The YGR194c (XKS1) gene encodes the xylulokinase from the budding yeast Saccharomyces cerevisiae

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Abstract

We report the finding of a Saccharomyces cerevisiae gene necessary for growth in culture media with D-xylulose as the sole carbon source. This gene corresponds to the YGR194c open reading frame that we have previously described, and it is renamed now XKS1. Data bank comparisons of the protein encoded by the XKS1 gene showed significant homology with different xylulokinases, indicating a possible role in xylulose phosphorylation. The wild-type gene in a centromeric plasmid complemented defective growth of xks1 S. cerevisiae mutant strains in xylulose. By contrast, overexpression negatively influenced cell growth in this carbon source. © 1998 Federation of European Microbiological Societies. Published by Elsevier Science B.V.

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1. Introduction

In the framework of the European BIOTECH program for sequencing of the Saccharomyces cerevisiae genome, we have recently sequenced a DNA fragment from the right arm of chromosome VII. An open reading frame (ORF) not previously described, named YGR194c, was identified in this fragment (EMBL Accession Number X82408) [1,2]. Ygr194c showed representative homology with bacterial D-xylulose kinases. This family of enzymes is involved in the phosphorylation of xylulose for the integration of the phosphorylated product in the pentose phosphate pathway (PPP) [3]. Several members of the xylulokinase family have been identified in bacteria [4–6]. However, in eukaryotic organisms, although xylulokinase activity has been detected in yeast [7,8] and a DNA fragment presumably involved in this function has been isolated from S. cerevisiae [9], to date no DNA or protein sequences have been reported to be related to this function.

The fermentation of pentose sugars has economic importance for the production of ethanol from lignocellulosic biomass, where the major pentose sugar is xylose. Although it is generally accepted that S. cerevisiae is unable to utilize xylose for growth, this ability has been described, at very low efficiency, in the presence of a mixture of substrates [10]. S. cerevisiae is unable to metabolize xylose due to the lack of the enzymes xylose reductase (XR) and xylitol

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dehydrogenase (XDH). As a solution, metabolic engineering has been used to improve the xylose-metabolizing pathway [3,11]. The product generated, xylulose, is readily utilized by many \textit{S. cerevisiae} strains [7,12], although at a rate considerably lower than when glucose is used, via phosphorylation to xylulose-5-P and incorporation into the PPP.

Sequence analysis of \textit{YGR194c} and related xylulokinases was carried out, showing the existence of several conserved domains characteristic of sugar kinases. To test if \textit{YGR194c} gene encodes for the putative \textit{S. cerevisiae} xylulokinase activity, we created strains specifically deleted in this ORF and characterized them for the ability of growing in xylulose as sole carbon source. The effect of overexpression of this gene in \textit{S. cerevisiae} was also studied.
2. Materials and methods

2.1. Construction of *S. cerevisiae* xks1 deletant strains

Disruptions were performed in parallel in two *S. cerevisiae* diploid strains, FY1679 (MATa; *ura3-52*/*ura3-52*; *his3A200*/*HIS3*; *leu2Δ1*/*LEU2*; *trplΔ63*/*TRP1*; GAL2/*GAL2*) and W303 (MATα; *ura3-311*/*ura3-1*; *his3-15*/*his3-11*; *I5*; *leu2-3, 112*/*leu2-3, 112*; *trpl-11trp1-1*; *ade2-1*/*ade2-1*; *can1-100*/*can1-100*). Gene disruption was performed by the LFH (Long Flanking Homology) polymerase chain reaction (PCR) technique [13], allowing substitution of the target ORF by the *kanMX4* marker [14], which confers resistance to geneticin (Gibco BRL). The oligonucleotides devised for this purpose were: L1, 5′-GGATCCGTCGACCTGCAGCGTACCATTAA-3′; L2, 5′-GGTATAGAAACTGATAGAAATG-3′; L3, 5′-AAGCAGCGTCAATTCATCTCATCCT-3′; and L4, 5′-CCCTGAGATG-ATTAAACAATA-3′, where the primer pair L1–L2 amplifies the DNA region directly upstream from the target gene coding region, and L3–L4 the downstream one. Yeast transformation by the lithium acetate protocol [15], modifying the final steps to allow the expression of the *kanMX4* cassette, afforded geneticin-resistant transformants. Cells were recovered, plated into YPD plates supplemented with 200 mg l−1 geneticin and grown at 28°C for 2–3 days until the appearance of stable geneticin-resistant clones. Sporulation and tetrad analyses were performed by standard micromanipulation procedures, to give wild-type and deleted mutant haploid strains.

2.2. Cloning of the XKS1 gene and construction of the overexpression cassette

Standard molecular biology techniques for DNA manipulation and bacterial transformation were performed by the usual procedures [16]. The *Escherichia coli* strain used as plasmid host was DH5α (supE44; ΔlacU169 (Δ80lacZAM15); hsdR17; recA1; endA1; gyrA46; thi1; relA1). The construction bearing the *XKS1* gene, pJV11, was constructed by ligating the *NsiI−PstI* 3.3-kbp fragment from pEGH059 cosmid subclone E1 [1] into *PstI*-cleaved centromeric *pRS416* vector [17]. The overexpression cassette was constructed in two consecutive steps; first, the pJV9 plasmid was obtained by ligating the promoterless 2.1-kbp fragment *XhoI−XhoI*, after generation of blunt ends, from pJV11 into *SmaI*-cleaved *pBlueScript* KS vector (Stratagene); then, pJV10 was constructed by ligating the *BamHI−EcoRV* insert from pJV9 into *BamHI−Pmel*-cleaved *pCM190* vector, which includes a tetracycline-regulatable promoter [18].

2.3. Growth in different carbon sources

These assays were carried out on solid and liquid media. Experiments on solid media were done in 4-well microplates (Nunclon), by addition of 1 ml of Minimal Medium (composed of 1.67 g l−1 yeast nitrogen base without amino acids, 5 g l−1 ammonium sulfate and the appropriate amount of amino acids except uracil, a carbon source if any (5 g l−1) and 20 g l−1 agar-agar (MM-Ura)) to each well. The plate was placed at 30°C to account for the latency period due to the slow consumption of xylulose. The plate was placed on the centre of each well and the plates were incubated at 30°C for 4 days.

For experiments developed in liquid media, a preinoculum was first obtained by growing yeast cells overnight at 30°C in MM-Ura. 105 cells were then inoculated in 96-well microtiter plates, achieving a final volume of 200 μl of MM-Ura plus the corresponding carbon source, xylulose, glucose, D-xylose, or without carbon source. The plates were incubated for 4 days at 30°C and periodically observed under the microscope.

For obtaining growth curves, a 96-well Integrated EIA Management System reader (Labsystems) was used. In these experiments, growth conditions were the same as indicated above for liquid media, but after inoculation the plate was incubated for 1 day at 30°C to account for the latency period due to the slow consumption of xylulose. The plate was placed into the optical density reader at 30°C, which took data in 2-h intervals over a period of 30 h.
3. Results and discussion

3.1. The protein encoded by YGR194c shows homology with xylulose kinases

YGR194c encodes a hypothetical protein of 600 amino acid residues. Comparisons with data base sequences gave 31% identity over a 531-amino acid (aa) overlap with the hypothetical protein RO8D7.7 of Caenorhabditis elegans chromosome III and to a lesser extent (25% identity over a 351-aa overlap) with different prokaryotic xylulokinases. On analyzing the amino acid sequence deduced from the YGR194c gene we found a set of conserved residues corresponding to the common ATPase domain of sugar kinases described by Bork et al. [19] (Fig. 1). In particular, Ygr194c was conserved in 78% within the PHOSPHATE 1 and in 94% within the PHOSPHATE 2 consensus regions, as well as in the adenosine-binding region (79%), all of these regions being involved in ATP binding [19]. Conserved regions include amino acids, such as D\textsuperscript{27} and G\textsuperscript{319}, which are common to all sugar kinases. D\textsuperscript{27} is even conserved in other protein families having in common an ATPase domain, such as those from the actin and Hsp70 families [19]. In addition, the residues G\textsuperscript{25}, T\textsuperscript{30} and K\textsuperscript{34} within the PHOSPHATE 1 domain, and the S (I/L) GTS consensus in the PHOSPHATE 2 domain are characteristic of all prokaryotic xylulokinases and are also conserved in the S. cerevisiae and C. elegans homologues (Fig. 1). However, the glycine following the S (I/L) GTS consensus in all the prokaryotic sequences is not conserved in the two putative eukaryotic homologues available (Fig. 1). Moreover, the localization of the PHOSPHATE 1 and PHOSPHATE 2 domains in the protein and the distance between them is constant in all the members of the xylulokinase family, including the two eukaryotic sequences (Fig. 1), suggesting a common three-dimensional structure. Taking into account these homology data, it is very likely that the YGR194c gene would in fact be a member of the xylulokinase family depicted here. Although this aspect should be confirmed by future biochemical studies, the genetic evidence presented below led us to rename this open reading frame \textit{XKS1}.

3.2. \textit{XKS1} is required for growth when \textit{d}-xylulose is provided as the sole carbon source, but its overexpression is deleterious

In order to test if \textit{XKS1} gene was critical for growth in \textit{d}-xylulose as the sole carbon source, \textit{S. cerevisiae} diploid strains, FY1679 and W303, were deleted specifically in this gene as described above. This strategy allowed the deletion of the \textit{XKS1} gene from nucleotide 4–1800.

The high cost of pure \textit{d}-xylulose precludes growth experiments in standard culture volumes. We therefore devised a growth system in micro-volumes using microtiter plates as described above. In preliminary assays we obtained equivalent results in FY1679 and W303 strains (data not shown), and so henceforth only the data obtained with the FY1679 strain will be referred to.

A haploid mutant segregant was independently transformed with the pJV11 plasmid (bearing the \textit{XKS1} gene) and the control pRS416 plasmid, while a wild-type segregant was separately transformed with pRS416 and the plasmid bearing the overexpression cassette (pJV10). The ability of these transformed strains to grow on glucose- or \textit{d}-xylulose-containing media, as well as in media lacking a carbon source, was tested as described in Section 2. All strains tested were seen to grow normally in glucose (Fig. 2). When xylulose was present as the sole carbon source, the xks1::kanMX4 (pRS416) mutant strain did not grow, or grew to the same extent as any of the tested strains on a medium lacking the carbon source (Fig. 2). However, the same mutant strain transformed with the plasmid bearing the wild-type \textit{XKS1} gene (pJV11) was able to grow on xylulose, confirming the participation of the protein encoded by \textit{XKS1} in xylulose metabolism. The complemented mutant strain grew on xylulose even better than the wild type. This could be explained by the fact that the number of copies of the pRS416 plasmid, even though this is a centromeric vector, may slightly increase the gene dosage, thus improving the utilization of xylulose by the cell. By contrast, its overexpression (strain bearing pJV10) afforded normal growth on glucose but surprisingly affected cell growth in a negative manner when xylulose was the only carbon source (Fig. 2). This could be understood in terms of the
availability of adenosine triphosphate (ATP) in the cell or the toxicity of the possible reaction product, xylulose-5-P. More studies will be required to clarify this point. When we tested xylulose-5-P (Sigma) as the carbon source to bypass the xylulose to xylulose 5-P step, neither the wild-type nor the mutant strains grew (data not shown), possibly due to the lack of a substrate transport system inside the cell or again due to the hypothetical toxicity of high xylulose-5-P levels.

Experiments performed in liquid medium confirmed the results indicated above. Neither mutant nor wild-type strains were able to grow in xylulose (data not shown), confirming previous reports [10]. Furthermore, only the wild-type strain and the complemented mutant strain were able to grow in xylulose, the latter showing more efficiency. In addition, growth curves were obtained from wild type (pRS416), xks1::kanMX4 (pJ11) and xks1::kanMX4 (pJV11) in MM-Ura containing glucose, xylulose and in the absence of carbon source. The experiment was carried out with three different transformants of each tested strain. The deletant strain was unable to assimilate xylulose, showing growth rates similar to those obtained in the absence of carbon source, whereas the latter showed more efficiency. In addition, growth curves were obtained from wild type (pRS416), xks1::kanMX4 (pJ11) and xks1::kanMX4 (pJV11) in MM-Ura containing glucose, xylulose and in the absence of carbon source. The experiment was carried out with three different transformants of each tested strain. The deletant strain was unable to assimilate xylulose, showing growth rates similar to those obtained in the absence of carbon source ($\mu = 0.087 \pm 0.009$ h$^{-1}$ in xylulose; $\mu = 0.085 \pm 0.004$ h$^{-1}$ without carbon source). The experiment also revealed that the phenotype studied is rescued by the wild-type gene in a centromeric plasmid, achieving slightly higher (but comparable) growth rates than the wild-type strain ($\mu = 0.225 \pm 0.05$ h$^{-1}$).

In summary, our data suggest that the XKS1 gene product belongs to the xylulokinase family. Consistent with this, Ho and coworkers [9,20] have isolated a S. cerevisiae DNA fragment able to complement a xylulokinase E. coli mutant and showed that a disrupted yeast strain displays reduced xylulokinase activity in terms of $\alpha$-xylulose disappearance, although no sequence was provided. Our data clearly suggest that the XKS1 gene, corresponding to the recently sequenced S. cerevisiae ORF YGR194c, encodes the budding yeast xylulokinase. Furthermore, overexpression of this gene, using a tetracycline-regulatable promoter, demonstrates that high levels of expression of this gene are toxic for the cells when grown with xylulose as the sole carbon source.

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