), and *KlERG6* (involved in ergosterol biosynthesis). *K. lactis* belongs to the species placed before the whole genome duplication proposed by Wolfe and Shields (1997). Most of the genes duplicated in *S. cerevisiae* are single in *K. lactis.* Inspection of the phylogenetic relationship of multidrug resistance-related ATP-binding cassette transporters in five yeast species (Gbelska et al. 2006) revealed that the *K. lactis* ORF KLLA0D03432g is a homolog of two closely related proteins from *S. cerevisiae* called *Sc*Snq2p and *Sc*Pdr18p. Coordinated activation of transcription of *ScPDR18* and several ergosterol biosynthetic genes (*ERG2–4, ERG6, ERG24*) was observed in stressed *S. cerevisiae* cells (Godinho et al. 2018). Thus, we suppose that the *K. lactis* ORF KLLA0D03432g represents a blend of the properties of both of its *S. cerevisiae* orthologs, *ScSNQ2* and *ScPDR18.*

Based on our results, we propose that there is a shared regulon between *KlPDR1* and *KlSTB5* in *K. lactis.* The genes upregulated by overexpression of *KlPDR1* are also upregulated in the absence of *KlSTB5. Kl*Stb5p, thus, functions as a negative regulator of genes involved in *K. lactis* multidrug resistance (*KlSNQ2, KlPDR5*). Similar conclusion was found in the work of Noble et al. (2013), who showed that *CgSTB5* gene encodes a transcriptional repressor of several genes implicated in azole resistance in *C. glabrata.*

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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