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The *Kluyveromyces lactis* CPY Homologous Genes – Cloning and Characterization of the *KlPCL1* Gene

D. STANEVA[a], D. UCCELLETTI[b], P. VENKOV[c], G. MILOSHEV[a], C. PALLESCHI[b]

[a]*Laboratory of Yeast Molecular Genetics, Institute of Molecular Biology, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria*
[b]*Department of Developmental and Cell Biology, University of Rome 'La Sapienza', 00185 Rome, Italy*
[c]*Department of Molecular Ecology, Institute of Cryobiology and Food Technology, 1407 Sofia, Bulgaria*
e-mail dstaneva@bio21.bas.bg

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ABSTRACT. A 3.85-kb genomic fragment containing the *KlPCL1* gene, with an open reading frame (ORF) of 1359 bp, was isolated from *Kluyveromyces lactis* genomic library by heterologous colony hybridization using the *Saccharomyces cerevisiae PRC1* (*ScPRC1*) gene as a probe. The *KlPCL1* nucleotide sequence was identical to the *KLLAOC17490g* ORF of *K. lactis* and showed >55 % identity with *S. cerevisiae YBR139w* and *PRC1* genes encoding carboxypeptidases. The deduced *Kl*Pcl1p amino acid sequence displayed strong similarities to yeast and higher eukaryotic carboxypeptidases. *In silico* analyses revealed that *Kl*Pcl1p contained several highly conserved regions characteristic of the serine-type carboxypeptidases, such as the catalytic triad in the active site and the LNGGPGCSS, FHIAGESYAGHYIP and ICNWLGN motifs involved in the substrate binding. All this suggests that the *KlPCL1* gene product belongs to the serine carboxypeptidase family. Sporulation and ascus dissection of a diploid strain heterozygous for single-copy disruption of *KlPCL1* revealed that this gene is not essential in *K. lactis*. Further analyses of haploid and diploid deletion mutants demonstrated that disruption of the *KlPCL1* gene neither impaired sporulation nor affected growth abilities of *K. lactis* cells under a variety of physiological conditions, *e.g.*, growth on different carbon sources, at various temperatures or pH of the medium, and under nitrogen depletion.

Abbreviations

CPY	carboxypeptidase Y	PCR	polymerase chain reaction
G418	geneticin	PRC	proteinase C
Kl	*Kluyveromyces lactis*	SM	synthetic medium
ME	malt extract (medium)	*Sc*	*Saccharomces cerevisiae*
MM	minimal medium	YP	yeast extract–peptone
ORF	open reading frame	YPD	yeast extract–peptone–dextrose
PCL	proteinase C-like		

The dairy yeast *Kluyveromyces lactis* is genetically closely related to the traditional baker's yeast *Saccharomyces cerevisiae* but exhibits distinct physiological characteristics (Weśolowski-Louvel *et al*. 1996). *K. lactis* is both an attractive system for scientific studies and an organism of choice for the commercial production of homologous and heterologous proteins on an industrial scale (Hussein *et al*. 1989; Van den Berg *et al*. 1990; van Ooyen *et al*. 2006). The genome of *K. lactis* has been fully sequenced (Dujon *et al*. 2004). Nevertheless, most of the *K. lactis* genes still remain uncharacterized.

Intracellular proteolytic activities are involved in post-translational maturation of precursors and activation of zymogens; in protein turnover, the catabolism of unfolded and mislocalized proteins, metabolism of exogenously supplied peptides, *etc*. Yeast contains several types of carboxypeptidases (exopeptidases removing amino acids from the carboxyl termini of proteins and peptides) with diverse functions. *S. cerevisiae* carboxypeptidase Y (*Sc*CPY; EC 3.4.16.5), also known as proteinase C (PRC), is a vacuolar glycoprotein encoded by the *ScPRC1* (*PRC* for proteinase C) gene. *Sc*CPY is a serine-type proteinase with broad substrate specificity (Hayashi *et al*. 1973) involved in general protein degradation in the vacuole, especially when cells are starved of nitrogen, and is required for degradation of proteins during sporulation (Jones *et al*. 1997; van den Hazel *et al*. 1996).

Major vacuolar carboxypeptidase activities, in particular CPY activity, have been detected and partially biochemically characterized in *K. lactis* (Flores *et al*. 1999); however, none of the three genes that would encode CPY homologues has been functionally studied so far.

Here, we report on the identification and initial characterization of the *KlPCL1* gene, whose predicted product exhibits high similarities to the *Sc*CPY and to carboxypeptidases from other eukaryotes. Our results demonstrated that disruption of the *KlPCL1* gene is not lethal for haploid *K. lactis* cells. No significant

phenotypic differences were detected between strains carrying either the disrupted or the wild-type *KlPCL1* allele.

MATERIALS AND METHODS

Strains and media. Table I lists the strains used. The cells were grown in YP-based medium (in %: yeast extract 1, peptone 2) or SM (0.67 % Yeast nitrogen base without amino acids (*Difco*) and 20 μg/mL of the appropriate auxotrophic requirements) containing 2 % (*W/V*) carbon source: glucose (YPD), galactose, lactose, glycerol or ethanol. Transformants were selected on YPD+G418 with 100 μg/mL of G418 (*Sigma*). Minimal medium (MM), used to test the growth on limited amount of nitrogen, consists of 0.17 % YNB without amino acids and diammonium sulfate (*Difco*), the required nutrition supplements (at 20 or 10 μg/L) and without or with 0.5, 0.1 or 0.025 % diammonium sulfate. For growth tests at different pH, the pH of the medium was adjusted to 3, 4, 5, 8 or 10 by addition of HCl or NaOH. ME medium (5 % malt extract, 3 % agar) was used for mating and sporulation.

Table I. List of strains used

Strain	Genotype	Constructed by	Source
	Kluyveromyces lactis		
MW270-7B	*MAT***a** *leu2 metA1-1 uraA1-1*		Billard *et al.* 1996
MW98-8C	*MAT*α *argA lysA rag1 rag2 uraA* K^+ $pKD1^0$		Bianchi *et al.* 1987
DNS1	*MAT***a**/*MAT*α *ARGA*/*argA LEU2*/*leu2 LYSA*/*lysA META*/*metA1-1 RAG1*/*rag1 RAG2*/*rag2 uraA1*/*uraA1*	mating MW270-7B and MW98-8C	this study
DNS3 (*PCL1*/Δ*pcl1*)	Isogenic to DNS1 except *KlPCL1*/*Klpcl1*Δ*427*::kanMX4	cassette integration	*ditto*
18B (*PCL1*)	*MAT***a** *leu2 metA1-1 uraA1 KlPCL1*	DNS3 meiotic segregant	*ditto*
19B (*PCL1*)	*MAT*α *argA lysA uraA1 KlPCL1*	*ditto*	*ditto*
5A (Δ*pcl1*)	*MAT*α *argA lysA uraA1 Klpcl1*Δ*427*::kanMX4	*ditto*	*ditto*
18D (Δ*pcl1*)	*MAT***a** *leu2 metA1-1 uraA1 Klpcl1*Δ*427*::kanMX4	*ditto*	*ditto*
DNS4 (*PCL1*/*PCL1*)	*MAT***a**/*MAT*α *ARGA*/*argA LEU2*/*leu2 LYSA*/*lysA META*/*metA1-1 uraA1*/*uraA1 KlPCL1*/*KlPCL1*	crossing 18B and 19B	*ditto*
DNS5 (Δ*pcl1*/Δ*pcl1*)	*MAT***a**/*MAT*α *ARGA*/*argA LEU2*/*leu2 LYSA*/*lysA META*/*metA1-1 uraA1*/*uraA1 Klpcl1*Δ*427*::kanMX4/*Klpcl1*Δ*427*::kanMX4	crossing 5A with 18D	*ditto*
	Saccharomyces cerevisiae		
FY3	*MAT***a** *ura3-52*		Winston *et al.* 1995
	Escherichia coli		
DH5α	ϕ80d*lac*ZΔM15 *rec*A1 *end*A1 *gyr*A96 *thi*-1 *hsd*R17 *sup*E44 *rel*A1 *deo*R Δ(*lac*ZYA–*arg*F)U169		Sambrook *et al*. 1989

E. coli DH5α cells were propagated in L-broth (in %: yeast extract 0.5, bacto-tryptone 1, NaCl 1), supplemented with 75 μg/mL ampicillin for selection of transformants.

DNA manipulations. Standard DNA techniques for generating recombinant DNAs, plasmid DNA preparations from *E. coli*, DNA restriction enzyme analyses and agarose-gel electrophoresis were done according to Sambrook *et al*. (1989). *E. coli* DH5α competent cells were prepared, stored and transformed according to Inoue *et al.* (1990). Yeast genomic DNA was prepared according to Hoffman and Winston (1987). *K. lactis* cells were transformed by electroporation (Lundblad *et al*. 1997) using a *Bio-Rad* gene pulser at 1.5 kV,

25 μF and 200 Ω. For PCR amplifications, four oligonucleotide sequences were used derived from the *KlPCL1* gene, *i.e*. F1 (5′-TCA ATA CCT ACC CGT TCT TCA-3′), R1 (5′-TCA TCG TGA AAG TGT TTG ACA-3′), F2 (5′-GAG CCG CAA ATT GCA AAT-3′) and R2 (5′-GTT TGC AAA GGG CTA AAT GAG-3′) and two primers for the kanMX4 module, K2 (5′-GTC GCA CCT GAT TGC CCG ACA-3′) and K3 (5′-TTG GAC GAG TCG GAA TCG CAG-3′) (*see* Fig. 2). The 1550 bp part of the *ScPRC1* ORF was PCR-amplified using primers scPRC1F (5′-GCC TGT CCA CTA CAC TCG CTA-3′) and scPRC1R (5′-ACC GTG GAT CCA TTC GTT AA-3′) and *S. cerevisiae* FY3 genomic DNA as a template. The PCR product was labeled with α-^{32}P-dATP using the Random-primed DNA labeling kit (*Roche*) as recommended by the manufacturer and employed as a probe in screening the *K. lactis* genomic library by colony hybridization and in Southern blot analysis.

Colony hybridization and Southern blot analysis. In general, the procedures were carried out according to Sambrook *et al*. (1989). *E. coli* DH5α competent cells were transformed with a *K. lactis* CBS2359/152 genomic library constructed on the KEp6 vector (Weśolowski-Louvel *et al.* 1988) and grown on ampicillin-containing L-broth plates (≈1200 colonies per plate, 27 plates in all) for 16 h at 37 °C. Colonies were replicated onto a Sartolon blotting membrane disc (*Sartorius*) by leaving the membrane on the plate for 1 min. The membranes, always with colonies side up, were further processed for cell lysis and DNA denaturation and fixation to the filter *in situ* (Sambrook *et al*. 1989). Hybridizations were performed at low-stringency (6× SSC, 0.1 % SDS; 50 °C) using the radioactively labeled *ScPRC1* probe, followed by two washes with 2× SSC, 0.1 % SDS at 50 °C for 20 min per wash.

Disruption of PCL1 *chromosomal gene in* K. lactis. To construct a cassette for disruption of *KlPCL1* ORF, the 2117 bp *Xho*I–*Hin*dIII fragment from the selected genomic subclone pBS-74-*Kl*PCL1-3.85 was cloned into the *Xho*I–*Hin*dIII-digested pBluescript II KS vector. Then the 427-bp *Sma*I–*Eco*RV fragment from the coding region of *KlPCL1* gene was replaced with the kanMX4 module, derived from pFA6-kanMX4 (Wach *et al.* 1994). The obtained construct, pBS-*Kl*pcl1Δ::kanMX4, was used as a template for PCR amplification of the *Klpcl1Δ427*::kanMX4 disruption cassette with primers F1 and R1 (*see* Fig. 2). Disruption of the chromosomal *KlPCL1* locus was verified using PCR.

Sporulation, ascus dissection and ascospore analysis. Diploid strains were sporulated by a 2-d cultivation on ME medium at 28 °C. Following treatment with NEE-154 Glusulase (*DuPont*) asci were dissected using the Singer® *MSM Instruments*. The phenotypes of segregants were examined on YPD medium containing G418 and on SM supplemented appropriately. The mating type of the segregants was determined according to Pearson *et al*. (1998).

Growth curve assay and phenotypic tests. The cells were pregrown in YPD overnight at 28 °C and then re-inoculated into fresh YPD medium at an A_{600} of 0.1. Samples were taken at intervals and the cell concentrations were recorded at 600 nm.

Heterozygous and homozygous diploid disruptants and the parental haploid disruptants were cultivated in YPD and 4 μL of equal, 10-fold serial dilutions of cells were spotted on plates containing differently supplemented media (*e.g*., YP-based or SM medium supplied with different carbon sources, MM with or without diammonium sulfate, media with pH ranging from 3 to 10). For comparison, haploid and diploid wild-type strains were also included. Plates were examined for growth after 1, 2, 3, and 5 d.

Computational analysis of nucleotide and derived amino acid sequences. The pairs of *S. cerevisiae* and *K. lactis* proteins were compared at Génolevures (**http://cbi.labri.fr/Genolevures/elt/KLLA/**) with the pairwise BLASTP (version 2.2.6). For multiple alignments we used the program DNAMAN version 4.13 (*Lynnon BioSoft*). The sequence of the *KlPCL1* gene is deposited in the *GenBank* library under accession no. AJ551275.

RESULTS AND DISCUSSION

Isolation of the KlPCL1 *gene and* in silico *analysis of the nucleotide sequence*. The *K. lactis* genome contains three ORFs that would encode a CPY homologue, *i.e*. *KLLA0C17490g*, *KLLA0E17897g* and *KLLA0A09977g*. To pick out *K. lactis* gene(s) related to the *ScPRC1* gene (coding for CPY), a *K. lactis* CBS2359/152 genomic library (Weśolowski-Louvel *et al.* 1988) was screened by bacterial colony hybridization using the *ScPRC1* gene as a probe. Screening ≈30 000 transformants, 18 positive colonies were initially identified. Southern analysis of plasmid DNA isolated from one of the selected colonies revealed the presence of ≈3.85 kb genomic insert which cross-hybridized specifically to the *ScPRC1* probe (*data not shown*). Sequencing of the isolated genomic clone disclosed an ORF of 1359 bp (*GenBank* accession no. AJ551275). Sequence comparisons indicate that this ORF is identical with the *K. lactis* ORF *KLLA0C17490g* and highly similar to the *S. cerevisiae YBR139W* (59 % identity) and *ScPRC1* (57 % identity) genes encoding CPYs,

and to *KLLA0E17897g* and *KLLA0A09977g* ORFs of *K. lactis* (59 and 57.6 % identity, respectively). The identified ORF was named *KlPCL1* (*PCL* for proteinase C-like).

Computational analysis of the deduced Kl*Pcl1p amino acid sequence*. Analysis of the *KlPCL1* gene product using SignalP 3.0 Server (Bendtsen *et al*. 2004) and PSORT II (**http://psort.nibb.ac.jp/**) indicated the presence of an N-terminal signal sequence and a putative peptidase cleavage site (Fig. 1). No localization signal was identified. Five putative *N*-glycosylation sites matching the consensus pattern NXS/T was found in the *Kl*Pcl1p sequence.

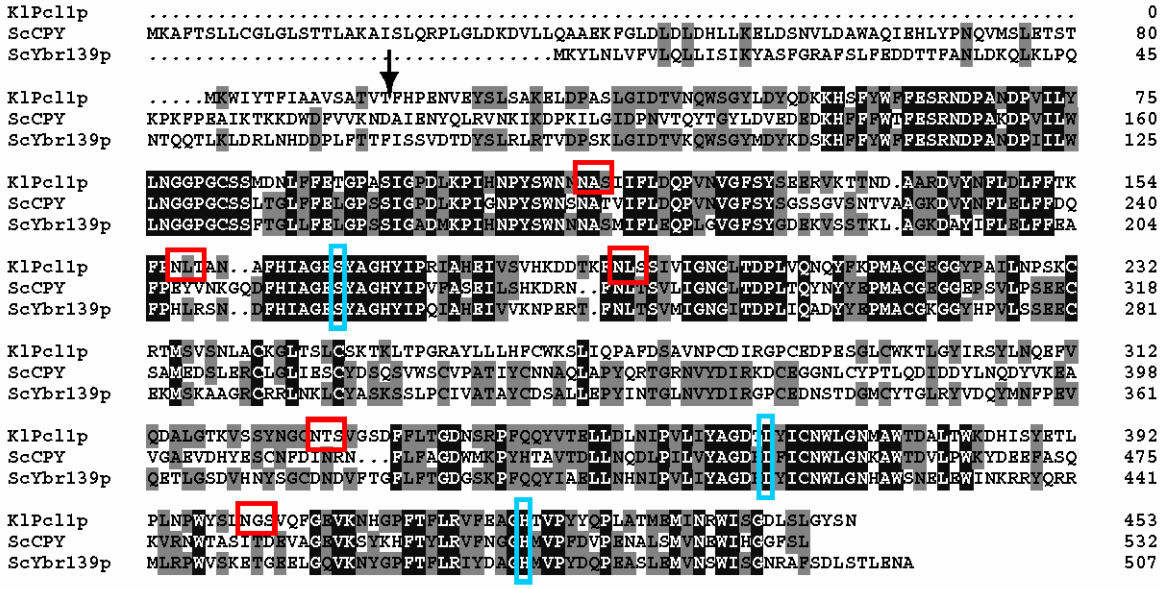

Fig. 1. Alignment of the deduced amino acid sequence of *Kl*Pcl1p with two *S. cerevisiae* serine-carboxypeptidases. The conserved residues of the catalytic triad S169, D366 and H425 in *Kl*Pcl1p are marked by *blue boxes*. The *arrow* indicates the predicted signal peptidase cleavage site. The five putative sites for *N*-linked glycosylation are *red framed*. Sequences shown are for *K. lactis* Pcl1p (*Kla*cPcl1p, accession nos. Q70SJ1), *S. cerevisiae* CPY (*Sc*CPY, P00729), and *S. cerevisiae* Ybr139Wp (*Sc*Ybr139p, P38109).

The amino acid sequence comparisons revealed that the predicted *Kl*Pcl1p polypeptide (UniProtKB Q70SJ1) was homologous with serine-type carboxypeptidases of different organisms, *e.g.*, with *S. cerevisiae* Ybr139Wp (56 % identity, 73 % similarity), with *Sc*CPY/*Sc*Prc1p (51 %), with *K. lactis KLLA0E17897g* and *KLLA0A09977g* predicted products (49 and 60 % identity, respectively) as well as with CPYs of other yeasts (52–49 % identity). The identity with non-PRC carboxypeptidases, such as *Sc*Kex1p, KLLAOF09999g, some plant carboxypeptidases and the human cathepsin A, was only 28–33 %. Several regions exhibiting extremely high conservation in *Kl*Pcl1p and carboxypeptidases from divergent organisms were identified. Such regions, shown to be crucial for the functioning of these proteins, included the three essential residues S, D and H that form the catalytic triad of serine-type carboxypeptidases (Jung *et al*. 1999; Liao and Remington 1990; Nasr *et al*. 1994). These residues can be identified in the *Kl*Pcl1p amino acid sequence as S-169, D-366 and H-425 (Fig. 1). The nonapeptide LNGGP GCSS (76–84), the sequence surrounding the active site serine FHIAG ESYAG HYIP (163–176) and the motif ICNWL GN (368–374) all showed significant conservation. Each of these motifs comprises components (underlined residues) of the substrate binding subsites, the oxyanion hole or the hydrogen bond network (Liao and Remington 1990; Nasr *et al*. 1994; Jung *et al*. 1999; Nakase *et al*. 2001).

Based on sequence similarities at nucleotide and amino acid levels and the existence of several motifs characteristic of serine-type carboxypeptidases one can propose that the *KlPCL1* gene codes for a serine carboxypeptidase. In the *S. cerevisiae* genome two genes, *ScPRC1* and *ScYBR139w*, encode serine-type carboxypeptidases (Stennicke *et al*. 1996; Baxter *et al*. 2004) while in the *K. lactis* genome three ORFs, *KLLA0A09977g*, *KLLA0E17897g* and *KLLA0C17490g*, showed homology to this protein family. It has been reported that the order of genes on chromosomes (synteny), in some short regions, had been preserved in *K. lactis* and *S. cerevisiae* (Byrne and Wolfe 2006). Shared synteny is one of the most reliable criteria for establishing the orthology of genomic regions in different species. We examined the genomic context around each of the *KLLA0A09977g*, *KlPCL1* (*KLLA0C17490g*) and *KLLA0E17897g* genes in *K. lactis* and *S. cerevisiae*. Based on sequence similarity and synteny data we concluded that *KLLA0A09977g* is the orthologue

of *ScPRC1*. For *KlPCL1* gene, comparison of the neighboring genomic regions revealed many chromosome rearrangements, therefore no definite orthologue can be drawn.

KlPCL1 *is a non-essential gene in* K. lactis. As a first step of *KlPCL1* functional analysis we disrupted the chromosomal *KlPCL1* gene in order to determine whether the gene is essential for the viability of *K. lactis* cells. The PCR-amplified disruption cassette *Klpcl1Δ427*::kanMX4 (*see Materials and Methods*, Fig. 2A) was used to transform a well-sporulating diploid strain DNS1 (Table I). Six stably G418-resistant

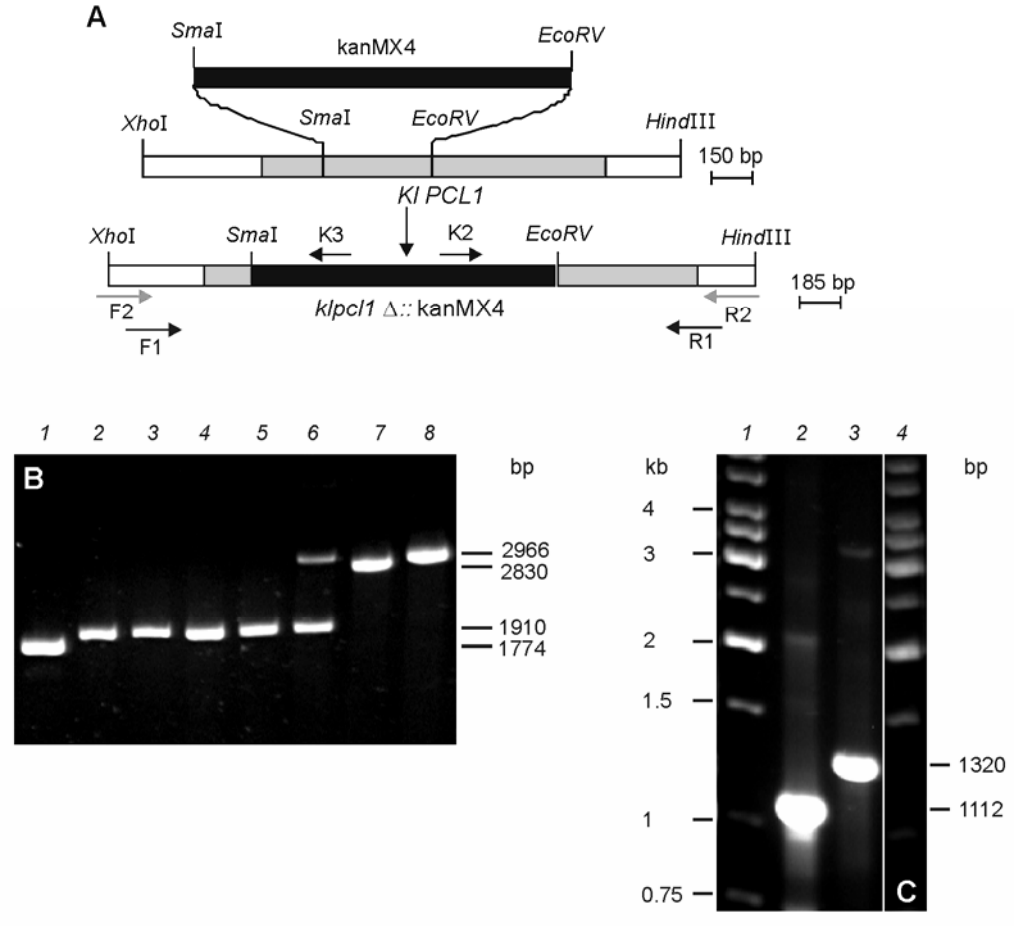

Fig. 2. Disruption of the *KlPCL1* gene. **A**: Scheme of the *KlPCL1* g gene disruption cassette and its integration into the *KlPCL1* chromosomal locus. The cassette contained the kanMX4 module, which replaced a region of 427 bp from the *KlPCL1* coding sequence. Primers used in PCR analysis are indicated as *arrows* and are derived from chromosomal DNA sequences (F1, R1, F2 and R2) or kanMX4 cassette (K2 and K3). **B**: PCR analysis of integrative transformants. Agarose gel electrophoresis of DNA fragments amplified from chromosomal DNA of *K. lactis* G418^R diploid transformants (lanes *3*–*6*), the wild-type strain MW270-7B (lanes *1*, *2*) and on the disruption cassette (lanes *7*, *8*). PCR was performed by using primer pairs F1–R1 (lanes *1*, *7*) and F2–R2 (lanes *2*, *3*–*6*, *8*). **C**: PCR analysis of the insertion boundaries. PCR amplification was performed employing chromosomal DNA from the diploid heterozygous strain DNS3 and the primer pairs F2–K3 (lane *2*) and K2–R2 (lane *3*). In lanes *1* and *4*, GeneRuler^{TM} 1-kb DNA ladder.

(G418^R) DNS1 transformants were selected and disruption of the *KlPCL1* chromosomal locus was verified by PCR analysis employing primer pairs F2–R2, F2–K3 and K2–R2 (Fig. 2). One out of six G418^R transformants displayed the expected 2966 bp fragment (corresponding to the disrupted allele *Klpcl1Δ427*::kanMX4) in addition to the 1910 bp (wild-type) amplicon using F2–R2 primer pair (Fig. 2B, lane 6), and the 1112 bp F2–K3 and the 1320 bp K2–R2 amplified fragments (kanMX4 integrated into *KlPCL1*) (Fig. 2C). This heterozygous strain *KlPCL1*/*Klpcl1Δ427*::kanMX4 was named DNS3 (Table I).

After sporulation of DNS3, 40 asci were dissected on YPD giving rise to 37 complete tetrads, containing four viable spores. $2^+:2^-$ segregation for all auxotrophic markers (except for uracil auxotrophy) and two G418^R:2 G418^S spores in each tetrad were detected (Fig. 3). The colonies resulting from dissection of some but not all asci varied in size and obviously this feature did not correlate directly to the distribution of the *KlPCL1* alleles (wild-type or disrupted). Most probably it is determined by a combination of factors as its segregation differs from 2:2. Segregants of three randomly chosen tetrads (numbers 5, 7 and 14) were

analyzed by PCR using the R2–F2 primer pair. The 2:2 segregation of the *KlPCL1* and *Klpcl1*Δ*427*::kanMX4 alleles (1910-bp and 2966-bp bands, respectively) in haploid descendants derived from one and the same tetrad is showh in Fig. 3. It is noteworthy that all G418^S spores carried the intact *KlPCL1* gene, while G418^R haploids all carried the disrupted allele *Klpcl1*Δ*427*::kanMX4. The ability of the haploid strains carrying the *Klpcl1*Δ*427*::kanMX4 allele to grow on YPD as well as the haploids carrying the wild-type allele demonstrated that disruption of the *KlPCL1* gene is not lethal. Therefore, the *KlPCL1*gene is not essential for viability of *K. lactis* cells.

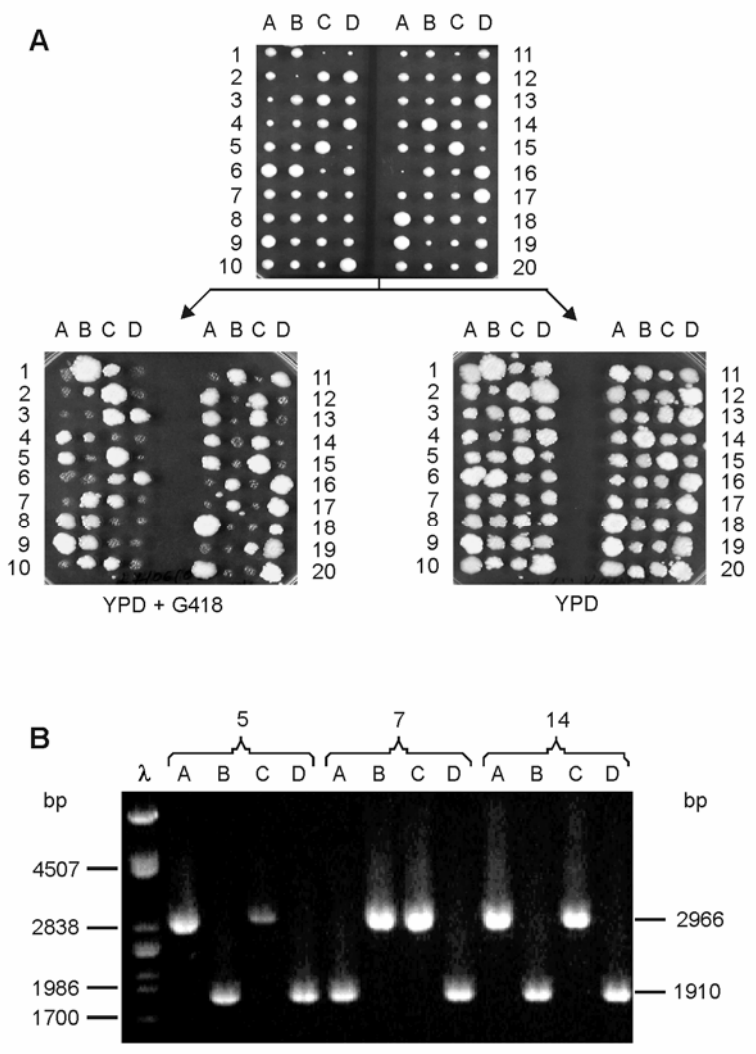

Fig. 3. Tetrad analysis of DNS3 heterozygous strain *KlPCL1*/*Klpcl1*Δ*427*::kanMX4. **A**: The *KlPCL1* is a non-essential gene. All four spores derived from one and the same ascus are viable. Segregation 2:2 was observed for G418^R:G418^S phenotypes in each tetrad. **B**: PCR analysis of genomic DNA isolated from each spore of three randomly chosen tetrads (numbers 5, 7, and 14) using primer pair F2–R2. All spores that contained the *Klpcl1*Δ*427*::kanMX4 allele (2966-bp band) were G418^R, while those carrying the *KlPCL1* allele (1910-bp band) were all G418^S; λ – λ DNA digested with *Pst*I.

Phenotypic effect of KlPCL1 *gene inactivation*. To further investigate the biological role of the *KlPCL1* gene product, we compared the growth abilities of strains carrying the disrupted allele with strains carrying the wild-type allele of *KlPCL1* under a variety of physiological conditions. From the growth curves (Fig. 4) it is obvious that the growth rates of 19B (*PCL1*) and 18D (Δ*pcl1*) haploid cell cultures almost overlap. Such similarity was observed for the growth of the heterozygous DNS3 (*PCL1*/Δ*pcl1*) as well as the homozygous DNS4 (*PCL1*/*PCL1*) and DNS5 (Δ*pcl1*/Δ*pcl1*) diploid cell cultures (*data not shown*). It has been demonstrated that *S. cerevisiae* genes *PRC1* and *YBR139w* are up-regulated as cells responded to environmental stress conditions including temperature shock and nitrogen depletion (Gasch *et al*. 2000; Brown *et al*. 2001). In addition, analyzing the promoter of *KlPCL1* for transcription regulatory sites we found three heat-shock elements. All this suggests the involvement of *KlPCL1* in response to elevated temperature.

However, haploid and diploid *KlPCL1* disruptants cultured on differently supplemented media at 15, 28 and 37 °C grew to the same extent as the control wild-type strains, irrespective of the cultivation temperature, the media (rich or synthetic) and the carbon source supplied, fermentable or non-fermentable (*data not shown*). Likewise, regardless of the genotype, all strains grew in a similar manner on limited amounts of ammonium ions and at different pH of the medium (*data not shown*). Taking into account the involvement of *Sc*CPY in the bulk protein degradation during sporulation and the presence of two predicted middle sporulation elements in the promoter region of *KlPCL1*, one can expect an impaired sporulation ability of the homozygous DNS5 disruptant. However, the diploid strain DNS5 showed the same sporulation capacity as that of the heterozygous diploid DNS3 and the homozygous wild-type strain DNS4, suggesting that the *KlPCL1* gene product is dispensable for sporulation in *K. lactis*.

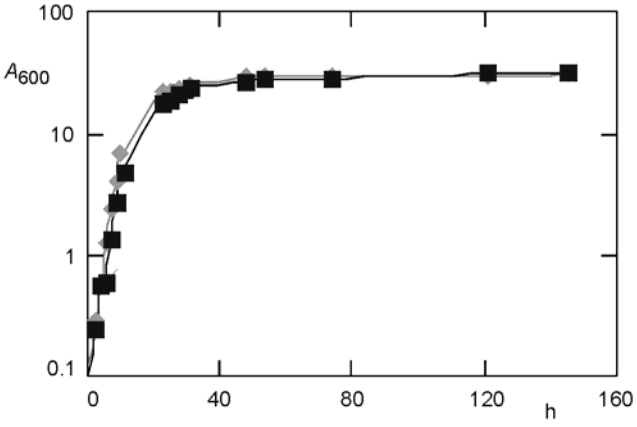

Fig. 4. Growth curves of haploid *K. lactis* strains 18D (Δ*pcl1*; *diamonds*) and 19B (*PCL1*; *squares*) carrying the disrupted or the wild-type allele of *KlPCL1* gene, respectively. The cells were grown in YPD medium and A_{600} was measured at the times (h) indicated; values are means of three independent experiments with >10 % variation. Δ*pcl1* – *Klpcl1Δ427*::kanMX4 allele, *PCL1* – *KlPCL1* allele.

Although that the deleted region of *KlPCL1* coding sequence, encompassing amino acids 81–222, comprises the predicted active site serine and components of the putative substrate binding motifs (Fig. 1), disruption of *KlPCL1* did not affect cell growth under the conditions tested and the sporulation capacity. Thus, it appears that *KlPCL1*, similarly to the *ScPRC1* and *ScYBR139w* genes of *S. cerevisiae* (Nasr *et al*. 1994; Winzeler *et al*. 1999), is a non-essential gene and its inactivation does not lead to an easily discernible phenotype in *K. lactis*.

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